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Review Article

# Assembly of mammalian oxidative phosphorylation complexes I–V and supercomplexes

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The assembly of the five oxidative phosphorylation system (OXPHOS) complexes in the inner mitochondrial membrane is an intricate process. The human enzymes comprise core proteins, performing the catalytic activities, and a large number of 'supernumerary' subunits that play essential roles in assembly, regulation and stability. The correct addition of prosthetic groups as well as chaperoning and incorporation of the structural components require a large number of factors, many of which have been found mutated in cases of mitochondrial disease. Nowadays, the mechanisms of assembly for each of the individual complexes are almost completely understood and the knowledge about the assembly factors involved is constantly increasing. On the other hand, it is now well established that complexes I, III and IV interact with each other, forming the so-called respiratory supercomplexes or 'respirasomes', although the pathways that lead to their formation are still not completely clear. This review is a summary of our current knowledge concerning the assembly of complexes I–V and of the supercomplexes.

## Introduction

The oxidative phosphorylation system (OXPHOS) of the mitochondrial inner membrane is composed of five enzymes (complexes I–V; cI–V). In mammals, they are all multimeric and, except for cII, have subunits encoded both in the mitochondrial genome (mtDNA) and the nuclear genome (nDNA). The mtDNA-encoded subunits are hydrophobic and their translation happens close to the inner membrane to facilitate their translocation [1]. The nuclear-encoded structural subunits and many other factors necessary for the correct biogenesis of OXPHOS are expressed in the cytoplasm and imported inside the organelle [2].

Assembly of mitochondrial complexes II–V has been extensively studied in *Saccharomyces cerevisiae* [3-7], whereas research concerning cI has been carried out in *Yarrowia lipolytica* [8] and *Neurospora crassa* [9]. Many factors and mechanisms are conserved in mammals, and this has helped to identify genetic mutations associated with mitochondrial disease. However, it is now evident that there are specific factors in higher animals that are also involved in OXPHOS biogenesis and efforts are being made to understand their exact functions and implications in disease (see article by Ghezzi and Zeviani in this issue [201]). Moreover, studying assembly defects both in human cells and mouse disease models, has given highly valuable information about the assembly pathways and the proteins involved [10].

The OXPHOS complexes can interact with each other forming higher order structures, called supercomplexes or 'respirasomes' [11-13], whose functional role and assembly are still not completely understood [14-18].

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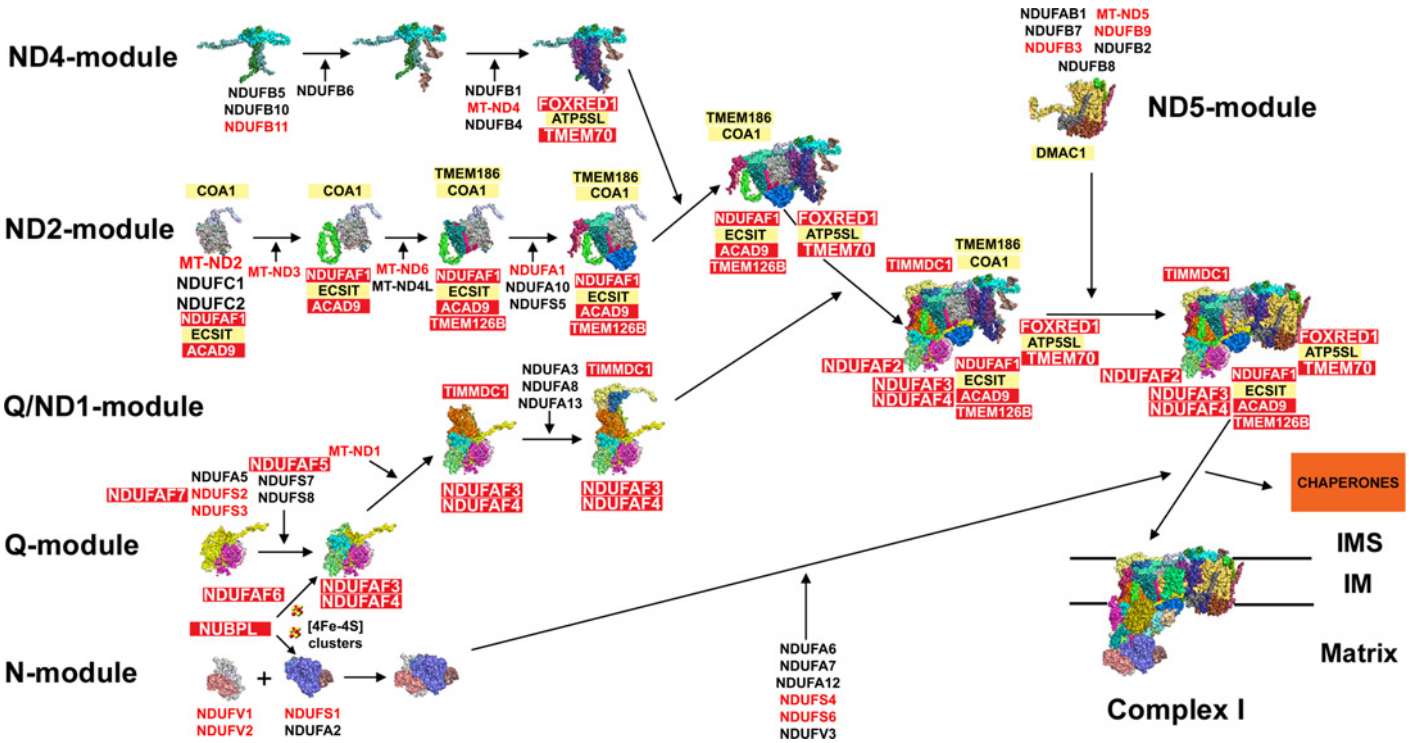


**Figure 1. Complex I assembly model (see main text for details) based on the bovine cI cryo-EM structure with Protein Data Bank (PDB) ID: 5LC5 [23] and the models proposed in references [33,34,199]**

Red colour indicates proteins with described pathological mutations. Abbreviations: IM, inner membrane; IMS, intermembrane space.

## Assembly of complex I

Complex I (EC 1.6.5.3) or NADH:ubiquinone reductase ($H^+$ translocating) with 45 subunits is the largest OXPHOS complex. It is an L-shaped enzyme with a hydrophilic arm protruding into the matrix, where electron transfer from NADH to coenzyme Q (CoQ) happens, and a proton translocating hydrophobic arm. The CoQ binding site is in the interface of both arms. Fourteen core subunits, conserved from bacteria to humans, perform the catalytic activities [19,20]. Seven core subunits in the hydrophilic arm contain the redox active centres: a non-covalently bound FMN and seven Fe–S clusters [21]. The other seven are all the cI subunits encoded in the mtDNA and are located in the hydrophobic arm, forming the proton channels [22]. The remaining 30 subunits are 'supernumerary' but important for assembly and stability [22-24].

Exhaustive research concerning human cI assembly has been carried out for 15 years [25-33]. However, several recent breakthroughs have granted a much deeper understanding about this process. Thus, we now know the complete mammalian cI structure [22,23] and how the subunits are organized in six modules (N, Q, ND1, ND2, ND4 and ND5) that, with the help of specific assembly factors, are brought together through five main subassemblies (Figure 1) [24,34].

The **N-module**, which is the tip of the hydrophilic arm and the last one to be incorporated [30,35], results from the assembly of NDUFV1, NDUFV2, NDUFS1 and NDUFA2 [34], to which NDUFA6, NDUFA7, NDUFA12, NDUFS4, NDUFS6 and NDUFV3 must be further associated with to complete the module [24].

The **Q-module** is built through the association of NDUFA5, NDUFS2 and NDUFS3 plus NDUFS7 and NDUFS8. The chaperones NDUFAF3/C3ORF60 and NDUFAF4/C6ORF66 [36,37] remain bound to this module until the final assembly steps [34]. NDUFAF6/C8ORF38 [38] also seems to participate in the assembly of the Q-module [24,39]. NDUFAF3, 4 and 6, are necessary to maintain normal MT-ND1 synthesis [40,41]. NDUFAF5 adds a hydroxyl group to $Arg^{73}$ of NDUFS7 [42] and NDUFAF7 dimethylates NDUFS2 in $Arg^{85}$ [43], an essential modification for cI assembly [44]. NUBPL/IND1 delivers [4Fe–4S] clusters specifically to the N- and Q-module subunits [45,46].

The **ND1-module** builds around the Q-module with the help of TIMMDC1/C3ORF1 [47,48], which remains bound to the Q/ND1 subassembly until the last maturation steps. MT-ND1 joins first and then NDUFA3, NDUFA8 and NDUFA13 are added [34].

The **ND2-module** is formed by an initial intermediate that contains MT-ND2, NDUFC1 and NDUFC2 bound to NDUFAF1/CIA30 [49,50], ECSIT [51] and ACAD9 [52,53]. Then, MT-ND3 is added together with TMEM126B [54], forming a larger intermediate to which subunits MT-ND6 and MT-ND4L bind. The latest assembly stages involve the incorporation of subunits NDUFA1, NDUFA10 and NDUFS5 [24,34]. The stable association of the assembly factors NDUFAF1 + ECSIT + ACAD9 + TMEM126 was denominated Mitochondrial Complex I Assembly (MCIA) complex [48,54]. Two other chaperones were found interacting with this module: TMEM186 and COA1 [34], the latter being a well-known cIV assembly factor [55,56].

The main **ND4-module** intermediate binds NDUFB1, NDUFB4, NDUFB5, NDUFB6, NDUFB10, NDUFB11 and MT-ND4 together with FOXRED1 [46,57-59], ATP5SL [24,47] and also TMEM70, described as a cV assembly factor [34,60,61].

The **ND5-module** corresponds to the distal part of the membrane arm and it is composed of MT-ND5, NDUFB2, NDUFB3, NDUFB7, NDUFB8, NDUFB9 and NDUFAB1 [24,34]. DMAC1/TMEM261 is implicated in its stabilization and/or assembly [24].

The ND2- and the ND4-modules get together first, with still all the chaperones bound to them. Later on, the Q/ND1 and the ND5-modules join the nascent complex. This intermediate only lacking the N-module is stabilized by NDUFAF2/NDUFA12L/B17.2L [24,35,62]. In the last step, the pre-assembled N-module becomes attached and the chaperones released [34].

## Assembly of complex II

Complex II (EC 1.3.5.1) or succinate dehydrogenase (quinone) is shared between the TCA cycle and the ETC and has no proton pumping activity. It is composed of four nDNA-encoded subunits. The two hydrophilic catalytic subunits are SDHA/SDH1 and SDHB/SDH2. Hydrophobic subunits SDHC/SDH3 and SDHD/SDH4 constitute the cII membrane anchor, containing a haem *b* group and two CoQ binding sites [63-65]. The two electrons from succinate oxidation are transferred to a FAD covalently bound to SDHA, then to the three different Fe–S clusters in SDHB and finally to CoQ [65,66].

Complex II assembly (Figure 2) happens through the independent maturation of SDHA, SDHB and SDHC + SDHD mediated by subunit-specific chaperones [7].

**SDHA** is flavinylated before assembly into cII, and SDHAF2/Sdh5 mediates this step [67,68]. Following FAD incorporation, SDHA binds to SDHAF4/Sdh8, which keeps the subunit stable and competent for assembly with SDHB, while protecting it from auto-oxidation [69].

**SDHB** also incorporates its Fe–S clusters before joining the rest of the subunits. Fe–S clusters are synthesized in the mitochondrial matrix [70,71] and then transferred to the apoprotein. This step is mediated by SDHAF1, necessary also for SDHB stability [72-74]. SDHAF3/ACN9/LYRM10 is another protein involved in SDHB stability and oxidative damage protection after insertion of the Fe–S clusters [7,75,76].

When both SDHA and SDHB acquire their respective prosthetic groups they join together, liberating SDHAF4 but keeping the binding with SDHAF1 and SDHAF3 [7,75].

**SDHC** and **SDHD** are assembled together in the inner membrane by a yet unknown mechanism. The haem *b* group, co-ordinated in the interface of both subunits, does not play any catalytic role but is required for their stability [77,78]. Another factor that influences the dimerization of SDHC and SDHD, as well as their stability, is the presence of both hydrophilic subunits [68,75].

