Chapter 4
Assembly Factors of Human Mitochondrial Respiratory Chain Complexes: Physiology and Pathophysiology

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Abstract Mitochondrial disorders are clinical syndromes associated with abnormalities of the oxidative phosphorylation (OXPHOS) system, the main responsible for the production of energy in the cell. OXPHOS is carried out in the inner mitochondrial membrane by the five enzymatic complexes of the mitochondrial respiratory chain (MRC). The subunits constituting these multimeric complexes have a dual genetic origin, mitochondrial or nuclear. Hence, mitochondrial syndromes can be due to mutations of mitochondrial DNA or to abnormalities in nuclear genes. The biogenesis of the MRC complexes is an intricate and finely tuned process. The recent discovery of several OXPHOS-related human genes, mutated in different clinical syndromes, indicates that the majority of the inherited mitochondrial disorders are due to nuclear genes, and many of them encode proteins necessary for the proper assembly/stability of the MRC complexes. The detailed mechanisms of these processes are not fully understood and the exact function of many such factors remains obscure.

We present an overview on the hypothesized assembly processes of the different MRC complexes, focusing on known assembly factors and their clinical importance.

4.1 Introduction

The mitochondrial respiratory chain (MRC) is composed of five multiheteromeric complexes (complex I, CI; complex II, CII; complex III, CIII; complex IV, CIV or cytochrome *c* oxidase, COX; complex V, CV, or ATP synthase), all embedded in the inner mitochondrial membrane, and two mobile electron shuttles, ubiquinone (Coenzyme Q, CoQ), a lipoidal quinone, and cytochrome *c* (cyt *c*), a heme-containing

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small polypeptide. Electron donors including reduced nicotinamide-adenine dinucleotide, $NADH^{+}$ (via CI), and reduced flavin adenine dinucleotide, $FADH_2$ (via CII and other flavoproteins, including electron transfer flavoprotein ubiquinone reductase or ETF-dehydrogenase (EC 1.5.5.1), the terminal component of fatty acid β-oxidation and ketogenic aminoacid oxidation pathways), supply electrons to CoQ, which donates them to CIII. CIII transfers one electron at a time to cyt *c*, which passes it to COX; COX eventually fixes four electrons to molecular oxygen with the formation of two molecules of water. This process, known as respiration, liberates energy that is partly converted by the proton pumping activity of CI, CIII, and CIV into an electrochemical potential (ΔμH) composed of an electrical gradient (ΔΨ) and a pH gradient, across the inner mitochondrial membrane. ΔμH constitutes the driving proton motive force not only for the phosphorylation of ADP to ATP, operated by CV, but also for a number of other processes, such as heat production, Ca^{++} import inside mitochondria, and protein translocation.

From a genetic standpoint, the MRC is unique, as it is formed through the complementation of two separate genetic systems: the nuclear and the mitochondrial genomes. Four of the five MRC complexes, namely CI, CIII, CIV, and CV, contain subunits encoded by the mitochondrial DNA, mtDNA, and synthesized in situ by the organelle-specific translation machinery. Thus, ad hoc replication, transcription and translation machineries, composed of hundreds of RNA and protein factors, have been maintained through evolution of eukaryotes to carry out the synthesis of a few, but essential, mtDNA-encoded proteins. In humans, seven (ND1, 2, 3, 4, 4L, 5, 6) are components of CI, one (cytochrome *b*) of CIII, three (COI, II, III) of CIV and two (ATPase 6 and 8) of CV.

Specific pathways are required for the assembly of each MRC complex, including the insertion of mtDNA-encoded subunits into the inner membrane of mitochondria, in concert with >80 nuclear DNA encoded subunits; the synthesis and incorporation of several prosthetic groups that form the catalytic redox cores of CI, CII, CIII, and CIV; and the ultimate formation of functionally active holocomplexes. Individual holocomplexes can also organize themselves in respiratory supercomplexes. Additional systems warrant the quality control of protein and non-protein components of the MRC complexes, thus contributing to the maintenance of their structural integrity, functional activity and turnover. Thus, a highly regulated, extremely complex process is at work in mitochondria to control the formation, stability, interactions, function, and plasticity of the MRC. Defects in genes encoding the components of these control and execution systems can compromise the function of the MRC, thus leading to faulty OXPHOS and disease. For instance, many mtDNA mutations affect the mtDNA genes encoding the RNA apparatus for in situ translation (tRNA and rRNA encoding genes) rather than those encoding structural MRC proteins. Likewise, in addition to mutations in nuclear DNA-encoded MRC subunits, a vast number of mitochondrial disorders are due to mutations in "ancillary" gene products controlling their assembly, function and turnover (Ghezzi and Zeviani 2011).

The term "mitochondrial disorders" is referred to (genetic) the defects of OXPHOS. The peculiar genetics of mtDNA, the intergenomic interactions converging on the formation of the MRC, and the structural and functional complexities of

the biochemical pathways underpinning OXPHOS, explain the extreme heterogeneity of inherited mitochondrial disorders, which include a vast range of symptoms, severity, age of onset, progression, and outcome (DiMauro and Davidzon 2005). Several modes of transmission have been reported: maternal or Mendelian (autosomal dominant, recessive, X-linked), depending whether the primary genetic defect resides in mtDNA or nuclear DNA. Sporadic conditions, presumably caused by de novo mutations, are also known, as for instance in single mtDNA deletions. Altogether, the prevalence of genetic OXPHOS defects is not less than 1:5,000 (Schaefer et al. 2004; Thorburn et al. 2004; Mancuso et al. 2007), and probably higher (Cree et al. 2009). Clinical manifestations range from lesions in single structures (i.e., the optic nerve in Leber's hereditary optic neuropathy, LHON), tissues or organs (e.g., myopathies, encephalopathies, cardiomyopathies), to multisystem syndromes, with multiple organ involvement, the onset varying from neonatal to adult life. The degree of organ failure is also variable, depending on tissue-specific energy demand, tissue-specific vs. ubiquitous expression of OXPHOS-related genes, mitotic segregation of heteroplasmic mtDNA mutations, the presence of a genetic bottleneck for mtDNA during embryonic development, and other, poorly defined, causes. Because OXPHOS is necessary for energy supply to virtually any cell, any organ can be affected by mitochondrial disease. However, the most common clinical presentations include the involvement of muscle, heart and brain, i.e., postmitotic, specialized tissues, with high metabolic requests (McFarland and Turnbull 2009).