## Assembly of complex III

Complex III (EC 1.10.2.2) or quinol-cytochrome *c* reductase performs electron transfer coupled to proton pumping using the 'Q-cycle' mechanism [79,80]. Structurally, it is a tightly bound symmetrical dimer ($cIII_2$), being each 'monomer' composed of three catalytic core (MT-CYB, CYC1 and UQCRFS1) and seven supernumerary subunits [81,82]. The 78-amino acid mitochondrial targeting sequence (MTS) cleaved off from UQCRFS1 was considered an extra subunit [81,83], but it needs to be cleared out to maintain $cIII_2$ structural and functional fitness [84,85]. MT-CYB contains two *b*-type haems with different redox potential as well as two CoQ binding sites. There is one [2Fe–2S] cluster inserted in the C-terminal end of UQCRFS1, and CYC1 binds a haem *c1* group that transfers the electrons to the mobile electron carrier cytochrome *c*. The supernumerary subunits are not involved in the catalysis, but are important for correct assembly and/or stability of the enzyme [86,87].

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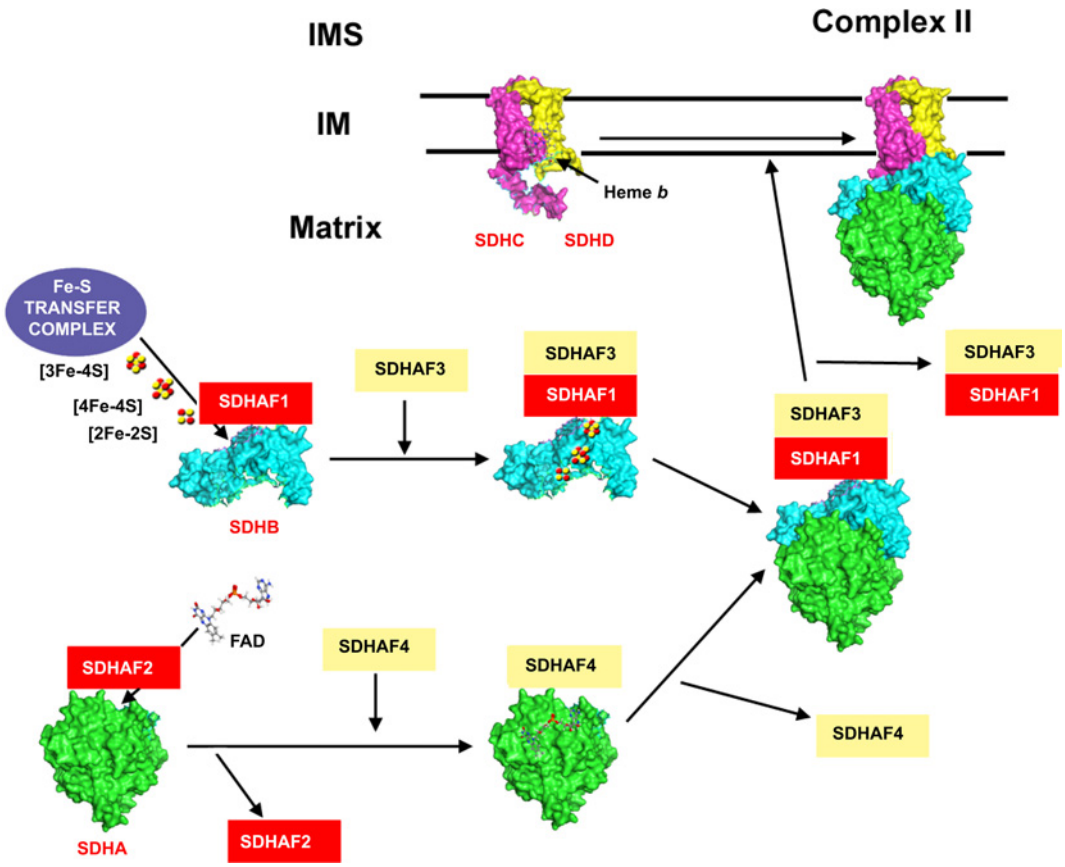


**Figure 2. Complex II assembly model (see main text for details) based on the porcine cII crystal structure with PDB ID: 1ZOY [65] and the model proposed in reference [7]**

Red colour indicates proteins with described pathological mutations. Abbreviations: IM, inner membrane; IMS, intermembrane space.

Yeast cIII assembly starts with the synthesis of **cytochrome *b*** (MT-CYB in human nomenclature) by mitochondrial ribosomes and its insertion into the inner membrane, mediated by Cbp3/UQCC1 and Cbp6/UQCC2 that remain bound to MT-CYB once it is completely synthesized. Cbp4/UQCC3 joins after the first haem-*b* ($b_L$) but before the second one ($b_H$) is incorporated [88-90]. Once the first structural subunits (UQCRB and UQCRQ) are incorporated, UQCC1-UQCC2 detach and go back to act as translational activators [88,89]. These first steps in cIII assembly (Figure 3) are supposedly conserved, because the three factors are present in humans and mutations in *UQCC2* produce deficient MT-CYB synthesis [91,92].

Maturation of cIII occurs, both in yeast and humans, with the addition of the **Rieske Fe–S protein** (Rip1/UQCRFS1) and of the smallest subunit (Qcr10/UQCR11) to an already dimeric pre-complex III (pre-$cIII_2$) [93-95]. After import into mitochondria, UQCRFS1 is bound and stabilized in the matrix by MZM1L/LYRM7 [96-98] that also mediates binding to the Fe–S cluster transfer complex [99]. Incorporation of UQCRFS1 to pre-$cIII_2$ is mediated by Bcs1/BCS1L [93,94,100,101]. In human and mouse mitochondria, TTC19 [102] binds fully assembled $cIII_2$ and favours the elimination of UQCRFS1 N-terminal fragments to maintain normal activity levels [84].

The intermediate steps of $cIII_2$ assembly are not known in humans. However, being that the initial and the final stages are the same and the assembly factors involved are orthologous proteins, it is assumed that they will share very many similarities [103]. The order of incorporation in *S. cerevisiae* was determined by creating yeast strains deleting one structural subunit at a time and studying the stability of the remaining cIII components [104-107]. Up to now, there are no described assembly factors involved in the incorporation or stabilization of $cIII_2$ intermediate subunits and transitional subcomplexes.

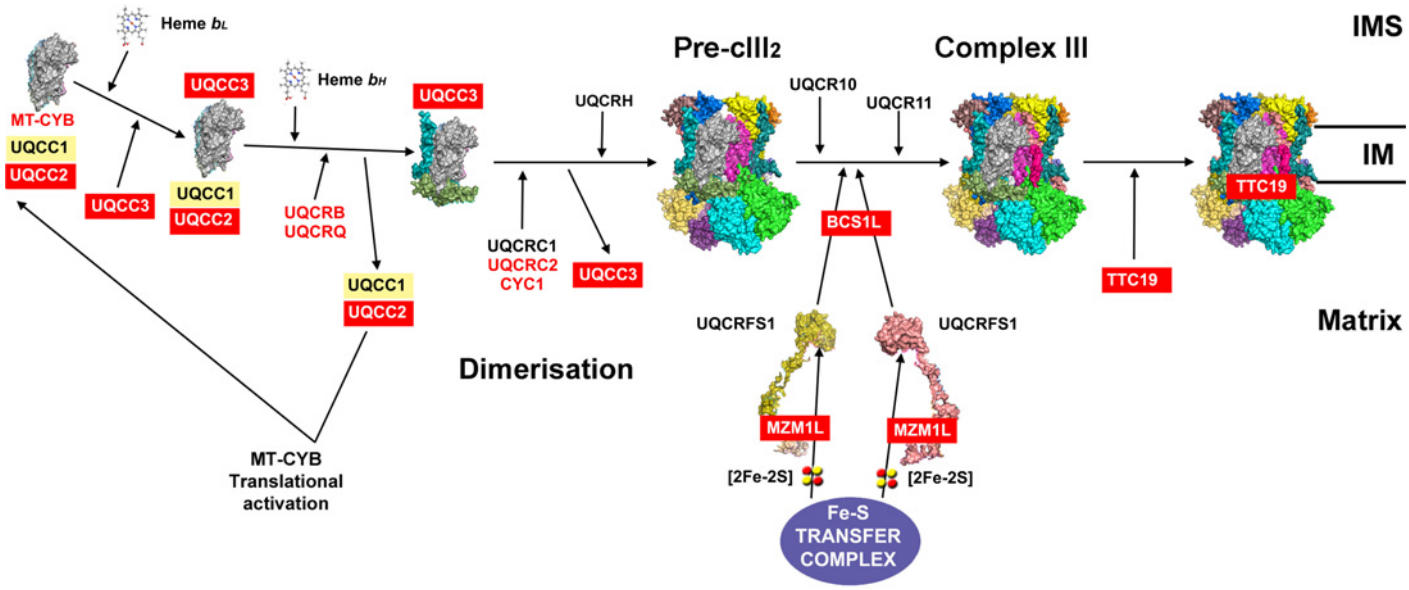


**Figure 3. Complex III assembly model (see main text for details) based on the bovine cIII$_2$ crystal structure with PDB ID: 1BGY [81] and the models proposed in references [85,103]**

Red colour indicates proteins with described pathological mutations. Abbreviations: IM, inner membrane; IMS, intermembrane space.

## Assembly of complex IV

Complex IV (EC 1.9.31) or cytochrome *c* oxidase (COX) catalyses the oxidation of cytochrome *c* and the reduction of oxygen to water, coupled to proton translocation [108]. Mammalian cIV contains 13 or 14 subunits [109-111]. MT-CO1 is the largest catalytic subunit containing a haem *a* group and a binuclear haem $a_3$-$Cu_B$ centre. MT-CO2 is the second core subunit and holds the $Cu_A$ centre. MT-CO3, the third core subunit, plays no direct catalytic role [108]. The rest of subunits (supernumerary) are thought to be important for the stabilization of the catalytic core and regulation of its activity [112-117]. Complex IV is the only OXPHOS complex containing tissue-specific and developmentally regulated isoforms [118,119], reflecting the importance of an exquisite regulation of COX activity.

The first model of subunit incorporation for human COX [120], basically still stands with minor modifications [115,121-124]. According to this model, **MT-CO1** is the 'seed' around which the rest of the subunits build up, starting with COX4I1 and COX5A. The stable subassemblies created during this process were named S1–S4, S4 being the fully assembled holoenzyme [10,120]. Proteomics analyses of a MT-CO3-deficient cell line, with a very prominent subcomplex accumulation, completed the view about subunit incorporation (Figure 4), which happens in groups or 'modules', defined by each one of the core subunits [123], as it does in yeast [125].

The **initial COX subassembly** appears to be the association of COX4I1 + COX5A [123]. This early subcomplex also contains HIGD1A [123], one of the human homologues of yeast Rcf1 [126-129].

The **MT-CO1 module** contains the many chaperones and assembly factors involved in its maturation and stabilization, and it is also known as 'MITRAC' for mitochondrial translation regulation assembly intermediate of cytochrome *c* oxidase [56,130]. COX14/C12ORF62 [55,131] and COA3/CCDC56/MITRAC12 [56,132] bind nascent MT-CO1 and are implicated in assembly regulation either by translational [133] or post-translational mechanisms [134]. In human mitochondria, MT-CO1 expression is especially sensitive to defects in the mitochondrial RNA-binding protein LRPPRC [135-137] and requires the specific translational activator TACO1 [138,139]. Later on, CMC1 binds MT-CO1 + COA3 + COX14 before or during addition of the prosthetic groups [134]. Haem A biosynthesis is carried out by COX10 [140,141] and COX15 [142]. The exact molecular function of SURF1 [143,144] remains unclear, but its involvement in haem A delivery has been proposed [124]. A role for PET117 in this same process has been suggested due to its interaction with COX15 [145]. $Cu_B$ assembly requires the metallochaperone COX11 [146,147], with COX17 donating the coppers [148,149], and COX19 maintaining COX11 in the right redox state [150]. CMC1 is released prior to the addition of COA1/C7ORF44/MITRAC15 [55,56,151] and SURF1. MITRAC7/SMIM20 is another factor described to stabilize MT-CO1 in early assembly stages [130].

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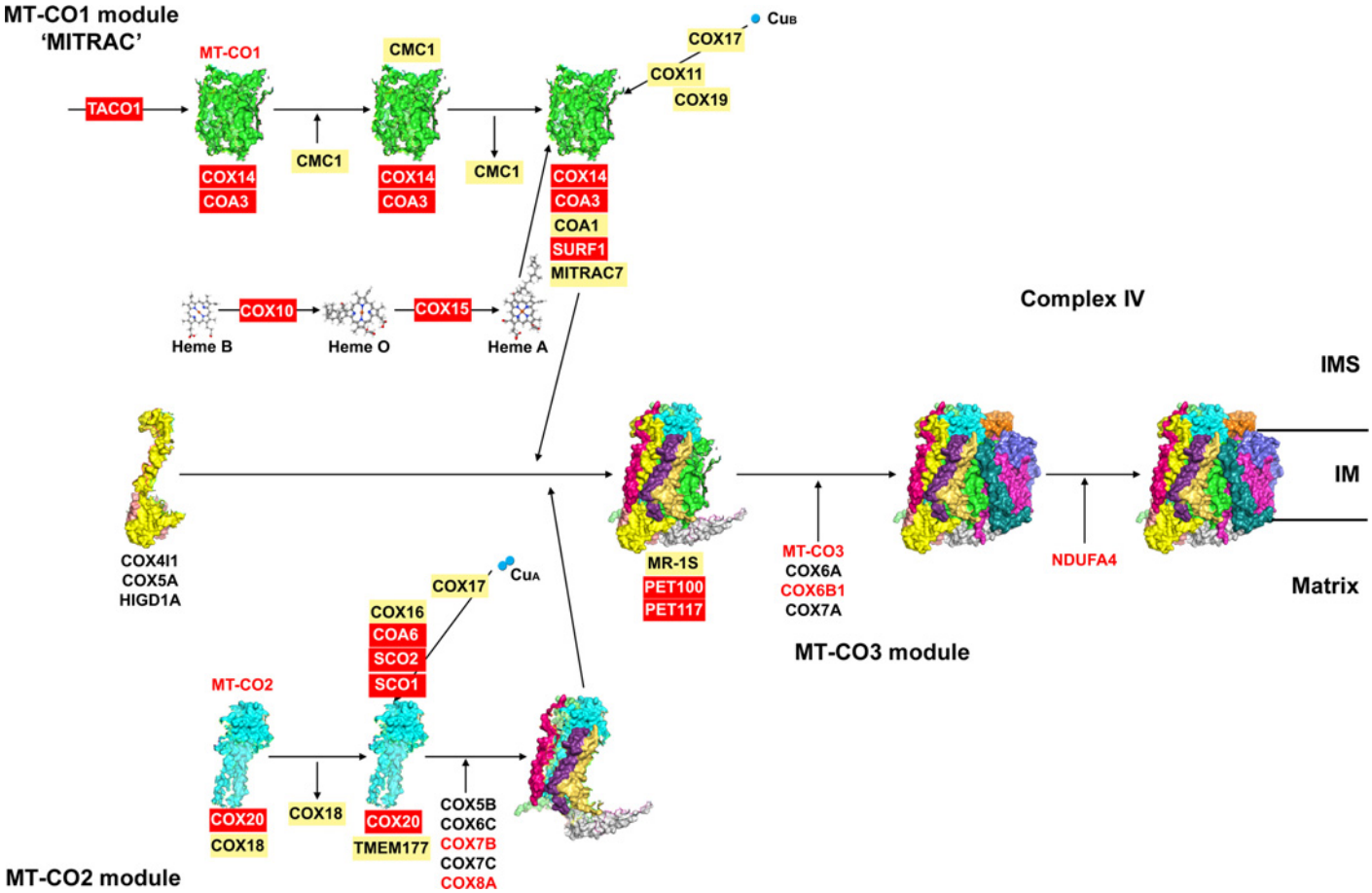


**Figure 4. Complex IV assembly model (see main text for details) based on the bovine cIV crystal structure with PDB ID: 2OCC [109] and the model proposed in reference [123]**

Red colour indicates proteins with described pathological mutations. Abbreviations: IM, inner membrane; IMS, intermembrane space.

The intermediate step in COX assembly is the joining of COX4I1 + COX5A, MT-CO1 and the **MT-CO2 module** (MT-CO2 + COX5B + COX6C + COX7C + COX8A and, most probably COX7B), equalling the 'S3' intermediary [120] minus MT-CO3 [123]. MT-CO2 requires COX18 for membrane translocation [152] and COX20/FAM36A and TMEM177 for stabilization [153-155]. Copper-binding proteins COX17, SCO1 and SCO2 [156-158] together with COA6 [159-161] and COX16 [162-164], are involved in the assembly of the $Cu_A$ centre. MR-1S is a vertebrate-specific COX chaperone that interacts with the highly conserved factors PET100 [165-167] and PET117 [168,169] during assembly of the MT-CO2 module [123].

The incorporation of the **MT-CO3 module** (MT-CO3 + COX6A1 + COX6B1 + COX7A2) completes the assembly of the 13 canonical COX subunits [109,123]. No specific assembly factors for this module are currently known.

The **last subunit** to be incorporated is NDUFA4, initially thought to be part of complex I [170], but later assigned to complex IV [110,117].

More proteins that those described here are required for cIV assembly [124] but their exact molecular role is still not understood.

## Assembly of complex V

Complex V (EC 3.6.14), $H^+$-transporting two-sector ATPase or $F_oF_1$-ATPase, is the enzyme that synthesizes ATP using the proton motive force generated by cI, III and IV. It is composed of two topological and functional distinct domains: membrane-extrinsic and matrix-facing $F_1$ plus membrane-intrinsic $F_o$, with a central axis and a peripheral stalk connecting them [171]. Subunits a (MT-ATP6) and A6L (MT-ATP8) of the $F_o$ domain are encoded in the

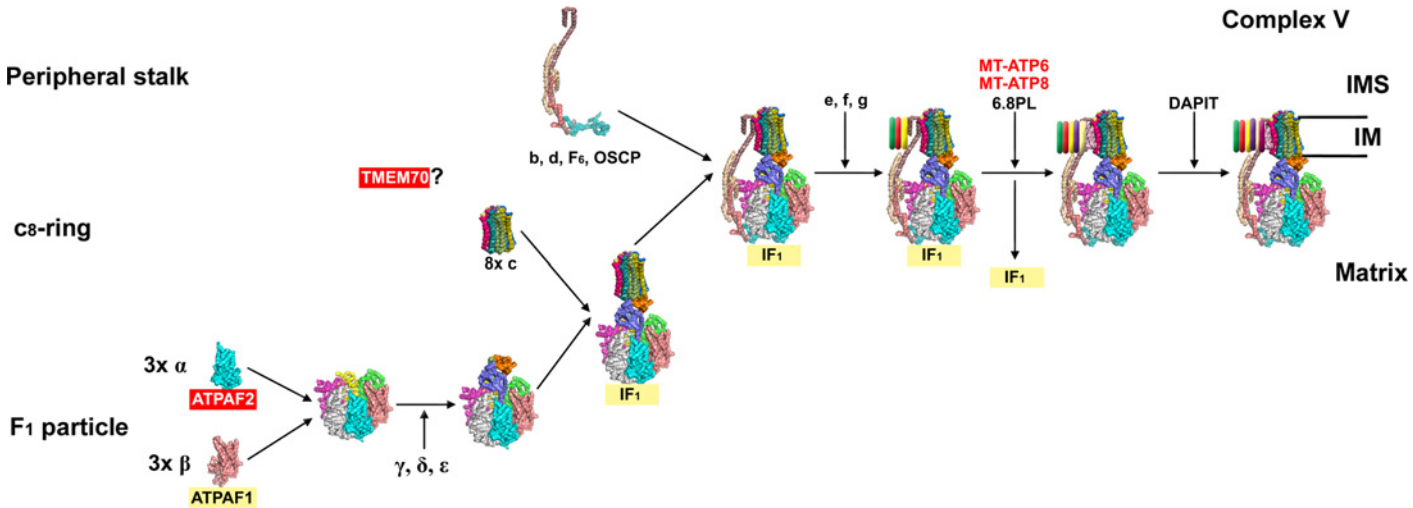


**Figure 5. Complex V assembly model (see main text for details) based on the bovine cV cryo-EM structure with PDB ID: 5ARA [200] and the model proposed in references [172,180]**

Red colour indicates proteins with described pathological mutations. Abbreviations: IM, inner membrane; IMS, intermembrane space.

mtDNA, whereas all the rest of cV components are nDNA encoded [172]. Protons coming back to the matrix through $F_o$ produce a rotational movement providing the energy for ADP+Pi condensation in the $F_1$ domain [171,173].

Assembly of cV has been studied using subunit incorporation dynamics [174], analysis of mtDNA-deficient cell lines [175,176] and more recently by creating knockout cell lines for specific cV subunits [177-180]. As depicted in Figure 5, this complex is also put together by assembling three pre-formed modules corresponding to: $F_1$ particle, $c_8$-ring (a ring composed by eight copies of the c-subunit) and peripheral stalk [172].

The **$F_1$ subcomplex**, composed of three copies of the α subunit/ATP5A1, three β subunits/ATP5B together with the central stalk subunits γ/ATP5C1, δ/ATP5D and ε/ATP5E, is assembled with the assistance of chaperones ATPAF1/ATP11 and ATPAF2/ATP12, which bind ATP5B and ATP5A1, respectively [181-185]. The **$c_8$-ring**, encoded by *ATPG1*, *ATPG2* and *ATPG3*, is assembled in the membrane by still unknown mechanisms [172]. A subcomplex containing subunits of the **peripheral stalk** is also pre-formed [177,180].

After the $c_8$-ring and the $F_1$ subcomplex come together, the peripheral stalk is incorporated in two steps: first subunits b/ATP5F1, d/ATPH, $F_6$/ATP5J and OSCP/ATP5O and then e/ATP5I, g/ATP5L and f/ATPJ2 [172,180]. The peripheral stalk can also join the $F_1$ subcomplex in absence of the $c_8$-ring [179,180]. During these initial steps, the inhibitor protein $IF_1$ is bound to the intermediates, being liberated with the insertion of the two mtDNA-encoded subunits [178-180]. In the cases in which a/MT-ATP6 and A6L/MT-ATP8 are missing, the previous assembly intermediate is readily accumulated [174,176,179]. The interaction of the last subunits is stabilized by 6.8L/MLQ/C14ORF2 and the peripheral subunit DAPIT/USMG5 is incorporated to finish cV assembly [180].

One of the few proteins known to be involved in cV biogenesis is TMEM70 and although its exact function is still not known, mutations in the gene encoding this factor have recurrently been associated with ATP synthase deficiency [60,186].

## Assembly of respiratory supercomplexes

The OXPHOS complexes interact with each other forming higher order structures, which have been called supercomplexes. Complexes IV and V can form dimers and oligomers [11,187,188]. In addition, defined associations of complexes I, III and IV are reproducibly found when mitochondrial membrane extracts are solubilized with digitonin and separated through Blue Native Gel Electrophoresis [11,12]. Thus, according to their molecular size and subunit composition, the main supercomplexes have been assigned the following stoichiometries: $III_2IV_1$, $I_1III_2$, $I_1III_2IV_1$, and $I_2III_2IV_{1-2}$. Supercomplex $I_1III_2IV_1$ is the 'respirasome' and supercomplex $I_2III_2IV_2$ has been named as 'respiratory megacomplex' [189]. High-resolution Cryo-EM structures of the respirasome of several mammalian species,

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including human, have been recently resolved [189-193]. The association of the individual complexes into these structurally defined supercomplexes is now very well established but their specific functional role still needs to be clarified [14-18].

Two alternative views exist to explain respirasome assembly. The first possibility is that the individual complexes are completely assembled before they join together in the supercomplexes [12,34]. This mode of action would permit the dynamic association-dissociation of the complexes to adapt to varying energy demands, if the role of the supercomplexes were to increase the efficiency of electron transfer, as proposed by the 'plasticity model' [12,194]. However, there are also evidences pointing to the co-assembly of subunits from the different complexes before completion of the single enzymes. Accordingly, maturation of cI would not happen unless $cIII_2$ and cIV are bound to a 'pre-cI' scaffold [195]. Also, incomplete complexes have been found assembled together in cultured cells and tissues from patients carrying mutations in different structural subunits and assembly factors implicated in the last steps of cI and cIII assembly [10]. The fact that COA1, a well-characterized cIV chaperone, is bound to cI assembly intermediates [34] could also reflect co-assembly of at least cI and cIV, although the authors of this report did not provide evidence as to whether MT-CO1 is also bound to the same subcomplexes.

COX7A2L/COX7R/SCAFI is an orthologue of the cIV structural subunit COX7A that was first described as a supercomplex assembly factor because of being necessary for the incorporation of cIV into supercomplex structures [194]. However, more recent evidences have demonstrated a role for this protein for the formation of $III_2IV_1$ but not for the incorporation of cIV into the respirasomes [188,196,197]. The dynamic interchange between the three types of COX7A proteins, COX7A2L (SCAFI), COX7A1 (muscle-type structural subunit) and COX7A2 (liver-type structural subunit) could potentially determine whether cIV stays as a monomer, oligomerizes or forms the $III_2IV_1$ supercomplex, as well as the mode of binding to cI [13,198].