Adhering to the title of our review, the following discussion will focus on factors involved in the assembly of human MRC complexes, mainly those reported to be abnormal in patients, causing faulty OXPHOS and disease (Table 4.1). In broad terms, an "assembly factor" of a given MRC component is any protein (or non-protein) species that plays a role in its formation or stability, but is not stably part of it. Investigation of assembly defects has not only been useful to understand the molecular pathogenesis of several mitochondrial disorders, but has also helped understand the biogenesis of MRC formation in mammals. However, in only a few cases has the detailed mechanism of action been elucidated, so that the definition of "assembly factor" remains largely observational, based on the association between an assembly defect of a given complex with mutations in a particular gene product. For example, BCS1L and SURF1 are important for the assembly of CIII and CIV, but the precise mechanism by which they accomplish this task remains unclear. Moreover, the manifestations of their defects could be due to a multiplicity of functions, some of which unrelated to the MRC. For instance, defects of BCS1L, a CIII assembly factor, are associated with iron overload and multivisceral failure with normal CIII activity in some patients (Visapaa et al. 2002), whereas in others the main abnormality is, as expected, a specific defect of CIII; mutations in SURF1, an assembly factor of CIV, are lethal in humans and flies, but are associated with prolonged longevity and neuroprotection from calcium-induced neuronal excitotoxicity in knockout mice (Dell'Agnello et al. 2007); SCO1 and SCO2, two other CIV assembly factors, are involved in cellular copper homeostasis (Leary et al. 2007), but SCO2 has also been proposed to participate in a p53-dependent checkpoint system that controls the switch from glycolysis to respiration as the main energy

Table 4.1 Assembly factors of the MRC and related mitochondrial disease

Protein	Predicted function/s	Phenotypes
CI assembly factors		
NDUFAF1	CI chaperone interacting transiently with early arm membrane intermediates	Cardiomyoencephalopathy, lactic acidosis
ECSIT	Associated with NDUFAF1	–
NDUFAF2	Stabilizer of late intermediate(s)	Leukoencephalopathy with vanishing white matter, Leigh syndrome
NDUFAF3	Interact with some CI subunits and with NDUFAF4	Variable phenotypes: macrocephaly, severe muscle weakness, myoclonic seizures, brain leukomalacia
NDUFAF4	Interact with some CI subunits and with NDUFAF3	Encephalopathy, antenatal cardiomyopathy
C20ORF7	Probable methyltransferase; early arm membrane assembly	Leigh syndrome, progressive spasticity
C8ORF38	Unclear role but necessary for CI function	Leigh syndrome
NUBPL	Facilitates the effective assembly of Fe–S cofactors and subunits	Leukodystrophy, myopathy, ataxia
ACAD9	Complex I assembler by the interaction with the mitochondrial inner membrane or with NDUFAF1/ECSIT	Cardiomyopathy, encephalopathy, lactic acidosis, exercise intolerance
FOXRED1	Functional role in electrons transfer reactions; aminoacid metabolism	Leigh syndrome
CII assembly factors		
SDHAF1	Fe/S clusters insertion or retention within the CII protein backbone	Encephalomyopathy
SDHAF2	Flavination of SDHA	Hereditary paraganglioma
CIII assembly factors		
BCS1L	Incorporation of RISP	GRACILE syndrome, Bjornstad syndrome, encephalopathy, proximal tubulopathy and liver failure
TTC19	Important in early assembly, interacts with fully assembled CIII	Progressive encephalopathy
HCCS	Synthesis of cyt *c1* and cyt *c*	MIDAS
PTCD2	Maturation and stabilization of cytochrome *b* mRNA	–
CIV assembly factors		
SURF1	Formation of the early subcomplexes of COX	Leigh syndrome
SCO1	Incorporation of copper atoms in the catalytic sites of the nascent complex	Infantile encephalopathy, neonatal hepatopathy, ketoacidotic comas

SCO2	Incorporation of copper atoms in the catalytic sites of the nascent complex	Infantile cardioencephalomyopathy
COX10	Heme A synthesis (conversion of heme *b* to heme *o*)	Leigh syndrome, proximal renal tubulopathy, hypertrophic cardiomyopathy, sensorineural deafness, metabolic acidosis
COX15	Heme A synthesis (conversion of heme *o* to heme *a*)	Infantile cardiomyopathy, Leigh syndrome
COX11	Biosynthesis of heme *a*	–
COX17	Copper recruitment	–
COX19	Copper translocation to mitochondria	–
LPPRC	RNA metabolism, regulation of mtDNA genes	French-Canadian Leigh syndrome
TACO1	COX subunit I translation	Slow progressive Leigh syndrome
C20ORF64	Involved in a very early step of the COX assembly	Fatal neonatal cardiomyopathy
CV assembly factors		
ATPAF1	F1 chaperon; essential for assembly of $\alpha+\beta$ heterooligomer	–
ATPAF2	F1 chaperon; essential for assembly of $\alpha+\beta$ heterooligomer	Degenerative encephalopathy, connatal lactic acidosis, methyl glutaconic aciduria
TMEM70	Assembly of F1; F1 interaction with some of the F0 subunits	Neonatal encephalocardiomyopathy

provider of the cell. Further complexity derives from the recent observation that individual respiratory complexes agglomerate into gigantic supercomplexes.

Studies on the facultative anaerobic yeast *Saccharomyces cerevisiae* have not only led to an understanding of the basic mechanisms underlying the assembly of complexes III, IV, and V, but also to the discovery of many assembly factors, some of which have orthologs in humans.

Because CI is missing in *S. cerevisiae*, other yeasts, such as *Neurospora crassa* and *Yarrowia lipolytica*, or mammals, such as *Bos taurus*, have served as models for the study of CI structure and assembly. Blue native gel electrophoresis (BNGE) is a fundamental technique used to analyze the molecular steps for MRC assembly in different organisms and conditions, including human disease (Schagger and von Jagow 1991; Wittig et al. 2006). For example, the identification of CI subcomplexes in patients has allowed investigators to identify consistent anomalous patterns and relate them to specific genetic defects. Furthermore, the study of subcomplexes in mutant cells, and in normal cells after inhibition of mitochondrial protein synthesis, has led to the delineation of the current models for CI assembly in humans and the refinement of those for CII, CIII, CIV, and CV.

4.2 Complex I

In mammalian mitochondria, CI (NADH ubiquinone oxidoreductase, EC 1.6.5.3) catalyzes the oxidation of reduced NADH by CoQ. CI is the largest complex in the OXPHOS system, consisting of ≈45 subunits in mammals, for a total molecular mass of ≈1 MDa (Carrol et al. 2006). Seven subunits (ND1–ND6, ND4L) are encoded by the mitochondrial genome, the remaining 38 by nuclear DNA (nDNA) genes. The seven mtDNA encoded subunits and seven nuclear-encoded subunits (NDUFV1, NDUFV2, NDUFS1, NDUFS2, NDUFS3, NDUFS7, NDUFS8) form the catalytic "core" structure of the complex (Koopman et al. 2010), conserved in virtually all organisms that contain a CI, including *Thermus thermophilus*. The remaining nuclear-DNA encoded subunits are possibly involved in CI assembly, stability, and regulation of activity.