## Final remarks

Assembly of the OXPHOS system is an intricate process that we still do not completely understand, despite the great efforts of many research teams and the spectacular advances described here. It is important to continue studying the processes governing the assembly of each of the complexes and of the supercomplexes, as well as the exact molecular role of the proteins involved in its basic assembly and fine regulation. This will help us understand the mechanisms regulating this central part of metabolism in health and disease. For a detailed explanation of the pathologies associated with mutations in the described assembly factors, see the accompanying article in this issue: 'Human diseases associated with defects in assembly of OXPHOS complexes' Ghezzi and Zeviani [201].

## Summary

- Assembly of the OXPHOS complexes requires a significant amount of ancillary proteins.
- Many assembly factors are conserved from yeast to humans, but some are specific for higher animals.
- Complex I is the largest OXPHOS enzyme and its assembly occurs through modules, each of which requires specific assembly factors.
- Despite being the smallest OXPHOS component, complex II assembly is assisted by, at least, four different chaperones.
- Up to now, only the first and last steps of complex III assembly are well understood.
- Complex IV assembly is highly regulated, with more than 30 known assembly factors, involved mainly in the maturation of the catalytic core.
- The order of incorporation of the 17 subunits of complex V is well known, but only 3 assembly factors have been identified so far.
- The OXPHOS complexes interact with each other in the supercomplexes or 'respirasomes', although the way they assemble together is still not known.

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The authors would like to dedicate this review to the memory of Leo Nijtmans.

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## Competing interests

The authors declare that there are no competing interests associated with the manuscript.

## Abbreviations

CoQ, coenzyme Q; COX, cytochrome *c* oxidase; MITRAC, mitochondrial translation regulation assembly intermediate of cytochrome *c* oxidase; MTS, mitochondrial targeting sequence; OXPHOS, oxidative phosphorylation system.

# References

1 Mai, N., Chrzanowska-Lightowlers, Z.M. and Lightowlers, R.N. (2017) The process of mammalian mitochondrial protein synthesis. *Cell Tissue Res.* **367**, 5–20, https://doi.org/10.1007/s00441-016-2456-0
2 Wasilewski, M., Chojnacka, K. and Chacinska, A. (2017) Protein trafficking at the crossroads to mitochondria. *Biochim. Biophys. Acta* **1864**, 125–137, https://doi.org/10.1016/j.bbamcr.2016.10.019
3 Tzagoloff, A. and Dieckmann, C.L. (1990) PET genes of *Saccharomyces cerevisiae*. *Microbiol. Rev.* **54**, 211–225
4 Barrientos, A. (2003) Yeast models of human mitochondrial diseases. *IUBMB Life* **55**, 83–95, https://doi.org/10.1002/tbmb.718540876
5 Fontanesi, F., Soto, I.C., Horn, D. and Barrientos, A. (2006) Assembly of mitochondrial cytochrome c-oxidase, a complicated and highly regulated cellular process. *Am. J. Physiol. Cell Physiol.* **291**, C1129–C47, https://doi.org/10.1152/ajpcell.00233.2006
6 Smith, P.M., Fox, J.L. and Winge, D.R. (2012) Biogenesis of the cytochrome bc(1) complex and role of assembly factors. *Biochim. Biophys. Acta* **1817**, 276–286, https://doi.org/10.1016/j.bbabio.2011.11.009
7 Van Vranken, J.G., Na, U., Winge, D.R. and Rutter, J. (2015) Protein-mediated assembly of succinate dehydrogenase and its cofactors. *Crit. Rev. Biochem. Mol. Biol.* **50**, 168–180, https://doi.org/10.3109/10409238.2014.990556
8 Kerscher, S., Drose, S., Zwicker, K., Zickermann, V. and Brandt, U. (2002) *Yarrowia lipolytica*, a yeast genetic system to study mitochondrial complex I. *Biochim. Biophys. Acta* **1555**, 83–91, https://doi.org/10.1016/S0005-2728(02)00259-1
9 Schulte, U. (2001) Biogenesis of respiratory complex I. *J. Bioenerg. Biomembr.* **33**, 205–212, https://doi.org/10.1023/A:1010730919074
10 Fernandez-Vizarra, E., Tiranti, V. and Zeviani, M. (2009) Assembly of the oxidative phosphorylation system in humans: what we have learned by studying its defects. *Biochim. Biophys. Acta* **1793**, 200–211, https://doi.org/10.1016/j.bbamcr.2008.05.028
11 Schagger, H. (2002) Respiratory chain supercomplexes of mitochondria and bacteria. *Biochim. Biophys. Acta* **1555**, 154–159, https://doi.org/10.1016/S0005-2728(02)00271-2
12 Acin-Perez, R., Fernandez-Silva, P., Peleato, M.L., Perez-Martos, A. and Enriquez, J.A. (2008) Respiratory active mitochondrial supercomplexes. *Mol. Cell* **32**, 529–539, https://doi.org/10.1016/j.molcel.2008.10.021
13 Letts, J.A. and Sazanov, L.A. (2017) Clarifying the supercomplex: the higher-order organization of the mitochondrial electron transport chain. *Nat. Struct. Mol. Biol.* **24**, 800–808, https://doi.org/10.1038/nsmb.3460
14 Barrientos, A. and Ugalde, C. (2013) I function, therefore i am: overcoming skepticism about mitochondrial supercomplexes. *Cell Metab.* **18**, 147–149, https://doi.org/10.1016/j.cmet.2013.07.010
15 Acin-Perez, R. and Enriquez, J.A. (2014) The function of the respiratory supercomplexes: the plasticity model. *Biochim. Biophys. Acta* **1837**, 444–450, https://doi.org/10.1016/j.bbabio.2013.12.009
16 Moreno-Loshuertos, R. and Enriquez, J.A. (2016) Respiratory supercomplexes and the functional segmentation of the CoQ pool. *Free Radic. Biol. Med.* **100**, 5–13, https://doi.org/10.1016/j.freeradbiomed.2016.04.018
17 Lobo-Jarne, T. and Ugalde, C. (2018) Respiratory chain supercomplexes: structures, function and biogenesis. *Semin. Cell Dev. Biol.* **76**, 179–190, https://doi.org/10.1016/j.semcdb.2017.07.021
18 Milenkovic, D., Blaza, J.N., Larsson, N.G. and Hirst, J. (2017) The enigma of the respiratory chain supercomplex. *Cell Metab.* **25**, 765–776, https://doi.org/10.1016/j.cmet.2017.03.009
19 Efremov, R.G., Baradaran, R. and Sazanov, L.A. (2010) The architecture of respiratory complex I. *Nature* **465**, 441–445, https://doi.org/10.1038/nature09066
20 Baradaran, R., Berrisford, J.M., Minhas, G.S. and Sazanov, L.A. (2013) Crystal structure of the entire respiratory complex I. *Nature* **494**, 443–448, https://doi.org/10.1038/nature11871
21 Hirst, J. and Roessler, M.M. (2016) Energy conversion, redox catalysis and generation of reactive oxygen species by respiratory complex I. *Biochim. Biophys. Acta* **1857**, 872–883, https://doi.org/10.1016/j.bbabio.2015.12.009
22 Vinothkumar, K.R., Zhu, J. and Hirst, J. (2014) Architecture of mammalian respiratory complex I. *Nature* **515**, 80–84, https://doi.org/10.1038/nature13686
23 Zhu, J., Vinothkumar, K.R. and Hirst, J. (2016) Structure of mammalian respiratory complex I. *Nature* **536**, 354–358, https://doi.org/10.1038/nature19095

24 Stroud, D.A., Surgenor, E.E., Formosa, L.E., Reljic, B., Frazier, A.E., Dibley, M.G. et al. (2016) Accessory subunits are integral for assembly and function of human mitochondrial complex I. *Nature* **538**, 123–126, https://doi.org/10.1038/nature19754
25 Antonicka, H., Ogilvie, I., Taivassalo, T., Anitori, R.P., Haller, R.G., Vissing, J. et al. (2003) Identification and characterization of a common set of complex I assembly intermediates in mitochondria from patients with complex I deficiency. *J. Biol. Chem.* **278**, 43081–43088, https://doi.org/10.1074/jbc.M304998200
26 Ugalde, C., Janssen, R.J., van den Heuvel, L.P., Smeitink, J.A. and Nijtmans, L.G. (2004) Differences in assembly or stability of complex I and other mitochondrial OXPHOS complexes in inherited complex I deficiency. *Hum. Mol. Genet.* **13**, 659–667, https://doi.org/10.1093/hmg/ddh071
27 Ugalde, C., Vogel, R., Huijbens, R., van den Heuvel, B., Smeitink, J. and Nijtmans, L. (2004) Human mitochondrial complex I assembles through the combination of evolutionary conserved modules: a framework to interpret complex I deficiencies. *Hum. Mol. Genet.* **13**, 2461–2472, https://doi.org/10.1093/hmg/ddh262
28 Vogel, R.O., Dieteren, C.E., van den Heuvel, L.P., Willems, P.H., Smeitink, J.A., Koopman, W.J. et al. (2007) Identification of mitochondrial complex I assembly intermediates by tracing tagged NDUFS3 demonstrates the entry point of mitochondrial subunits. *J. Biol. Chem.* **282**, 7582–7590, https://doi.org/10.1074/jbc.M609410200
29 Vogel, R.O., Smeitink, J.A. and Nijtmans, L.G. (2007) Human mitochondrial complex I assembly: A dynamic and versatile process. *Biochim. Biophys. Acta* **1767**, 1215–1227, https://doi.org/10.1016/j.bbabio.2007.07.008
30 Lazarou, M., McKenzie, M., Ohtake, A., Thorburn, D.R. and Ryan, M.T. (2007) Analysis of the assembly profiles for mitochondrial- and nuclear-DNA-encoded subunits into complex I. *Mol. Cell Biol.* **27**, 4228–4237, https://doi.org/10.1128/MCB.00074-07
31 Lazarou, M., Thorburn, D.R., Ryan, M.T. and McKenzie, M. (2009) Assembly of mitochondrial complex I and defects in disease. *Biochim. Biophys. Acta* **1793**, 78–88, https://doi.org/10.1016/j.bbamcr.2008.04.015
32 Mimaki, M., Wang, X., McKenzie, M., Thorburn, D.R. and Ryan, M.T. (2012) Understanding mitochondrial complex I assembly in health and disease. *Biochim. Biophys. Acta* **1817**, 851–862, https://doi.org/10.1016/j.bbabio.2011.08.010
33 Sanchez-Caballero, L., Guerrero-Castillo, S. and Nijtmans, L. (2016) Unraveling the complexity of mitochondrial complex I assembly: a dynamic process. *Biochim. Biophys. Acta* **1857**, 980–990, https://doi.org/10.1016/j.bbabio.2016.03.031
34 Guerrero-Castillo, S., Baertling, F., Kownatzki, D., Wessels, H.J., Arnold, S., Brandt, U. et al. (2017) The assembly pathway of mitochondrial respiratory chain complex I. *Cell Metab.* **25**, 128–139, https://doi.org/10.1016/j.cmet.2016.09.002
35 Vogel, R.O., van den Brand, M.A., Rodenburg, R.J., van den Heuvel, L.P., Tsuneoka, M., Smeitink, J.A. et al. (2007) Investigation of the complex I assembly chaperones B17.2L and NDUFAF1 in a cohort of CI deficient patients. *Mol. Genet. Metab.* **91**, 176–182, https://doi.org/10.1016/j.ymgme.2007.02.007
36 Saada, A., Edvardson, S., Rapoport, M., Shaag, A., Amry, K., Miller, C. et al. (2008) C6ORF66 is an assembly factor of mitochondrial complex I. *Am. J. Hum. Genet.* **82**, 32–38, https://doi.org/10.1016/j.ajhg.2007.08.003
37 Saada, A., Vogel, R.O., Hoefs, S.J., van den Brand, M.A., Wessels, H.J., Willems, P.H. et al. (2009) Mutations in NDUFAF3 (C3ORF60), encoding an NDUFAF4 (C6ORF66)-interacting complex I assembly protein, cause fatal neonatal mitochondrial disease. *Am. J. Hum. Genet.* **84**, 718–727, https://doi.org/10.1016/j.ajhg.2009.04.020
38 Pagliarini, D.J., Calvo, S.E., Chang, B., Sheth, S.A., Vafai, S.B., Ong, S.E. et al. (2008) A mitochondrial protein compendium elucidates complex I disease biology. *Cell* **134**, 112–123, https://doi.org/10.1016/j.cell.2008.06.016
39 Bianciardi, L., Imperatore, V., Fernandez-Vizarra, E., Lopomo, A., Falabella, M., Furini, S. et al. (2016) Exome sequencing coupled with mRNA analysis identifies NDUFAF6 as a Leigh gene. *Mol. Genet. Metab.*, https://doi.org/10.1016/j.ymgme.2016.09.001
40 McKenzie, M., Tucker, E.J., Compton, A.G., Lazarou, M., George, C., Thorburn, D.R. et al. (2011) Mutations in the gene encoding C8orf38 block complex i assembly by inhibiting production of the mitochondria-encoded subunit ND1. *J. Mol. Biol.* **414**, 413–426, https://doi.org/10.1016/j.jmb.2011.10.012
41 Zurita Rendon, O. and Shoubridge, E.A. (2012) Early complex I assembly defects result in rapid turnover of the ND1 subunit. *Hum. Mol. Genet.* **21**, 3815–3824, https://doi.org/10.1093/hmg/dds209
42 Rhein, V.F., Carroll, J., Ding, S., Fearnley, I.M. and Walker, J.E. (2016) NDUFAF5 hydroxylates NDUFS7 at an early stage in the assembly of human complex I. *J. Biol. Chem.* **291**, 14851–14860, https://doi.org/10.1074/jbc.M116.734970
43 Rhein, V.F., Carroll, J., Ding, S., Fearnley, I.M. and Walker, J.E. (2013) NDUFAF7 methylates arginine 85 in the NDUFS2 subunit of human complex I. *J. Biol. Chem.* **288**, 33016–33026, https://doi.org/10.1074/jbc.M113.518803
44 Zurita Rendon, O., Silva Neiva, L., Sasarman, F. and Shoubridge, E.A. (2014) The arginine methyltransferase NDUFAF7 is essential for complex I assembly and early vertebrate embryogenesis. *Hum. Mol. Genet.* **23**, 5159–5170, https://doi.org/10.1093/hmg/ddu239
45 Sheftel, A.D., Stehling, O., Pierik, A.J., Netz, D.J., Kerscher, S., Elsasser, H.P. et al. (2009) Human ind1, an iron-sulfur cluster assembly factor for respiratory complex I. *Mol. Cell. Biol.* **29**, 6059–6073, https://doi.org/10.1128/MCB.00817-09
46 Calvo, S.E., Tucker, E.J., Compton, A.G., Kirby, D.M., Crawford, G., Burtt, N.P. et al. (2010) High-throughput, pooled sequencing identifies mutations in NUBPL and FOXRED1 in human complex I deficiency. *Nat. Genet.* **42**, 851–858, https://doi.org/10.1038/ng.659
47 Andrews, B., Carroll, J., Ding, S., Fearnley, I.M. and Walker, J.E. (2013) Assembly factors for the membrane arm of human complex I. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 18934–18939, https://doi.org/10.1073/pnas.1319247110
48 Guarani, V., Paulo, J., Zhai, B., Huttlin, E.L., Gygi, S.P. and Harper, J.W. (2014) TIMMDC1/C3orf1 functions as a membrane-embedded mitochondrial complex I assembly factor through association with the MCIA complex. *Mol. Cell. Biol.* **34**, 847–861, https://doi.org/10.1128/MCB.01551-13
49 Vogel, R.O., Janssen, R.J., Ugalde, C., Grovenstein, M., Huijbens, R.J., Visch, H.J. et al. (2005) Human mitochondrial complex I assembly is mediated by NDUFAF1. *FEBS J.* **272**, 5317–5326, https://doi.org/10.1111/j.1742-4658.2005.04928.x
50 Dunning, C.J., McKenzie, M., Sugiana, C., Lazarou, M., Silke, J., Connelly, A. et al. (2007) Human CIA30 is involved in the early assembly of mitochondrial complex I and mutations in its gene cause disease. *EMBO J.* **26**, 3227–3237, https://doi.org/10.1038/sj.emboj.7601748