Ultrastructural studies of purified CI revealed an "L" shaped object, consisting of two arms, a hydrophilic "peripheral arm" protruding into the matrix, and a hydrophobic "membrane" arm, embedded in the mitochondrial inner membrane, with an angle between the two arms of about 100°. The value of the angle can slightly vary, since the two arms change their reciprocal positions during the catalytic cycle of the complex (Janssen et al. 2006). The two arms harbor three functional modules: (1) the N module, in the peripheral arm, contains the dehydrogenase site, formed by a flavin-mononucleotide (FMN) moiety, responsible for the oxidation of NADH to NAD+; (2) the Q module, in the hinge region between the two arms, contains the CoQ reduction site; (3) the P module, or proton translocation module, constitutes the membrane arm, and includes the seven mtDNA-encoded ND subunits (Vogel et al. 2007). Eight iron–sulfur (Fe–S) clusters are distributed in the N module (n. 5) and the Q module (n. 3); seven clusters form a redox chain, through which electrons

flow from FMN to CoQ, whereas the role of the eighth cluster, contained in the N module, is unclear.

The crystal structures of CI from *Thermus thermophilus* (Efremov et al. 2010) and *Yarrowia lipolytica* (Hunte et al. 2010) have provided important information on the mechanisms of electron flow, CoQ reduction, and proton pumping. In particular, the membrane arm, which largely coincides with the P module, includes 63 trans-membrane α-helices distributed among the seven core subunits encoded by mtDNA (Efremov et al. 2010). The P module is composed of two domains of similar size, proximal (P_p) and distal (P_D), connected by a narrow bridge consisting of a long α-helix, which is possibly part of ND5 (Hunte et al. 2010). The driving force for proton translocation is generated by the electrons extracted from NADH, flowing through the seven iron–sulfur cluster centers in the peripheral arm, distributed in the N and Q modules, and eventually converging onto ubiquinone, to form ubiquinol. Energy liberated by the electron flow is transferred from the Q to the P modules where it prompts the P_P–P_D protein bridge to move like a piston through the P_P–P_D domains, thus causing the nearby transmembrane helices to tilt and force proton translocation (Efremov et al. 2010).

4.2.1 Human Diseases Associated with Complex I Deficiency (MIM 252010)

Isolated CI deficiency is frequent in mitochondrial disorders (Distelmaier et al. 2009), being responsible for about one-third of all cases (Janssen et al. 2006). The primary genetic defect may be either in mtDNA or in nuclear DNA genes. Given the complexity of this huge enzyme, its dual genetic origin, and the incomplete information about its assembly, turnover, and regulation, it is not surprising that about half of the patients are still genetically undefined (Thorburn et al. 2004), and clinical presentations are so heterogeneous, including, for children, Leigh syndrome (LS), neonatal cardiomyopathy with lactic acidosis, fatal infantile lactic acidosis (FILA), macrocystic leukoencephalopathy, or pure myopathy (Loeffen et al. 2000; Bugiani et al. 2004). CI defects or CI-related mutations, particularly in mtDNA, include Leber's hereditary optic neuropathy (LHON), MELAS (mitochondrial encephalopathy with lactic acidosis and stroke-like episodes), or MELAS/LHON overlap syndromes (Lenaz et al. 2004). Several additional, poorly defined, syndromes include neurological signs, such as ophthalmoparesis, optic atrophy, epilepsy, ataxia and dystonia; gastrointestinal problems, such as pseudo-occlusion and gastroparesis; hepatopathy; cardiomyopathy; and renal dysfunctions, such as tubulopathy or proteinuria (Janssen et al. 2006).

4.2.2 CI Assembly Model and Assembly Factors

The complexity of CI assembly reflects its gigantic size, huge number of subunits, double genetics, multiple functions (e.g., redox vs. proton translocation), and numerous prosthetic groups. As mentioned above, there is no CI in the user-friendly

yeast *Saccharomyces cerevisiae*. This has been a major hurdle in the definition of the molecular steps of, and players in, CI assembly. Together, these facts explain why CI assembly is still poorly defined; however, observations from CI-defective patients, siRNA experiments in mammalian cells (Remacle et al. 2008; Scheffler et al. 2004; Schneider et al. 2008), and gene targeting in CI-dependent fungi, such as *Neurospora crassa*, have contributed to outline a module-based general model of assembly (Vogel et al. 2007; Lazarou et al. 2009) (Fig. 4.1). Accordingly, the earliest stage consists in the formation of an evolutionarily conserved hydrogenase (Q) module composed of subunits NDUFS2, NDUFS3, NDUFS7, and NDUFS8. After the addition of NDUFA9 (and possibly other subunits), this peripheral-arm intermediate is anchored to the mitochondrial inner membrane, where it is assembled with a membrane arm intermediate containing ND1. The resulting 400 kDa subcomplex is then assembled with a 460 kDa membrane-bound subcomplex, formed by hydrophobic subunits ND2, ND3, ND6, and NDUFB6. Next, subunits ND4 and ND5 are incorporated to form an intermediate, largely consisting of the Q and P modules. Eventually, a pre-formed N module composed of subunits NDUFV1, NDUFV2, NDUFV3, NDUFV6, NDUFS1, NDUFS4, and NDUFS6, join the Q–P intermediate, together with additional peripheral subunits, to form mature CI (McKenzie and Ryan 2010). Accordingly, the composition and size of several CI intermediates, characterized in CI-defective patients, suggest that the peripheral and membrane arms are joined together before the completion of each arm (Lazarou et al. 2007). Many important details are still missing, including, for instance, the role of several single-trans-membrane ancillary subunits, which are supposed to form a scaffold structure "holding" the giant complex during its oscillatory movements; or when and how the several prosthetic groups are incorporated into the complex.

By analogy to complex IV, for which >20 factors cooperate to assemble a 13 subunit complex, at least 10–20 additional CI assembly factors are expected to be found, besides the ones that will be discussed below (Calvo et al. 2010). The list is in fact expanding, thanks to the implementation of a number of strategies, including systematic exome sequencing of CI-defective patients and in silico analysis of interspecific genomic variance. For instance, differential genome subtraction is a powerful approach that exploits what has been considered a drawback, i.e., the absence of CI in *S. cerevisiae,* to select genes that are missing in this organism but present in CI-proficient organisms, including *N. crassa, Yarrowia lipolytica*, and higher eukaryotes, to test the hypothesis that some of them must be functionally related to CI function, including assembly. This strategy has been successfully applied for the identification of a new CI assembly factor, *B17.2* (*NDUFAF2*) (Ogilvie et al. 2005), and has later been implemented to identify 19 proteins that share common ancestry with a large subset of CI proteins. A causative mutation in one of these genes, C8ORF38, has been identified in a family with inherited CI deficiency (Pagliarini et al. 2008).

Some of the known CI assembly factors are physically associated with CI assembly intermediates, e.g., *N. crassa* CIA84 and CIA30 (Kuffner et al. 1998), and their human ortholog NDUFAF1 (Dunning et al. 2007); or ECSIT, a human signaling molecule interacting with NDUFAF1 (Vogel et al. 2007).

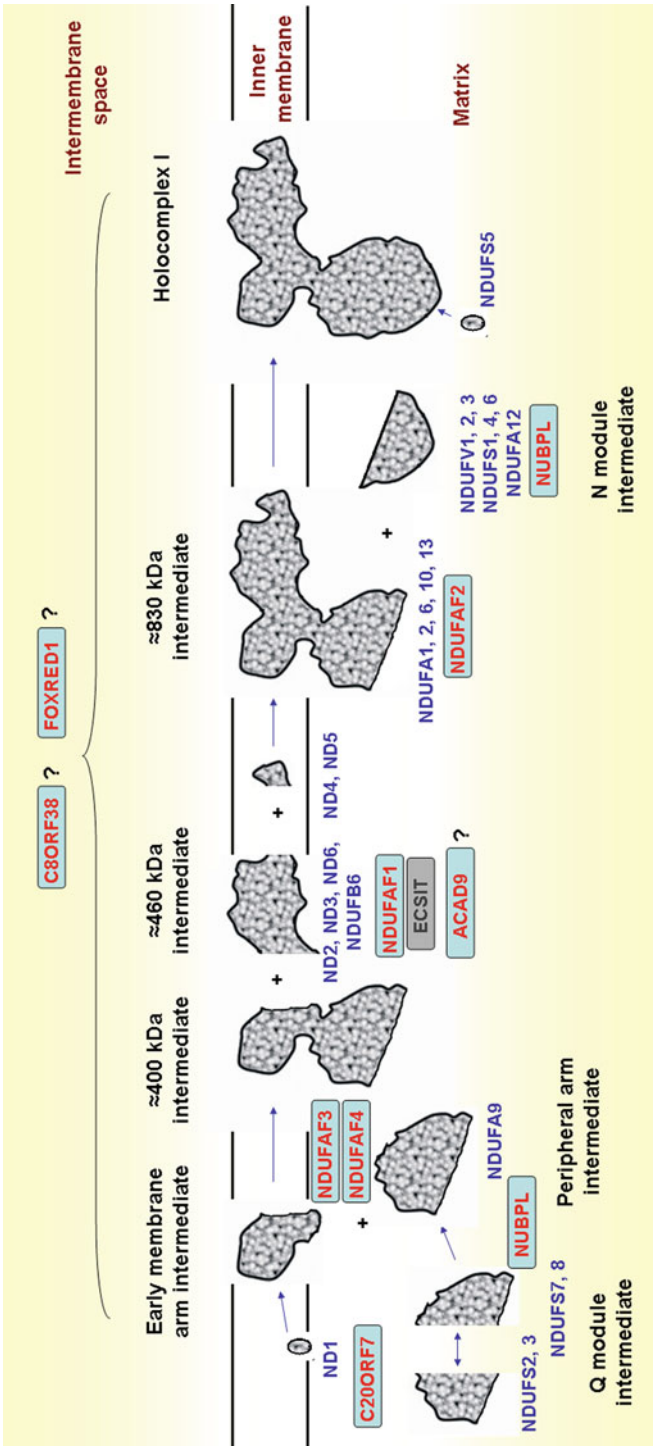

Fig. 4.1 Schematic outline of CI assembly. Assembly factors are *boxed*; in *red* are those associated with human disease, whereas the others are in *grey*. See text for details

Similar to other CI defective conditions, mutations in CI assembly factors cause a wide range of clinical disorders.

4.2.2.1 NDUFAF1/CIA30 (MIM 606934)

Studies in the aerobic fungus *N. crassa* led to the identification of two proteins associated with CI assembly intermediates, CIA30 and CIA84. siRNA-mediated knockdown of *NDUFAF1*, the human homologue of *CIA30*, resulted in decreased CI activity and amount. NDUFAF1 interacts with mitochondrial and nuclear CI subunits (Dunning et al. 2007) and is physically associated with two assembly intermediates (Bych et al. 2008). Based on these results, NDUFAF1 has been proposed as a chaperone transiently interacting with CI intermediates (Kuffner et al. 1998; Vogel et al. 2007), but mechanistic details are missing. Mutations in this protein were reported in a patient with cardiomyoencephalopathy, lactic acidosis and reduced levels of CI (Dunning et al. 2007). Little is known on the role of CIA84 and its human ortholog, the pentatricopeptide protein PTCD1, for which no mutation has been reported so far.

4.2.2.2 ECSIT (MIM 608388)

By two-dimensional (2-D) BNGE analysis and affinity purification, the mitochondrial isoform of ECSIT (standing for evolutionary conserved signaling intermediate in Toll pathway) was found in 500–600 kDa and 830 kDa assembly intermediates of CI, associated with CIA30. The knockdown of *ECSIT* in human cells reduces the levels of NDUFAF1, determining impaired CI assembly and activity (Vogel et al. 2007). ECSIT may play a role in mitochondrial production of reactive oxygen species (ROS) and in the macrophage-mediated antibacterial response: after stimulation of the innate immune system, a Toll-like receptor signaling adaptor (TRAF6) translocates to mitochondria, where it engages ECSIT. This interaction leads to ECSIT ubiquitination and migration to the mitochondrial periphery, resulting in increased mitochondrial and cellular ROS generation (West et al. 2011).

4.2.2.3 NDUFAF2/B17.2L (MIM 609653)

NDUFAF2 (*B17*.2L or *NDUFA12L*) was identified by differential genome subtraction of facultative vs. obligatory aerobic yeasts (Ogilvie et al. 2005). However, while in *Y. lipolytica* B17.2 is a structural subunit located in the peripheral arm of CI, its human ortholog, *NDUFAF2,* is a putative assembly factor that does not take part in the CI holocomplex.

A stop mutation of *NDUFAF2* was detected in a patient with progressive leukoencephalopathy with vanishing white matter, and impaired CI assembly (Ogilvie et al. 2005). Another mutation causing the complete absence of the protein was

found in two infants with hypotonia, nystagmus, and ataxia (Barghuti et al. 2008) associated with 30% residual CI activity in muscle mitochondria. Additional homozygous *NDUFAF2* mutations, associated with the complete absence of the protein, were identified in three LS patients (Calvo et al. 2010). In human heart mitochondria, NDUFAF2 co-immunoprecipitates with several CI subunits, and is associated with a late ≈830-kDa CI subassembly intermediate in several CI-deficient patients, but not in control samples (Vogel et al. 2007). Taken together, these studies demonstrate that the complete absence of NDUFAF2 does not prevent the formation of some fully assembled CI holocomplex, indicating that this protein could stabilize late CI intermediate(s) or help late subunits to be incorporated into the nascent complex.

4.2.2.4 NDUFAF3/C3ORF60 (MIM 612911)

Studies on CI-defective patients led to the identification of NDUFAF3 and NDUFAF4 (Saada et al. 2009). Mutations in *NDUFAF3* were found in three families with CI deficiency associated with a spectrum of severe phenotypes: a fulminant syndrome dominated by muscle hypertonia in the first, macrocephaly and severe muscle weakness in the second, and myoclonic epilepsy and leukomalacia in the third. All patients died before 6 months of age. The knockdown of *NDUFAF3* in HeLa cells shows decreased CI amount and activity, and a significant reduction of another assembly factor, NDUFAF4. NDUFAF3 and NDUFAF4 co-migrate in the same CI assembly intermediates and interact with the same set of CI subunits (Saada et al. 2009).