51 Vogel, R.O., Janssen, R.J., van den Brand, M.A., Dieteren, C.E., Verkaart, S., Koopman, W.J. et al. (2007) Cytosolic signaling protein Ecsit also localizes to mitochondria where it interacts with chaperone NDUFAF1 and functions in complex I assembly. *Genes Dev.* **21**, 615–624, https://doi.org/10.1101/gad.408407
52 Nouws, J., Nijtmans, L., Houten, S.M., van den Brand, M., Huynen, M., Venselaar, H. et al. (2010) Acyl-CoA dehydrogenase 9 is required for the biogenesis of oxidative phosphorylation complex I. *Cell Metab.* **12**, 283–294, https://doi.org/10.1016/j.cmet.2010.08.002
53 Haack, T.B., Danhauser, K., Haberberger, B., Hoser, J., Strecker, V., Boehm, D. et al. (2010) Exome sequencing identifies ACAD9 mutations as a cause of complex I deficiency. *Nat. Genet.* **42**, 1131–1134, https://doi.org/10.1038/ng.706
54 Heide, H., Bleier, L., Steger, M., Ackermann, J., Drose, S., Schwamb, B. et al. (2012) Complexome profiling identifies TMEM126B as a component of the mitochondrial complex I assembly complex. *Cell Metab.* **16**, 538–549, https://doi.org/10.1016/j.cmet.2012.08.009
55 Szklarczyk, R., Wanschers, B.F., Cuypers, T.D., Esseling, J.J., Riemersma, M., van den Brand, M.A. 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, https://doi.org/10.1186/gb-2012-13-2-r12
56 Mick, D.U., Dennerlein, S., Wiese, H., Reinhold, R., Pacheu-Grau, D., Lorenzi, I. et al. (2012) MITRAC links mitochondrial protein translocation to respiratory-chain assembly and translational regulation. *Cell* **151**, 1528–1541, https://doi.org/10.1016/j.cell.2012.11.053
57 Fassone, E., Duncan, A.J., Taanman, J.W., Pagnamenta, A.T., Sadowski, M.I., Holand, T. et al. (2010) FOXRED1, encoding an FAD-dependent oxidoreductase complex-I-specific molecular chaperone, is mutated in infantile-onset mitochondrial encephalopathy. *Hum. Mol. Genet.* **19**, 4837–4847, https://doi.org/10.1093/hmg/ddq414
58 Formosa, L.E., Mimaki, M., Frazier, A.E., McKenzie, M., Stait, T.L., Thorburn, D.R. et al. (2015) Characterization of mitochondrial FOXRED1 in the assembly of respiratory chain complex I. *Hum. Mol. Genet.* **24**, 2952–2965, https://doi.org/10.1093/hmg/ddv058
59 Zurita Rendon, O., Antonicka, H., Horvath, R. and Shoubridge, E.A. (2016) A mutation in the FAD-dependent oxidoreductase FOXRED1 results in cell-type specific assembly defects in oxidative phosphorylation complexes I and II. *Mol. Cell. Biol.*, https://doi.org/10.1128/MCB.00066-16
60 Cizkova, A., Stranecky, V., Mayr, J.A., Tesarova, M., Havlickova, V., Paul, J. et al. (2008) TMEM70 mutations cause isolated ATP synthase deficiency and neonatal mitochondrial encephalocardiomyopathy. *Nat. Genet.* **40**, 1288–1290, https://doi.org/10.1038/ng.246
61 Hejzlarova, K., Mracek, T., Vrbacky, M., Kaplanova, V., Karbanova, V., Nuskova, H. et al. (2014) Nuclear genetic defects of mitochondrial ATP synthase. *Physiol. Res.* **63**, S57–S71
62 Ogilvie, I., Kennaway, N.G. and Shoubridge, E.A. (2005) A molecular chaperone for mitochondrial complex I assembly is mutated in a progressive encephalopathy. *J. Clin. Invest.* **115**, 2784–2792, https://doi.org/10.1172/JCI26020
63 Oyedotun, K.S. and Lemire, B.D. (2001) The quinone-binding sites of the Saccharomyces cervisiae succinate-ubiquinone oxidoreductase. *J. Biol. Chem.* **276**, 16936–16943, https://doi.org/10.1074/jbc.M100184200
64 Yankovskaya, V., Horsefield, R., Tornroth, S., Luna-Chavez, C., Miyoshi, H., Leger, C. et al. (2003) Architecture of succinate dehydrogenase and reactive oxygen species generation. *Science* **299**, 700–704, https://doi.org/10.1126/science.1079605
65 Sun, F., Huo, X., Zhai, Y., Wang, A., Xu, J., Su, D. et al. (2005) Crystal structure of mitochondrial respiratory membrane protein complex II. *Cell* **121**, 1043–1057, https://doi.org/10.1016/j.cell.2005.05.025
66 Oyedotun, K.S., Sit, C.S. and Lemire, B.D. (2007) The *Saccharomyces cerevisiae* succinate dehydrogenase does not require heme for ubiquinone reduction. *Biochim. Biophys. Acta* **1767**, 1436–1445, https://doi.org/10.1016/j.bbabio.2007.09.008
67 Hao, H.X., Khalimonchuk, O., Schraders, M., Dephoure, N., Bayley, J.P., Kunst, H. et al. (2009) SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. *Science* **325**, 1139–1142, https://doi.org/10.1126/science.1175689
68 Kim, H.J., Jeong, M.Y., Na, U. and Winge, D.R. (2012) Flavinylation and assembly of succinate dehydrogenase are dependent on the C-terminal tail of the flavoprotein subunit. *J. Biol. Chem.* **287**, 40670–40679, https://doi.org/10.1074/jbc.M112.405704
69 Van Vranken, J.G., Bricker, D.K., Dephoure, N., Gygi, S.P., Cox, J.E., Thummel, C.S. et al. (2014) SDHAF4 promotes mitochondrial succinate dehydrogenase activity and prevents neurodegeneration. *Cell Metab* **20**, 241–252, https://doi.org/10.1016/j.cmet.2014.05.012
70 Braymer, J.J. and Lill, R. (2017) Iron-sulfur cluster biogenesis and trafficking in mitochondria. *J. Biol. Chem.* **292**, 12754–12763, https://doi.org/10.1074/jbc.R117.787101
71 Rouault, T.A. and Maio, N. (2017) Biogenesis and functions of mammalian iron-sulfur proteins in the regulation of iron homeostasis and pivotal metabolic pathways. *J. Biol. Chem.* **292**, 12744–12753, https://doi.org/10.1074/jbc.R117.789537
72 Ghezzi, D., Goffrini, P., Uziel, G., Horvath, R., Klopstock, T., Lochmuller, H. et al. (2009) SDHAF1, encoding a LYR complex-II specific assembly factor, is mutated in SDH-defective infantile leukoencephalopathy. *Nat. Genet.* **41**, 654–656, https://doi.org/10.1038/ng.378
73 Maio, N., Singh, A., Uhrigshardt, H., Saxena, N., Tong, W.H. and Rouault, T.A. (2014) Cochaperone binding to LYR motifs confers specificity of iron sulfur cluster delivery. *Cell Metab.* **19**, 445–457, https://doi.org/10.1016/j.cmet.2014.01.015
74 Maio, N., Ghezzi, D., Verrigni, D., Rizza, T., Bertini, E., Martinelli, D. et al. (2016) Disease-Causing SDHAF1 Mutations Impair Transfer of Fe-S Clusters to SDHB. *Cell Metab.* **23**, 292–302, https://doi.org/10.1016/j.cmet.2015.12.005
75 Na, U., Yu, W., Cox, J., Bricker, D.K., Brockmann, K., Rutter, J. et al. (2014) The LYR factors SDHAF1 and SDHAF3 mediate maturation of the iron-sulfur subunit of succinate dehydrogenase. *Cell Metab.* **20**, 253–266, https://doi.org/10.1016/j.cmet.2014.05.014
76 Dwight, T., Na, U., Kim, E., Zhu, Y., Richardson, A.L., Robinson, B.G. et al. (2017) Analysis of SDHAF3 in familial and sporadic pheochromocytoma and paraganglioma. *BMC Cancer* **17**, 497, https://doi.org/10.1186/s12885-017-3486-z
77 Lemarie, A. and Grimm, S. (2009) Mutations in the heme b-binding residue of SDHC inhibit assembly of respiratory chain complex II in mammalian cells. *Mitochondrion* **9**, 254–260, https://doi.org/10.1016/j.mito.2009.03.004
78 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, https://doi.org/10.1016/j.bbamcr.2012.04.008