4.2.2.5 NDUFAF4/C6ORF66 (MIM 611776)

A homozygous mutation in *NDUFAF4* was associated with severe CI deficiency in five consanguineous patients presenting with infantile encephalopathy and in one unrelated case of antenatal cardiomyopathy. BNGE analysis of mutant muscle mitochondria revealed reduction of fully assembled CI and accumulation of assembly intermediates (Saada et al. 2008). Similar to NDUFAF3, siRNA knockdown of *NDUFAF4* results in decreased levels of both CI holocomplex and NDUFAF3 (Saada et al. 2009), suggesting physical interaction and functional interdependence.

4.2.2.6 C20ORF7 (MIM 612360)

A homozygous mutation in an anonymous gene, *C20ORF7*, was identified in a lethal neonatal form of CI deficiency by homozygosity mapping followed by candidate gene analysis (Sugiana et al. 2008). Knockdown of *C20ORF7* decreased CI activity in cells and a role for C20ORF7 was demonstrated in the

assembly or stability of an early CI assembly intermediate that contains ND1 but not ND2 subunits. A second mutation was later found in affected members from one family, presenting with LS; CI activity was reduced in blood lymphocytes and muscle. However, the genetic etiology was unclear, since a mutation in a second gene, *CRLS1*, encoding cardiolipin synthase 1, was also found in these patients (Gerards et al. 2010). Recently, a *C20ORF7* variant has been reported in children with LS from Ashkenazi Jewish families; in this population the carrier rate is 1:290. Interestingly, these patients show combined deficiency of CI and CIV but expression of the wildtype (wt) gene in mutant cells restored CI activity to normal whereas recovery of CIV was incomplete (Saada et al. 2012). Decreased CIV activity was also observed in human fibroblasts after *C20ORF7* knockdown (Sugiana et al. 2008), suggesting for C20ORF7 an additional role in CIV assembly or in the formation of CI–CIV supercomplexes. For instance, C20ORF7 resembles, and possibly is, a methyltransferase, that could play a role in the posttranscriptional modification of proteins necessary for CI and CIV function and/or expression, or for the stability of the CI–CIV respirasome species (Saada et al. 2012).

4.2.2.7 C8ORF38 (MIM 612392)

Genome subtraction helped identify C8ORF38 as a protein necessary for CI function. A homozygous missense mutation in a conserved residue was associated with LS with isolated CI deficiency. Knockdown of *C8ORF38* resulted in reduced levels and activity of CI, but its exact function remains unclear (Pagliarini et al. 2008).

4.2.2.8 NUBPL/Ind1 (MIM 613621)

Fe–S clusters are present in CI, CII and CIII, and several enzymes and chaperones are required for their biosynthesis (see Sect. 4.6). In addition, Ind1 is a Fe–S cluster binding protein with a specific role in the incorporation of Fe–S centers in CI. Deletion of the *Ind1* gene in *Y. lipolytica* results in a very specific defect of CI, without affecting other mitochondrial Fe–S enzymes (Bych et al. 2008; Sheftel et al. 2009). Knockdown of the human ortholog, *NUBPL*, in cultured cells causes a specific decrease in the amount of CI holocomplex and the formation of an assembly intermediate missing the peripheral arm, which harbors all the Fe–S clusters, but containing the membrane-arm NDUFB6 subunit (Sheftel et al. 2009). These findings suggest that NUBPL/Ind1 specifically facilitates the incorporation of Fe–S clusters into CI peripheral arm.

In a high-throughput screening of CI deficient patients, compound heterozygous mutations in this protein were identified in a single case, presenting with mitochondrial encephalopathy (Calvo et al. 2010).

4.2.2.9 ACAD9 (MIM 611103)

ACAD9 was discovered as an essential CI assembly factor by different approaches: traditional homozygosity mapping and candidate gene screening, exome sequencing, and tandem mass analysis on proteins interacting with known assembly factors. ACAD9 interacts with NDUFAF1 and ECSIT and co-migrates with the same CI assembly intermediates that contain these two factors. Knockdown of ACAD9, NDUFAF1 or ECSIT in cultured cells determines the decrease of all three proteins, and of CI holocomplex as well (Gerards et al. 2011).

Mutations in ACAD9 are associated with infantile hypertrophic cardiomyopathy, encephalopathy and lactic acidosis (Haack et al. 2010; Nouws et al. 2010), with exercise intolerance (Gerards et al. 2011). All patients had a reduction of CI enzymatic activity and assembly. ACAD9 mutant cells and patients respond to riboflavin treatment, with partial correction of CI deficiency and clinical improvement (Haack et al. 2010; Gerards et al. 2011), possibly because ACAD9 is a $FADH_2$ dependent acyl-CoA dehydrogenase. However, although ACAD9 displays a β-oxidative activity in vitro, fatty acid β-oxidation seems normal in ACAD9 mutant patients, the only functional impairment being connected to CI assembly.

4.2.2.10 FOXRED1 (MIM613622)

FOXRED1 was identified by gene screening of CI-defective LS patients (Fassone et al. 2010; Calvo et al. 2010). Knockdown of *FOXRED1* decreases the levels of CI in control fibroblasts. The presence of a FAD-binding domain suggests a role for this protein as a redox enzyme, but its exact function remains elusive (Fassone et al. 2010).

4.3 Complex II

Complex II (succinate dehydrogenase ubiquinone–ubiquinol reductase, E.C. 1.3.5.1) is the only membrane-bound member of the tricarboxylic acid (TCA) cycle, where it functions as a succinate dehydrogenase (SDH), catalyzing the oxidation and dehydration of succinate to fumarate. CII takes part in the MRC by coupling this reaction to the reduction of ubiquinone to ubiquinol, that in turn funnels electrons to CIII (Ernster and Dallner 1995). CII is the smallest MRC complex (123 kDa), consisting of four subunits, encoded by *SDHA*, *SDHB*, *SDHC*, and *SDHD* nuclear genes. SDHA contains a FAD moiety, whereas three Fe–S centers are bound to SDHB. These two hydrophilic subunits are linked to SDHC and SDHD, two small, hydrophobic polypeptides that contain a heme *b* moiety and anchor the complex to the inner mitochondrial membrane (Sun et al. 2005). The crystal structure of porcine heart CII consists of a hydrophilic head protruding into the matrix, a hydrophobic tail embedded in the

inner membrane, and a short segment projecting into the intermembrane space (Yankovskaya et al. 2003; Sun et al. 2005). Mitochondrial CII shows close homology with a number of bacterial succinate ubiquinone reductases (SQRs), especially those of α-proteobacteria, from which mitochondria are supposed to derive (Andersson et al. 1998). The aminoacid sequences of the flavin and Fe–S binding domains of CII are highly conserved. The membrane domain is less conserved, although a four-helix bundle motif is ubiquitously present across species.

4.3.1 Human Diseases Associated with CII Deficiency (MIM 252011)

Mitochondrial disease with isolated impairment of CII is rare, encompassing 2–8% of OXPHOS defective cases (Munnich and Rustin 2001; Ghezzi et al. 2009). Two main clinical presentations are known: mitochondrial encephalomyopathy and familial paragangliomas (tumors of chromaffin cells).