79 Trumpower, B.L. (1990) The protonmotive Q cycle. Energy transduction by coupling of proton translocation to electron transfer by the cytochrome bc1 complex. *J. Biol. Chem.* **265**, 11409–11412
80 Crofts, A.R., Holland, J.T., Victoria, D., Kolling, D.R., Dikanov, S.A., Gilbreth, R. et al. (2008) The Q-cycle reviewed: How well does a monomeric mechanism of the bc(1) complex account for the function of a dimeric complex? *Biochim. Biophys. Acta* **1777**, 1001–1019, https://doi.org/10.1016/j.bbabio.2008.04.037
81 Iwata, S., Lee, J.W., Okada, K., Lee, J.K., Iwata, M., Rasmussen, B. et al. (1998) Complete structure of the 11-subunit bovine mitochondrial cytochrome bc1 complex. *Science* **281**, 64–71, https://doi.org/10.1126/science.281.5373.64
82 Hunte, C., Koepke, J., Lange, C., Rossmanith, T. and Michel, H. (2000) Structure at 2.3 A resolution of the cytochrome bc(1) complex from the yeast Saccharomyces cerevisiae co-crystallized with an antibody Fv fragment. *Structure* **8**, 669–684, https://doi.org/10.1016/S0969-2126(00)00152-0
83 Brandt, U., Yu, L., Yu, C.A. and Trumpower, B.L. (1993) The mitochondrial targeting presequence of the Rieske iron-sulfur protein is processed in a single step after insertion into the cytochrome bc1 complex in mammals and retained as a subunit in the complex. *J. Biol. Chem.* **268**, 8387–8390
84 Bottani, E., Cerutti, R., Harbour, M.E., Ravaglia, S., Dogan, S.A., Giordano, C. et al. (2017) TTC19 plays a husbandry role on UQCRFS1 turnover in the biogenesis of mitochondrial respiratory complex III. *Mol. Cell.* **67**, 96.e4–105.e4, https://doi.org/10.1016/j.molcel.2017.06.001
85 Fernandez-Vizarra, E. and Zeviani, M. (2018) Mitochondrial complex III Rieske Fe-S protein processing and assembly. *Cell Cycle* **17**, 681–687, https://doi.org/10.1080/15384101.2017.
86 Haut, S., Brivet, M., Touati, G., Rustin, P., Lebon, S., Garcia-Cazorla, A. et al. (2003) A deletion in the human QP-C gene causes a complex III deficiency resulting in hypoglycaemia and lactic acidosis. *Hum. Genet.* **113**, 118–122
87 Barel, O., Shorer, Z., Flusser, H., Ofir, R., Narkis, G., Finer, G. et al. (2008) Mitochondrial complex III deficiency associated with a homozygous mutation in UQCRQ. *Am. J. Hum. Genet.* **82**, 1211–1216, https://doi.org/10.1016/j.ajhg.2008.03.020
88 Gruschke, S., Kehrein, K., Rompler, K., Grone, K., Israel, L., Imhof, A. et al. (2011) Cbp3-Cbp6 interacts with the yeast mitochondrial ribosomal tunnel exit and promotes cytochrome b synthesis and assembly. *J. Cell Biol.* **193**, 1101–1114, https://doi.org/10.1083/jcb.201103132
89 Gruschke, S., Rompler, K., Hildenbeutel, M., Kehrein, K., Kuhl, I., Bonnefoy, N. et al. (2012) The Cbp3-Cbp6 complex coordinates cytochrome b synthesis with bc(1) complex assembly in yeast mitochondria. *J. Cell Biol.* **199**, 137–150, https://doi.org/10.1083/jcb.201206040
90 Hildenbeutel, M., Hegg, E.L., Stephan, K., Gruschke, S., Meunier, B. and Ott, M. (2014) Assembly factors monitor sequential hemylation of cytochrome b to regulate mitochondrial translation. *J. Cell Biol.* **205**, 511–524, https://doi.org/10.1083/jcb.201401009
91 Tucker, E.J., Wanschers, B.F., Szklarczyk, R., Mountford, H.S., Wijeyeratne, X.W., van den Brand, M.A. et al. (2013) Mutations in the UQCC1-interacting protein, UQCC2, cause human complex III deficiency associated with perturbed cytochrome b protein expression. *PLoS Genet.* **9**, e1004034, https://doi.org/10.1371/journal.pgen.1004034
92 Wanschers, B.F., Szklarczyk, R., van den Brand, M.A., Jonckheere, A., Suijskens, J., Smeets, R. et al. (2014) A mutation in the human CBP4 ortholog UQCC3 impairs complex III assembly, activity and cytochrome b stability. *Hum. Mol. Genet.* **23**, 6356–6365, https://doi.org/10.1093/hmg/ddu357
93 Cruciat, C.M., Hell, K., Folsch, H., Neupert, W. and Stuart, R.A. (1999) Bcs1p, an AAA-family member, is a chaperone for the assembly of the cytochrome bc(1) complex. *EMBO J.* **18**, 5226–5233, https://doi.org/10.1093/emboj/18.19.5226
94 Fernandez-Vizarra, E., Bugiani, M., Goffrini, P., Carrara, F., Farina, L., Procopio, E. et al. (2007) Impaired complex III assembly associated with BCS1L gene mutations in isolated mitochondrial encephalopathy. *Hum. Mol. Genet.* **16**, 1241–1252, https://doi.org/10.1093/hmg/ddm072
95 Conte, A., Papa, B., Ferramosca, A. and Zara, V. (2015) The dimerization of the yeast cytochrome bc1 complex is an early event and is independent of Rip1. *Biochim. Biophys. Acta* **1853**, 987–995, https://doi.org/10.1016/j.bbamcr.2015.02.006
96 Atkinson, A., Smith, P., Fox, J.L., Cui, T.Z., Khalimonchuk, O. and Winge, D.R. (2011) The LYR protein Mzm1 functions in the insertion of the Rieske Fe/S protein in yeast mitochondria. *Mol. Cell. Biol.* **31**, 3988–3996, https://doi.org/10.1128/MCB.05673-11
97 Cui, T.Z., Smith, P.M., Fox, J.L., Khalimonchuk, O. and Winge, D.R. (2012) Late-stage maturation of the Rieske Fe/S protein: Mzm1 stabilizes Rip1 but does not facilitate its translocation by the AAA ATPase Bcs1. *Mol. Cell. Biol.* **32**, 4400–4409, https://doi.org/10.1128/MCB.00441-12
98 Sanchez, E., Lobo, T., Fox, J.L., Zeviani, M., Winge, D.R. and Fernandez-Vizarra, E. (2013) LYRM7/MZM1L is a UQCRFS1 chaperone involved in the last steps of mitochondrial Complex III assembly in human cells. *Biochim. Biophys. Acta* **1827**, 285–293, https://doi.org/10.1016/j.bbabio.2012.11.003
99 Maio, N., Kim, K.S., Singh, A. and Rouault, T.A. (2017) A single adaptable Cochaperone-Scaffold complex delivers nascent iron-sulfur clusters to mammalian respiratory chain complexes I-III. *Cell Metab.* **25**, 945e6–953e6, https://doi.org/10.1016/j.cmet.2017.03.010
100 de Lonlay, P., Valnot, I., Barrientos, A., Gorbatyuk, M., Tzagoloff, A., Taanman, J.W. et al. (2001) A mutant mitochondrial respiratory chain assembly protein causes complex III deficiency in patients with tubulopathy, encephalopathy and liver failure. *Nat. Genet.* **29**, 57–60, https://doi.org/10.1038/ng706
101 Wagener, N., Ackermann, M., Funes, S. and Neupert, W. (2011) A pathway of protein translocation in mitochondria mediated by the AAA-ATPase Bcs1. *Mol. Cell* **44**, 191–202, https://doi.org/10.1016/j.molcel.2011.07.036
102 Ghezzi, D., Arzuffi, P., Zordan, M., Da Re, C., Lamperti, C., Benna, C. et al. (2011) Mutations in TTC19 cause mitochondrial complex III deficiency and neurological impairment in humans and flies. *Nat. Genet.* **43**, 259–263, https://doi.org/10.1038/ng.761
103 Fernandez-Vizarra, E. and Zeviani, M. (2015) Nuclear gene mutations as the cause of mitochondrial complex III deficiency. *Front. Genet.* **6**, 134, https://doi.org/10.3389/fgene.2015.00134
104 Zara, V., Palmisano, I., Conte, L. and Trumpower, B.L. (2004) Further insights into the assembly of the yeast cytochrome bc1 complex based on analysis of single and double deletion mutants lacking supernumerary subunits and cytochrome b. *Eur. J. Biochem.* **271**, 1209–1218, https://doi.org/10.1111/j.1432-1033.2004.04024.x
105 Zara, V., Conte, L. and Trumpower, B.L. (2007) Identification and characterization of cytochrome bc(1) subcomplexes in mitochondria from yeast with single and double deletions of genes encoding cytochrome bc(1) subunits. *FEBS J.* **274**, 4526–4539, https://doi.org/10.1111/j.1742-4658.2007.05982.x

106 Zara, V., Conte, L. and Trumpower, B.L. (2009) Evidence that the assembly of the yeast cytochrome bc1 complex involves the formation of a large core structure in the inner mitochondrial membrane. *FEBS J.* **276**, 1900–1914, https://doi.org/10.1111/j.1742-4658.2009.06916.x
107 Zara, V., Conte, L. and Trumpower, B.L. (2009) Biogenesis of the yeast cytochrome bc1 complex. *Biochim. Biophys. Acta* **1793**, 89–96, https://doi.org/10.1016/j.bbamcr.2008.04.011
108 Wikstrom, M., Krab, K. and Sharma, V. (2018) Oxygen activation and energy conservation by cytochrome c oxidase. *Chem. Rev.*, https://doi.org/10.1021/acs.chemrev.7b00664
109 Yoshikawa, S., Shinzawa-Itoh, K. and Tsukihara, T. (1998) Crystal structure of bovine heart cytochrome c oxidase at 2.8 A resolution. *J. Bioenerg. Biomembr.* **30**, 7–14, https://doi.org/10.1023/A:1020595108560
110 Balsa, E., Marco, R., Perales-Clemente, E., Szklarczyk, R., Calvo, E., Landazuri, M.O. et al. (2012) NDUFA4 is a subunit of complex IV of the mammalian electron transport chain. *Cell Metab.* **16**, 378–386, https://doi.org/10.1016/j.cmet.2012.07.015
111 Kadenbach, B. (2017) Regulation of mammalian 13-subunit cytochrome c oxidase and binding of other proteins: role of NDUFA4. *Trends Endocrinol. Metab.* **28**, 761–770, https://doi.org/10.1016/j.tem.2017.09.003
112 Arnold, S., Goglia, F. and Kadenbach, B. (1998) 3,5-Diiodothyronine binds to subunit Va of cytochrome-c oxidase and abolishes the allosteric inhibition of respiration by ATP. *Eur. J. Biochem.* **252**, 325–330, https://doi.org/10.1046/j.1432-1327.1998.2520325.x
113 Arnold, S. and Kadenbach, B. (1997) Cell respiration is controlled by ATP, an allosteric inhibitor of cytochrome-c oxidase. *Eur. J. Biochem.* **249**, 350–354, https://doi.org/10.1111/j.1432-1033.1997.t01-1-00350.x
114 Kadenbach, B. and Arnold, S. (1999) A second mechanism of respiratory control. *FEBS Lett.* **447**, 131–134, https://doi.org/10.1016/S0014-5793(99)00229-X
115 Massa, V., Fernandez-Vizarra, E., Alshahwan, S., Bakhsh, E., Goffrini, P., Ferrero, I. et al. (2008) Severe infantile encephalomyopathy caused by a mutation in COX6B1, a nucleus-encoded subunit of cytochrome c oxidase. *Am. J. Hum. Genet.* **82**, 1281–1289, https://doi.org/10.1016/j.ajhg.2008.05.002
116 Fornuskova, D., Stiburek, L., Wenchich, L., Vinsova, K., Hansikova, H. and Zeman, J. (2010) Novel insights into the assembly and function of human nuclear-encoded cytochrome c oxidase subunits 4, 5a, 6a, 7a and 7b. *Biochem. J.* **428**, 363–374, https://doi.org/10.1042/BJ20091714
117 Pitceathly, R.D., Rahman, S., Wedatilake, Y., Polke, J.M., Cirak, S., Foley, A.R. et al. (2013) NDUFA4 mutations underlie dysfunction of a cytochrome c oxidase subunit linked to human neurological disease. *Cell Rep.* **3**, 1795–1805, https://doi.org/10.1016/j.celrep.2013.05.005
118 Huttemann, M., Kadenbach, B. and Grossman, L.I. (2001) Mammalian subunit IV isoforms of cytochrome c oxidase. *Gene* **267**, 111–123, https://doi.org/10.1016/S0378-1119(01)00385-7
119 Sinkler, C.A., Kalpage, H., Shay, J., Lee, I., Malek, M.H., Grossman, L.I. et al. (2017) Tissue- and condition-specific isoforms of mammalian cytochrome c oxidase subunits: from function to human disease. *Oxid. Med. Cell Longev.* **2017**, 1534056, https://doi.org/10.1155/2017/1534056
120 Nijtmans, L.G., Taanman, J.W., Muijsers, A.O., Speijer, D. and Van den Bogert, C. (1998) Assembly of cytochrome-c oxidase in cultured human cells. *Eur. J. Biochem.* **254**, 389–394, https://doi.org/10.1046/j.1432-1327.1998.2540389.x
121 Stiburek, L., Hansikova, H., Tesarova, M., Cerna, L. and Zeman, J. (2006) Biogenesis of eukaryotic cytochrome c oxidase. *Physiol. Res.* **55** (Suppl. 2), S27–S41
122 Stiburek, L., Vesela, K., Hansikova, H., Pecina, P., Tesarova, M., Cerna, L. et al. (2005) Tissue-specific cytochrome c oxidase assembly defects due to mutations in SCO2 and SURF1. *Biochem. J.* **392**, 625–632, https://doi.org/10.1042/BJ20050807
123 Vidoni, S., Harbour, M.E., Guerrero-Castillo, S., Signes, A., Ding, S., Fearnley, I.M. et al. (2017) MR-1S interacts with PET100 and PET117 in module-based assembly of human cytochrome c oxidase. *Cell Rep.* **18**, 1727–1738, https://doi.org/10.1016/j.celrep.2017.01.044
124 Timon-Gomez, A., Nyvltova, E., Abriata, L.A., Vila, A.J., Hosler, J. and Barrientos, A. (2018) Mitochondrial cytochrome c oxidase biogenesis: recent developments. *Semin. Cell Dev. Biol.* **76**, 163–178, https://doi.org/10.106/j.semcdb.2017.08.055
125 McStay, G.P., Su, C.H. and Tzagoloff, A. (2013) Modular assembly of yeast cytochrome oxidase. *Mol. Biol. Cell* **24**, 440–452, https://doi.org/10.1091/mbc.e12-10-0749
126 Hayashi, T., Asano, Y., Shintani, Y., Aoyama, H., Kioka, H., Tsukamoto, O. et al. (2015) Higd1a is a positive regulator of cytochrome c oxidase. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 1553–1558, https://doi.org/10.1073/pnas.1419767112
127 Lundin, C., von Ballmoos, C., Ott, M., Adelroth, P. and Brzezinski, P. (2016) Regulatory role of the respiratory supercomplex factors in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* **113**, E4476–E4485, https://doi.org/10.1073/pnas.1601196113
128 Strogolova, V., Furness, A., Robb-McGrath, M., Garlich, J. and Stuart, R.A. (2012) Rcf1 and Rcf2, members of the hypoxia-induced gene 1 protein family, are critical components of the mitochondrial cytochrome bc1-cytochrome c oxidase supercomplex. *Mol. Cell. Biol.* **32**, 1363–1373, https://doi.org/10.1128/MCB.06369-11
129 Vukotic, M., Oeljeklaus, S., Wiese, S., Vogtle, F.N., Meisinger, C., Meyer, H.E. et al. (2012) Rcf1 mediates cytochrome oxidase assembly and respirasome formation, revealing heterogeneity of the enzyme complex. *Cell Metab.* **15**, 336–347, https://doi.org/10.1016/j.cmet.2012.01.016
130 Dennerlein, S., Oeljeklaus, S., Jans, D., Hellwig, C., Bareth, B., Jakobs, S. et al. (2015) MITRAC7 acts as a COX1-specific chaperone and reveals a checkpoint during cytochrome c oxidase assembly. *Cell Rep.* **12**, 1644–1655, https://doi.org/10.1016/j.celrep.2015.08.009
131 Weraarpachai, W., Sasarman, F., Nishimura, T., Antonicka, H., Aure, K., Rotig, A. et al. (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, https://doi.org/10.1016/j.ajhg.2011.11.027
132 Clemente, P., Peralta, S., Cruz-Bermudez, A., Echevarria, L., Fontanesi, F., Barrientos, A. et al. (2013) hCOA3 stabilizes cytochrome c oxidase 1 (COX1) and promotes cytochrome c oxidase assembly in human mitochondria. *J. Biol. Chem.* **288**, 8321–8331, https://doi.org/10.1074/jbc.M112.422220
133 Richter-Dennerlein, R., Oeljeklaus, S., Lorenzi, I., Ronsor, C., Bareth, B., Schendzielorz, A.B. et al. (2016) Mitochondrial protein synthesis adapts to influx of nuclear-encoded protein. *Cell* **167**, 471e10–483e10, https://doi.org/10.1016/j.cell.2016.09.003

134 Bourens, M. and Barrientos, A. (2017) A CMC1-knockout reveals translation-independent control of human mitochondrial complex IV biogenesis. *EMBO Rep.* **18**, 477–494, https://doi.org/10.15252/embr.201643103
135 Mootha, V.K., Lepage, P., Miller, K., Bunkenborg, J., Reich, M., Hjerrild, M. et al. (2003) Identification of a gene causing human cytochrome c oxidase deficiency by integrative genomics. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 605–610, https://doi.org/10.1073/pnas.242716699
136 Xu, F., Morin, C., Mitchell, G., Ackerley, C. and Robinson, B.H. (2004) The role of the LRPPRC (leucine-rich pentatricopeptide repeat cassette) gene in cytochrome oxidase assembly: mutation causes lowered levels of COX (cytochrome c oxidase) I and COX III mRNA. *Biochem. J.* **382**, 331–336, https://doi.org/10.1042/BJ20040469
137 Ruzzenente, B., Metodiev, M.D., Wredenberg, A., Bratic, A., Park, C.B., Camara, Y. et al. (2012) LRPPRC is necessary for polyadenylation and coordination of translation of mitochondrial mRNAs. *EMBO J.* **31**, 443–456, https://doi.org/10.1038/emboj.2011.392
138 Weraarpachai, W., Antonicka, H., Sasarman, F., Seeger, J., Schrank, B., Kolesar, J.E. 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, https://doi.org/10.1038/ng.390
139 Richman, T.R., Spahr, H., Ermer, J.A., Davies, S.M., Viola, H.M., Bates, K.A. et al. (2016) Loss of the RNA-binding protein TACO1 causes late-onset mitochondrial dysfunction in mice. *Nat. Commun.* **7**, 11884, https://doi.org/10.1038/ncomms11884
140 Antonicka, H., Leary, S.C., Guercin, G.H., Agar, J.N., Horvath, R., Kennaway, N.G. et al. (2003) 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**, 2693–2702, https://doi.org/10.1093/hmg/ddg284
141 Diaz, F., Thomas, C.K., Garcia, S., Hernandez, D. and Moraes, C.T. (2005) Mice lacking COX10 in skeletal muscle recapitulate the phenotype of progressive mitochondrial myopathies associated with cytochrome c oxidase deficiency. *Hum. Mol. Genet.* **14**, 2737–2748, https://doi.org/10.1093/hmg/ddi307
142 Antonicka, H., Mattman, A., Carlson, C.G., Glerum, D.M., Hoffbuhr, K.C., Leary, S.C. et al. (2003) Mutations in COX15 produce a defect in the mitochondrial heme biosynthetic pathway, causing early-onset fatal hypertrophic cardiomyopathy. *Am. J. Hum. Genet.* **72**, 101–114, https://doi.org/10.1086/345489
143 Tiranti, V., Hoertnagel, K., Carrozzo, R., Galimberti, C., Munaro, M., Granatiero, M. et al. (1998) Mutations of SURF-1 in Leigh disease associated with cytochrome c oxidase deficiency. *Am. J. Hum. Genet.* **63**, 1609–1621, https://doi.org/10.1086/302150
144 Zhu, Z., Yao, J., Johns, T., Fu, K., De Bie, I., Macmillan, C. et al. (1998) SURF1, encoding a factor involved in the biogenesis of cytochrome c oxidase, is mutated in Leigh syndrome. *Nat. Genet.* **20**, 337–343, https://doi.org/10.1038/3804
145 Taylor, N.G., Swenson, S., Harris, N.J., Germany, E.M., Fox, J.L. and Khalimonchuk, O. (2017) The assembly factor Pet117 couples heme a synthase activity to cytochrome oxidase assembly. *J. Biol. Chem.* **292**, 1815–1825, https://doi.org/10.1074/jbc.M116.766980
146 Hiser, L., Di Valentin, M., Hamer, A.G. and Hosler, J.P. (2000) Cox11p is required for stable formation of the Cu(B) and magnesium centers of cytochrome c oxidase. *J. Biol. Chem.* **275**, 619–623, https://doi.org/10.1074/jbc.275.1.619
147 Banci, L., Bertini, I., Cantini, F., Ciofi-Baffoni, S., Gonnelli, L. and Mangani, S. (2004) Solution structure of Cox11, a novel type of beta-immunoglobulin-like fold involved in CuB site formation of cytochrome c oxidase. *J. Biol. Chem.* **279**, 34833–34839, https://doi.org/10.1074/jbc.M403655200
148 Cobine, P.A., Pierrel, F. and Winge, D.R. (2006) Copper trafficking to the mitochondrion and assembly of copper metalloenzymes. *Biochim. Biophys. Acta* **1763**, 759–772, https://doi.org/10.1016/j.bbamcr.2006.03.002
149 Glerum, D.M., Shtanko, A. and Tzagoloff, A. (1996) Characterization of COX17, a yeast gene involved in copper metabolism and assembly of cytochrome oxidase. *J. Biol. Chem.* **271**, 14504–14509, https://doi.org/10.1074/jbc.271.24.14504
150 Bode, M., Woellhaf, M.W., Bohnert, M., van der Laan, M., Sommer, F., Jung, M. et al. (2015) Redox-regulated dynamic interplay between Cox19 and the copper-binding protein Cox11 in the intermembrane space of mitochondria facilitates biogenesis of cytochrome c oxidase. *Mol. Biol. Cell* **26**, 2385–2401, https://doi.org/10.1091/mbc.e14-11-1526
151 Pierrel, F., Bestwick, M.L., Cobine, P.A., Khalimonchuk, O., Cricco, J.A. and Winge, D.R. (2007) Coa1 links the Mss51 post-translational function to Cox1 cofactor insertion in cytochrome c oxidase assembly. *EMBO J.* **26**, 4335–4346, https://doi.org/10.1038/sj.emboj.7601861
152 Bourens, M. and Barrientos, A. (2017) Human mitochondrial cytochrome c oxidase assembly factor COX18 acts transiently as a membrane insertase within the subunit 2 maturation module. *J. Biol. Chem.* **292**, 7774–7783, https://doi.org/10.1074/jbc.M117.778514
153 Szklarczyk, R., Wanschers, B.F., Nijtmans, L.G., Rodenburg, R.J., Zschocke, J., Dikow, N. et al. (2013) A mutation in the FAM36A gene, the human ortholog of COX20, impairs cytochrome c oxidase assembly and is associated with ataxia and muscle hypotonia. *Hum. Mol. Genet.* **22**, 656–667, https://doi.org/10.1093/hmg/dds473
154 Bourens, M., Boulet, A., Leary, S.C. and Barrientos, A. (2014) Human COX20 cooperates with SCO1 and SCO2 to mature COX2 and promote the assembly of cytochrome c oxidase. *Hum. Mol. Genet.* **23**, 2901–2913, https://doi.org/10.1093/hmg/ddu003
155 Lorenzi, I., Oeljeklaus, S., Aich, A., Ronsor, C., Callegari, S., Dudek, J. et al. (2018) The mitochondrial TMEM177 associates with COX20 during COX2 biogenesis. *Biochim. Biophys. Acta* **1865**, 323–333, https://doi.org/10.1016/j.bbamcr.2017.11.010
156 Leary, S.C., Kaufman, B.A., Pellecchia, G., Guercin, G.H., Mattman, A., Jaksch, M. et al. (2004) Human SCO1 and SCO2 have independent, cooperative functions in copper delivery to cytochrome c oxidase. *Hum. Mol. Genet.* **13**, 1839–1848, https://doi.org/10.1093/hmg/ddh197
157 Leary, S.C., Cobine, P.A., Kaufman, B.A., Guercin, G.H., Mattman, A., Palaty, J. et al. (2007) The human cytochrome c oxidase assembly factors SCO1 and SCO2 have regulatory roles in the maintenance of cellular copper homeostasis. *Cell Metab.* **5**, 9–20, https://doi.org/10.1016/j.cmet.2006.12.001
158 Leary, S.C., Sasarman, F., Nishimura, T. and Shoubridge, E.A. (2009) Human SCO2 is required for the synthesis of CO II and as a thiol-disulphide oxidoreductase for SCO1. *Hum. Mol. Genet.* **18**, 2230–2240, https://doi.org/10.1093/hmg/ddp158
159 Pacheu-Grau, D., Bareth, B., Dudek, J., Juris, L., Vogtle, F.N., Wissel, M. et al. (2015) Cooperation between COA6 and SCO2 in COX2 maturation during cytochrome c oxidase assembly links two mitochondrial cardiomyopathies. *Cell Metab.* **21**, 823–833, https://doi.org/10.1016/j.cmet.2015.04.012