In the first group, LS is the most common clinical and neuropathological presentation, but myopathy, encephalopathy, leukodystrophy and isolated cardiomyopathy have also been reported. Mutations in *SDHA*, *SDHD*, and *SDHAF1* genes have been identified in a minority of CII defective patients.

The pathogenesis of CII-associated paragangliomas remains to be explained. The most widely accepted hypothesis is based on induction of the hypoxia program that switches energy metabolism from mitochondrial respiration to glycolysis. This adaptive mechanism would occur through the stabilization of the hypoxia-inducible transcription factor 1 (HIF1) as a result of increased concentration of succinate, the SDH substrate (Bayley and Devilee 2010). The activation of the hypoxic program includes increased cellular uptake of glucose, activation of glycolysis, and promotion of angiogenesis.

The four genetic loci associated with hereditary paragangliomas correspond to *SDHD* (PGL1), *SDHC* (PGL3), *SDHB* (PGL4), and *SDHAF2* (PGL2) genes.

4.3.2 CII Assembly Model and Assembly Factors

The low frequency of human conditions associated with CII deficiency and the absence of a proton pumping activity and mtDNA-encoded subunits may explain the scarcity of information on CII assembly, in spite of the fact that CII is the smallest and simplest MRC complex.

In *E. coli*, an active, soluble SDH is composed of SDHA and SDHB ortholog subunits (SdhA, B), independent of the SDHC and SDHD orthologs (SdhC, D) (Nakamura et al. 1996). However, there are no data in eukaryotes suggesting that the CII hydrophilic module, composed of SDHA and SDHB, builds up independent

of the membrane-embedded module, formed by SDHC and SDHD. SDHB seems to play a central role in the stabilization of the human holocomplex, since mutations in each and every SDH subunits cause the loss of SDHB (van Nederveen et al. 2009). CII also contains several prosthetic groups, including one heme *b* moiety, one FAD, and three Fe–S clusters. The function of the single heme *b* moiety is unknown. In *B. subtilis* SQR (Hagerhall 1997; Hederstedt 2002), the absence of heme *b* prevents the hydrophilic subunits to assemble with the membrane-bound subunits. The crystal structure of SQR-related prokaryotic enzymes (e.g., the quinol-fumarate reductase of *W. succinogenes*) shows physical interaction between four of the transmembrane helices and the heme *b* moiety (Lancaster et al. 1999). The histidyl ligands for heme *b* are conserved throughout the species and site-directed mutagenesis of *E. coli* residues involved in heme *b* binding induces the catalytic subunits to dissociate from the membrane-bound subunits. Taken together, these observations support a role for heme *b* in assembly and stability of the complex (Hagerhall and Hederstedt 1996; Nakamura et al. 1996). The current model for CII assembly starts with heme *b* binding to SdhD, followed by linking with SdhC (Lenaz and Genova 2010), to form the membrane-bound module. Heme *b* is also essential to link the hydrophilic module (SdhA + SdhB) to the membrane anchor subunits (SdhC + SdhD). While defects in factors involved in FAD supply (e.g., Flx1) (Tzagoloff et al. 1996) or Fe–S cluster synthesis (e.g., Tcm62) (Dibrov et al. 1998) can impair assembly and activity of CII, together with other Fe–S or FAD-dependent enzymes, only two specific CII assembly factors are presently known, SDHAF1 (Ghezzi et al. 2009) and SDHAF2 (Hao et al. 2009).

4.3.2.1 SDHAF1 (MIM612848)

SDHAF1, standing for SDH Assembly Factor 1, is a small protein containing a tripeptide sequence, LYR, a proposed signature for proteins involved in Fe–S metabolism. Hence, SDHAF1 could play a role in the insertion or retention of the Fe–S clusters within the protein backbone of CII, but this hypothesis needs further experimental evidence.

Although SDHAF1 resides in the mitochondrial matrix, while CII is membrane bound, mutations in this protein are associated with a drastic decrease of CII activity and content in both humans and yeast. A homozygous missense mutation in *SDHAF1* has been identified in related infants affected by leukoencephalopathy with the accumulation of lactate and succinate in the white matter, and severe reduction of CII activity and amount in muscle and fibroblasts (Ghezzi et al. 2009). Complementation assays in both human cells and a yeast model have confirmed the pathogenicity of the mutations. An additional homozygous stop mutation has later been identified in a CII-defective baby girl with leukoencephalopathy, born from consanguineous parents (R. Carrozzo; poster 251 Euromit8 Meeting 2011). To date, no mutation in SDHAF1 has been reported in patients with paraganglioma (Feichtinger et al. 2010).

4.3.2.2 SDHAF2 (MIM 613019)

The function of SDHAF2, standing for SDH assembly factor 2, is likely related to the incorporation of FAD into SDHA (Sdh1 in yeast), since Sdhaf2-less yeast strains, as well as SDHAF2 mutant human fibroblasts, have normal amounts of total Sdh1/SDHA protein but very low levels of the flavinated form (Hao et al. 2009). The binding of FAD to SDHA is probably a self-catalytic process, but requires that the imported SDHA subunit is properly refolded, forming the FAD-binding pouch. Sdhaf2/SDHAF2 could be a chaperone responsible for this step, in cooperation with hsp60 in *S. cerevisiae* (Robinson and Lemire 1996).

A germline missense mutation in SDHAF2, G78R, has been reported in two families with hereditary, multiple head and neck paragangliomas (PGL2). The G78 residue is part of the most conserved region of the protein, and the mutant R78 impairs its interaction with the SDHA subunit (Hao et al. 2009). The same mutation has later been found in a Spanish family, characterized by earlier onset of head and neck PGL. Haplotype analysis indicates that the G78R occurred independently in the two families (Bayley et al. 2010).

4.4 Complex III

CIII (ubiquinol–cytochrome *c* reductase, E.C. 1.10.2.2) catalyzes the electron transfer from reduced CoQH2, (ubiquinol) to cytochrome *c*. CIII is made up of 11 subunits (Iwata et al. 1998), only one (cytochrome *b*, cyt *b*) being encoded by mtDNA. Nuclear genes encode the remaining subunits: apo-cytochrome *c*1, cyt *c*1, the Rieske iron–sulfur protein (RISP or UQCRFS1), two relatively large "core" subunits, Core 1 (UQCRC1) and Core 2 (UQCRC2), and six additional, smaller proteins (UQCR6-11), the functions of which are unknown. In addition to the protein backbone, prosthetic groups of CIII include the two Fe-containing heme moieties of cyt *b* and *c1*, and the Fe–S cluster of RISP; these three subunits form the catalytic redox core of CIII. The CIII monomer is likely a transient form, which quickly converts into a stable, catalytically active homodimer.

4.4.1 Human Diseases Associated with CIII Deficiency (MIM 124000)

CIII defects are rare, compared to those of CI or CIV, the most frequent being caused by mutations in cyt *b*. Most of the cyt *b* mutations are sporadic and cause a mitochondrial myopathy with high plasma levels of creatine kinase and episodic myoglobinuria. CIII deficiency is also found in mutations of two assembly factors: *BCS1L* and *TTC19*.

4.4.2 CIII Assembly Model and Assembly Factors

Studies in *S. cerevisiae* have outlined a module-based assembly model for CIII, which is likely the same in mammals. The assembly starts with the formation of three different modules (1) cyt *b*+Qcr7+Qcr8, (2) cyt *c1*+Qcr6+Qcr9, and (3) Core1+Core2. The three modules assemble together, forming a precomplex to which RISP and Qcr10 are then added, leading to the formation of enzymatically active CIII (Zara et al. 2007). In humans, an additional subunit corresponds to the cleaved presequence of RISP (Brandt et al. 1993), which is inserted in the very last stage of CIII assembly. CIII dimerization occurs in the pre-complex stage, before the incorporation of the last subunits.

More recently, 2D-BNGE analysis of yeast CIII mutant strains has shown the existence of additional *bc1* sub-complexes, suggesting some corrections of the model (Zara et al. 2009) (Fig. 4.2). Accordingly, an initial module, containing cyt *b*+Qcr7+Qcr8, incorporates a second module composed of Core 1+Core 2+cyt *c*1, to form a 500 kDa dimerized sub-complex, to which Qcr6, Qcr9, RISP and Qcr10 are sequentially added.

Only two CIII-specific assembly factors are currently known in humans: BCS1L and TTC19.

4.4.2.1 BCS1L (MIM 603647)

BCS1L is the human ortholog of yeast Bcs1 (standing for b–c synthesis 1). A member of the AAA family, Bcs1 is essential for the incorporation of RISP, and possibly subunit 10, into CIII in the last steps of CIII assembly (Cruciat et al. 1999; Nobrega et al. 1992). Bcs1 seems to interact with the 500 kDa dimeric CIII pre-complex, playing a chaperone-like role to maintain the pre-complex in a conformation suitable for RISP incorporation. A similar function is predicted for BCS1L, since human cells from BCS1L mutant patients show accumulation of the CIII pre-complex, with hardly any trace of either incorporated RISP or fully assembled CIII (Zara et al. 2007). BCS1L is also found in a high molecular weight structure that does not contain any CIII-specific subunit (Fernandez-Vizarra et al. 2007; Cruciat et al. 1999). The latter is probably a membrane integral ring-shaped BCS1L homohexamer, a structure typical of AAA family members.

Several *BCS1L* gene mutations have been reported in CIII deficiency, associated with different clinical presentations: neonatal proximal tubulopathy, hepatopathy and encephalopathy (De Lonlay et al. 2001), and isolated progressive infantile encephalopathy. The acronym GRACILE stands for growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis and early death, and designates an infantile condition caused by a specific BCS1L mutation, S78G, which is part of the Finnish disease heritage. A second less-severe disease associated with BCS1L missense mutations is Björnstad syndrome, characterized by neurosensory hearing loss and abnormally curly and brittle hair (*pili torti*). The clinical heterogeneity can

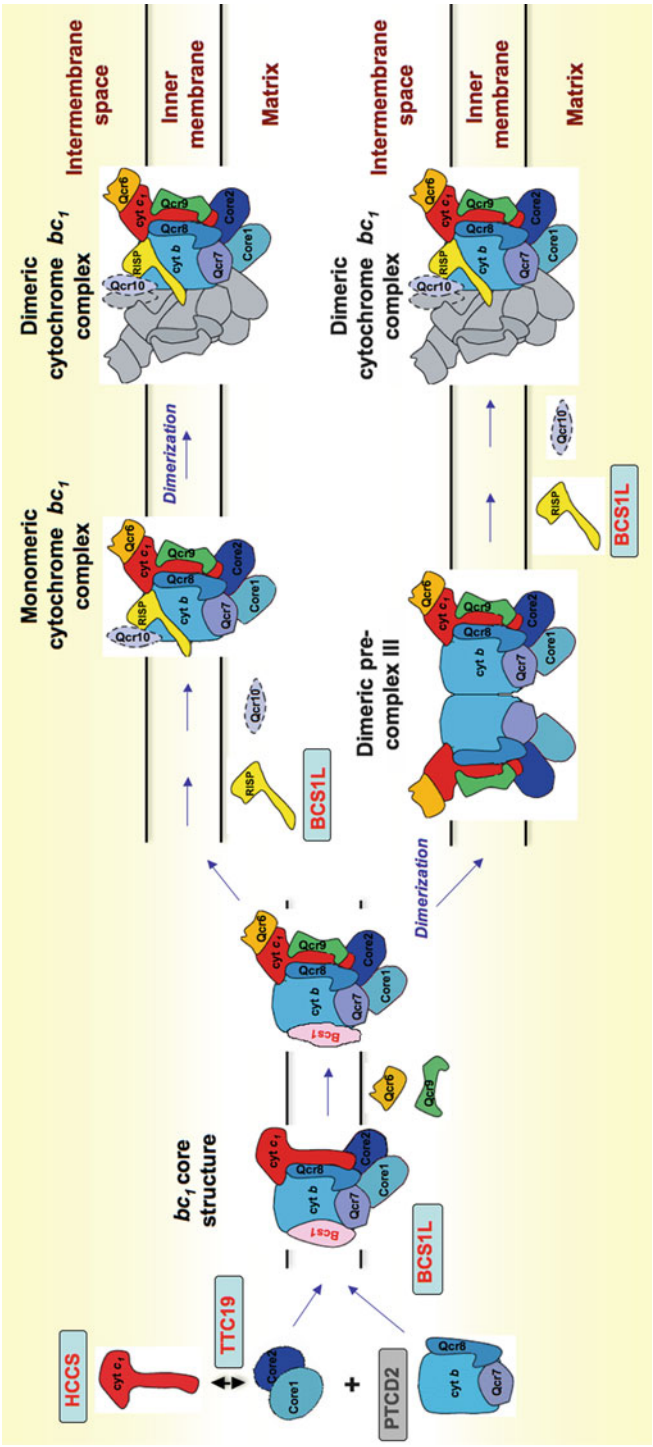

Fig. 4.2 Schematic outline of CIII assembly. Assembly factors are *boxed*; in *red* are those associated with human disease, whereas the others are in *grey*. Two possible pathways are depicted. The top pathway is characterized by monomeric CIII assembly (Zara et al. 2007); the bottom pathway is characterized by early dimerization of CIII pre-complex (Fernandez-Vizarra, 2009). See text for details

be linked to the functional domain affected by the different mutations. In the BCS1L protein there are three different domains: an N-terminal import domain, a BCS1L-specific domain and a C-terminal AAA, ATPase domain. For instance, while mutations have been reported in all domains, those in the ATPase domain are associated with a more severe phenotype (Hinson et al. 2007). A mouse recombinant model carrying the GRACILE mutation reproduces the main clinical features of the human disease, displaying CIII deficiency, with liver and kidney involvement. Interestingly, the clinical manifestations, and the CIII defect, including impaired RISP incorporation, start a few weeks after birth, suggesting the existence of another factor that plays the same role of Bcs1l during embryonic development and neonatal period (Levéen et al. 2011).