160 Stroud, D.A., Maher, M.J., Lindau, C., Vogtle, F.N., Frazier, A.E., Surgenor, E. et al. (2015) COA6 is a mitochondrial complex IV assembly factor critical for biogenesis of mtDNA-encoded COX2. *Hum. Mol. Genet.* **24**, 5404–5415, https://doi.org/10.1093/hmg/ddv265
161 Ghosh, A., Pratt, A.T., Soma, S., Theriault, S.G., Griffin, A.T., Trivedi, P.P. et al. (2016) Mitochondrial disease genes COA6, COX6B and SCO2 have overlapping roles in COX2 biogenesis. *Hum. Mol. Genet.* **25**, 660–671, https://doi.org/10.1093/hmg/ddv503
162 Carlson, C.G., Barrientos, A., Tzagoloff, A. and Glerum, D.M. (2003) COX16 encodes a novel protein required for the assembly of cytochrome oxidase in Saccharomyces cerevisiae. *J. Biol. Chem.* **278**, 3770–3775, https://doi.org/10.1074/jbc.M209893200
163 Aich, A., Wang, C., Chowdhury, A., Ronsor, C., Pacheu-Grau, D., Richter-Dennerlein, R. et al. (2018) COX16 promotes COX2 metallation and assembly during respiratory complex IV biogenesis. *Elife* **7**, https://doi.org/10.7554/eLife.32572
164 Cerqua, C., Morbidoni, V., Desbats, M.A., Doimo, M., Frasson, C., Sacconi, S. et al. (2018) COX16 is required for assembly of cytochrome c oxidase in human cells and is involved in copper delivery to COX2. *Biochim. Biophys. Acta* **1859**, 244–252, https://doi.org/10.1016/j.bbabio.2018.01.004
165 Church, C., Goehring, B., Forsha, D., Wazny, P. and Poyton, R.O. (2005) A role for Pet100p in the assembly of yeast cytochrome c oxidase: interaction with a subassembly that accumulates in a pet100 mutant. *J. Biol. Chem.* **280**, 1854–1863, https://doi.org/10.1074/jbc.M410726200
166 Lim, S.C., Smith, K.R., Stroud, D.A., Compton, A.G., Tucker, E.J., Dasvarma, A. 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, https://doi.org/10.1016/j.ajhg.2013.12.015
167 Olahova, M., Haack, T.B., Alston, C.L., Houghton, J.A., He, L., Morris, A.A. et al. (2015) A truncating PET100 variant causing fatal infantile lactic acidosis and isolated cytochrome c oxidase deficiency. *Eur. J. Hum. Genet.* **23**, 935–939, https://doi.org/10.1038/ejhg.2014.214
168 McEwen, J.E., Hong, K.H., Park, S. and Preciado, G.T. (1993) Sequence and chromosomal localization of two PET genes required for cytochrome c oxidase assembly in *Saccharomyces cerevisiae*. *Curr. Genet.* **23**, 9–14, https://doi.org/10.1007/BF00336742
169 Renkema, G.H., Visser, G., Baertling, F., Wintjes, L.T., Wolters, V.M., van Montfrans, J. et al. (2017) Mutated PET117 causes complex IV deficiency and is associated with neurodevelopmental regression and medulla oblongata lesions. *Hum. Genet.* **136**, 759–769, https://doi.org/10.1007/s00439-017-1794-7
170 Carroll, J., Fearnley, I.M., Skehel, J.M., Shannon, R.J., Hirst, J. and Walker, J.E. (2006) Bovine complex I is a complex of 45 different subunits. *J. Biol. Chem.* **281**, 32724–32727, https://doi.org/10.1074/jbc.M607135200
171 Walker, J.E. (2013) The ATP synthase: the understood, the uncertain and the unknown. *Biochem. Soc. Trans.* **41**, 1–16, https://doi.org/10.1042/BST20110773
172 Jonckheere, A.I., Smeitink, J.A. and Rodenburg, R.J. (2012) Mitochondrial ATP synthase: architecture, function and pathology. *J. Inherit. Metab. Dis.* **35**, 211–225, https://doi.org/10.1007/s10545-011-9382-9
173 Watt, I.N., Montgomery, M.G., Runswick, M.J., Leslie, A.G. and Walker, J.E. (2010) Bioenergetic cost of making an adenosine triphosphate molecule in animal mitochondria. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 16823–16827, https://doi.org/10.1073/pnas.1011099107
174 Nijtmans, L.G., Klement, P., Houstek, J. and Van den Bogert, C. (1995) Assembly of mitochondrial ATP synthase in cultured human cells: implications for mitochondrial diseases. *Biochim. Biophys. Acta* **1272**, 190–198, https://doi.org/10.1016/0925-4439(95)00087-9
175 Carrozzo, R., Wittig, I., Santorelli, F.M., Bertini, E., Hofmann, S., Brandt, U. et al. (2006) Subcomplexes of human ATP synthase mark mitochondrial biosynthesis disorders. *Ann. Neurol.* **59**, 265–275, https://doi.org/10.1002/ana.20729
176 Wittig, I., Meyer, B., Heide, H., Steger, M., Bleier, L., Wumaier, Z. et al. (2010) Assembly and oligomerization of human ATP synthase lacking mitochondrial subunits a and A6L. *Biochim. Biophys. Acta* **1797**, 1004–1011, https://doi.org/10.1016/j.bbabio.2010.02.021
177 Fujikawa, M., Sugawara, K., Tanabe, T. and Yoshida, M. (2015) Assembly of human mitochondrial ATP synthase through two separate intermediates, F1-c-ring and b-e-g complex. *FEBS Lett.* **589**, 2707–2712, https://doi.org/10.1016/j.febslet.2015.08.006
178 He, J., Carroll, J., Ding, S., Fearnley, I.M. and Walker, J.E. (2017) Permeability transition in human mitochondria persists in the absence of peripheral stalk subunits of ATP synthase. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 9086–9091, https://doi.org/10.1073/pnas.1711201114
179 He, J., Ford, H.C., Carroll, J., Ding, S., Fearnley, I.M. and Walker, J.E. (2017) Persistence of the mitochondrial permeability transition in the absence of subunit c of human ATP synthase. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 3409–3414, https://doi.org/10.1073/pnas.1702357114
180 He, J., Ford, H.C., Carroll, J., Douglas, C., Gonzales, E., Ding, S. et al. (2018) Assembly of the membrane domain of ATP synthase in human mitochondria. *Proc. Natl. Acad. Sci. U.S.A.*, https://doi.org/10.1073/pnas.1722086115
181 Ackerman, S.H. and Tzagoloff, A. (1990) Identification of two nuclear genes (ATP11, ATP12) required for assembly of the yeast F1-ATPase. *Proc. Natl Acad. Sci. U.S.A.* **87**, 4986–4990, https://doi.org/10.1073/pnas.87.13.4986
182 Wang, Z.G. and Ackerman, S.H. (2000) The assembly factor Atp11p binds to the beta-subunit of the mitochondrial F(1)-ATPase. *J. Biol. Chem.* **275**, 5767–5772, https://doi.org/10.1074/jbc.275.8.5767
183 Wang, Z.G., Sheluho, D., Gatti, D.L. and Ackerman, S.H. (2000) The alpha-subunit of the mitochondrial F(1) ATPase interacts directly with the assembly factor Atp12p. *EMBO J.* **19**, 1486–1493, https://doi.org/10.1093/emboj/19.7.1486
184 Wang, Z.G., White, P.S. and Ackerman, S.H. (2001) Atp11p and Atp12p are assembly factors for the F(1)-ATPase in human mitochondria. *J. Biol. Chem.* **276**, 30773–30778, https://doi.org/10.1074/jbc.M104133200
185 De Meirleir, L., Seneca, S., Lissens, W., De Clercq, I., Eyskens, F., Gerlo, E. et al. (2004) Respiratory chain complex V deficiency due to a mutation in the assembly gene ATP12. *J. Med. Genet.* **41**, 120–124, https://doi.org/10.1136/jmg.2003.012047
186 Magner, M., Dvorakova, V., Tesarova, M., Mazurova, S., Hansikova, H., Zahorec, M. et al. (2015) TMEM70 deficiency: long-term outcome of 48 patients. *J. Inherit. Metab. Dis.* **38**, 417–426, https://doi.org/10.1007/s10545-014-9774-8
187 Wittig, I. and Schagger, H. (2008) Structural organization of mitochondrial ATP synthase. *Biochim. Biophys. Acta* **1777**, 592–598, https://doi.org/10.1016/j.bbabio.2008.04.027
188 Mourier, A., Matic, S., Ruzzenente, B., Larsson, N.G. and Milenkovic, D. (2014) The respiratory chain supercomplex organization is independent of COX7a2l isoforms. *Cell Metab.* **20**, 1069–1075, https://doi.org/10.1016/j.cmet.2014.11.005

*Essays in Biochemistry* (2018) **62** 255–270
https://doi.org/10.1042/EBC20170098

189 Guo, R., Zong, S., Wu, M., Gu, J. and Yang, M. (2017) Architecture of human mitochondrial respiratory megacomplex I2III2IV2. *Cell* **170**, 1247.e12–1257.e12, https://doi.org/10.1016/j.cell.2017.07.050

190 Gu, J., Wu, M., Guo, R., Yan, K., Lei, J., Gao, N. et al. (2016) The architecture of the mammalian respirasome. *Nature* **537**, 639–643, https://doi.org/10.1038/nature19359

191 Wu, M., Gu, J., Guo, R., Huang, Y. and Yang, M. (2016) Structure of mammalian respiratory supercomplex I1III2IV1. *Cell* **167**, 1598.e10–609.e10, https://doi.org/10.1016/j.cell.2016.11.012

192 Letts, J.A., Fiedorczuk, K. and Sazanov, L.A. (2016) The architecture of respiratory supercomplexes. *Nature* **537**, 644–648, https://doi.org/10.1038/nature19774

193 Sousa, J.S., Mills, D.J., Vonck, J. and Kuhlbrandt, W. (2016) Functional asymmetry and electron flow in the bovine respirasome. *Elife* **5**, https://doi.org/10.7554/eLife.21290

194 Lapuente-Brun, E., Moreno-Loshuertos, R., Acin-Perez, R., Latorre-Pellicer, A., Colas, C., Balsa, E. et al. (2013) Supercomplex assembly determines electron flux in the mitochondrial electron transport chain. *Science* **340**, 1567–1570, https://doi.org/10.1126/science.1230381

195 Moreno-Lastres, D., Fontanesi, F., Garcia-Consuegra, I., Martin, M.A., Arenas, J., Barrientos, A. et al. (2012) Mitochondrial complex I plays an essential role in human respirasome assembly. *Cell Metab.* **15**, 324–335, https://doi.org/10.1016/j.cmet.2012.01.015

196 Williams, E.G., Wu, Y., Jha, P., Dubuis, S., Blattmann, P., Argmann, C.A. et al. (2016) Systems proteomics of liver mitochondria function. *Science* **352**, aad0189, https://doi.org/10.1126/science.aad0189

197 Perez-Perez, R., Lobo-Jarne, T., Milenkovic, D., Mourier, A., Bratic, A., Garcia-Bartolome, A. et al. (2016) COX7A2L is a mitochondrial complex III binding protein that stabilizes the III2+IV supercomplex without affecting respirasome formation. *Cell Rep.* **16**, 2387–2398, https://doi.org/10.1016/j.celrep.2016.07.081

198 Cogliati, S., Calvo, E., Loureiro, M., Guaras, A.M., Nieto-Arellano, R., Garcia-Poyatos, C. et al. (2016) Mechanism of super-assembly of respiratory complexes III and IV. *Nature* **539**, 579–582, https://doi.org/10.1038/nature20157

199 Formosa, L.E., Dibley, M.G., Stroud, D.A. and Ryan, M.T. (2018) Building a complex complex: assembly of mitochondrial respiratory chain complex I. *Semin. Cell Dev. Biol.* **76**, 154–162

200 Zhou, A., Rohou, A., Schep, D.G., Bason, J.V., Montgomery, M.G., Walker, J.E. et al. (2015) Structure and conformational states of the bovine mitochondrial ATP synthase by cryo-EM. *Elife* **4**, e10180, https://doi.org/10.7554/eLife.10180

201 Ghezzi, D. and Zeviani, M. (2018) Human diseases associated with defects in assembly of OXPHOS complexes. *Essays Biochem.* **62**, 271–286, https://doi.org/10.1042/EBC20170099