4.4.2.2 TTC19 (MIM 613814)

A second, putative CIII assembly factor, TTC19 (tetratricopeptide repeats 19) has recently been discovered, by investigating a CIII-defective, progressive mitochondrial encephalopathy (Ghezzi et al. 2011). All affected individuals carried *TTC19* nonsense mutations, resulting in the absence of the protein. TTC19 protein is localized in the inner mitochondrial membrane, where it takes part in two high molecular weight complexes, 500 kDa and ≥1 MDa in size. While the smaller complex corresponds to $CIII_2$, the larger one is also detected in rho^0 cells (that lack mtDNA and hence CIII) suggesting for TTC19 additional functions, unrelated to the MRC. The TTC19-mutant muscle accumulates CIII assembly intermediates that contain Core 1–2, but not RISP, suggesting that TTC19 is important in early CIII assembly. The tetratricopeptide motif is a protein–protein interaction module found in a number of functionally different proteins that facilitates specific interactions with partner proteins (Blatch and Lassle 1999).

TTC19 has no ortholog in yeast but a *TTC19* knockout model of *D. melanogaster* is characterized by neurological abnormalities associated with CIII deficiency. Similar to what reported in the *Bcs1l* knockin "GRACILE" mouse, normal development and CIII activity of mutant *D. melanogaster* larvae, and the late disease onset of mutant patients, suggest tissue-specific, age-related and developmentally dependent regulation of both TTC19 functions and CIII activity in different cell types and organisms.

4.4.2.3 HCCS (MIM 300056)

In humans, a single heme lyase, holocytochrome *c* synthase (HCCS), catalyzes the covalent binding of heme moieties to both apocytochromes *c* and *c*1 (Bernard et al. 2003). Catalytically active holocytochrome *c*1 is part of CIII, whereas holocytochrome *c* is a mobile cytochrome shuttling electrons from CIII to CIV, and taking part in other important cellular processes, for instance mitochondrial apoptosis (Jiang and Wang 2004).

Mutations in the *HCCS* gene are associated with MIDAS (microphthalmia, dermal aplasia and sclerocornea, MIM 309801), an X-linked dominant trait exclusively found in heterozygous mutant females, while hemizygous mutant males die at the embryonic stage.

4.4.2.4 PTCD2

A factor related to cytochrome *b* was found in a knockout mouse model obtained by random mutagenesis. A member of the pentatricopeptide protein family, PTCD2 (pentatricopeptide repeat domain protein 2) is a mitochondrial RNA-binding protein that participates in the maturation and stabilization of cytochrome *b* mRNA (Xu et al. 2008).

Several steps in human CIII assembly are still missing, including the exact sequence of incorporation for both protein subunits and prosthetic groups, the mechanism of formation of the CIII dimer, the details of BCS1L–RISP interaction and the exact role and interactions of TTC19 with CIII components. Developmental regulation of CIII assembly is suggested by BCS1L and TTC19 animal models. As for other MRC complexes, additional factors are likely involved in the various stages of CIII assembly.

4.5 Complex IV

Complex IV (cytochrome *c* oxidase, COX, E.C. 1.9.3.1), the terminal component of MRC, transfers electrons from reduced cytochrome *c* to molecular oxygen. This reaction is coupled to proton pumping across the inner mitochondrial membrane. Mammalian COX is composed of 13 subunits (Tsukihara et al. 1996; Yoshikawa et al. 1998), the three largest being encoded by mtDNA genes (MTCO1, MTCO2, MTCO3), whereas the remaining subunits are encoded by nuclear genes. The redox catalytic core is composed of subunits MTCO1 and MTCO2, which harbor two iron-containing heme *a* moieties (*a* and a_3) and two copper centers (CuA and CuB), responsible for the electron transfer. The third mtDNA-encoded subunit, MTCO3, is part of the structural core, being possibly involved in proton pumping. The function of the ten nuclear-encoded subunits (COX4, COX5A, COX5B, COX6A, COX6B, COX6C, COX7A, COX7B, COX7C and COX8) is currently unknown, but they may play regulatory (Arnold and Kadenbach 1997) and stabilization roles. In humans, COX displays multiple tissue-specific isoforms: for instance, COX6A and COX7A have a heart (H) isoform, present in skeletal and cardiac muscle, and a liver (L) isoform, present in extra-muscle tissues (Grossman and Lomax 1997); a testis-specific COX6B2 isoform and a lung-specific COX4 have also been identified. These alternative isoforms confer different kinetic properties to the enzyme, endowing it with remarkable functional plasticity.

4.5.1 Human Diseases Associated with CIV Deficiency (MIM 220110)

In humans, defects of COX activity are nearly as common as CI defects. In infancy, the most frequent manifestation of isolated, profound COX deficiency is LS, but other phenotypes include severe cardiomyopathy and encephalocardiomyopathy. Maternally inherited COX defects are associated with several mutations of mtDNA tRNA genes. Conversely, only a few mutations in the three COX-encoding mtDNA genes and a single mutation in a nuclear-encoded COX subunit, COX6B (Massa et al. 2008), have been reported to date, suggesting that most of the mutations in structural components of CIV are incompatible with extra-uterine life. Accordingly, the most common nuclear-DNA gene defects of COX are due to mutations in the assembly factors.

4.5.2 CIV Assembly Model and Assembly Factors

Many COX assembly factors are conserved between yeast and mammals, although half of them are missing in the latter organisms; in turn, mammalian COX includes subunits that are absent in yeast (i.e., COX7b and COX8). This explains not only the relatively greater wealth of information gained on CIV assembly, compared to other MRC complexes, but also the many details that are still missing.

In summary (Fig. 4.3), COX subunits are sequentially incorporated to form the nascent complex, starting from the insertion of newly synthesized MTCO1 into the inner mitochondrial membrane. This first, crucial step (S1) is followed by the incorporation of subunits COX4 and COX5A, to form a second assembly intermediate, S2. Insertion of heme *a* is likely to occur just after the formation of S1 or during the formation of S2, and proceeds together with the insertion of CuB and heme a_3 into MTCO1. The formation of the MTCO2-associated CuA center is followed by the incorporation of MTCO2 into the S2 intermediate. In yeast, this step requires Cox18 (MIM 610428), a factor also present in humans, catalyzing the insertion of the MTCO2 C-terminal tail into the mitochondrial inner membrane. Next, rapid sequential incorporation of MTCO3 and of smaller nuclear encoded subunits, including COX5b and COX8, to form S3, leads to the formation of a quasi-complete assembly intermediate (also named subcomplex b) (Williams et al. 2004; Stiburek et al. 2005). The addition of a few remaining subunits, including COX6A, COX6B, COX7A and COX7B (Nijtmans et al. 1998; Massa et al. 2008), all converging on the surface of the complex core, results in the formation of a holocomplex monomer (S4). Finally, monomeric COX dimerizes in an active structure that contains the cytochrome *c* binding site (Tsukihara et al. 1996), where two molecules of cytochrome *c* cooperatively bind at the interface between the two COX monomers (Birchmeier et al. 1976; Darley-Usmar et al. 1984) formed by contacts among MTCO1, COX6A, COX6B, and COX5B (Lee et al. 2001).

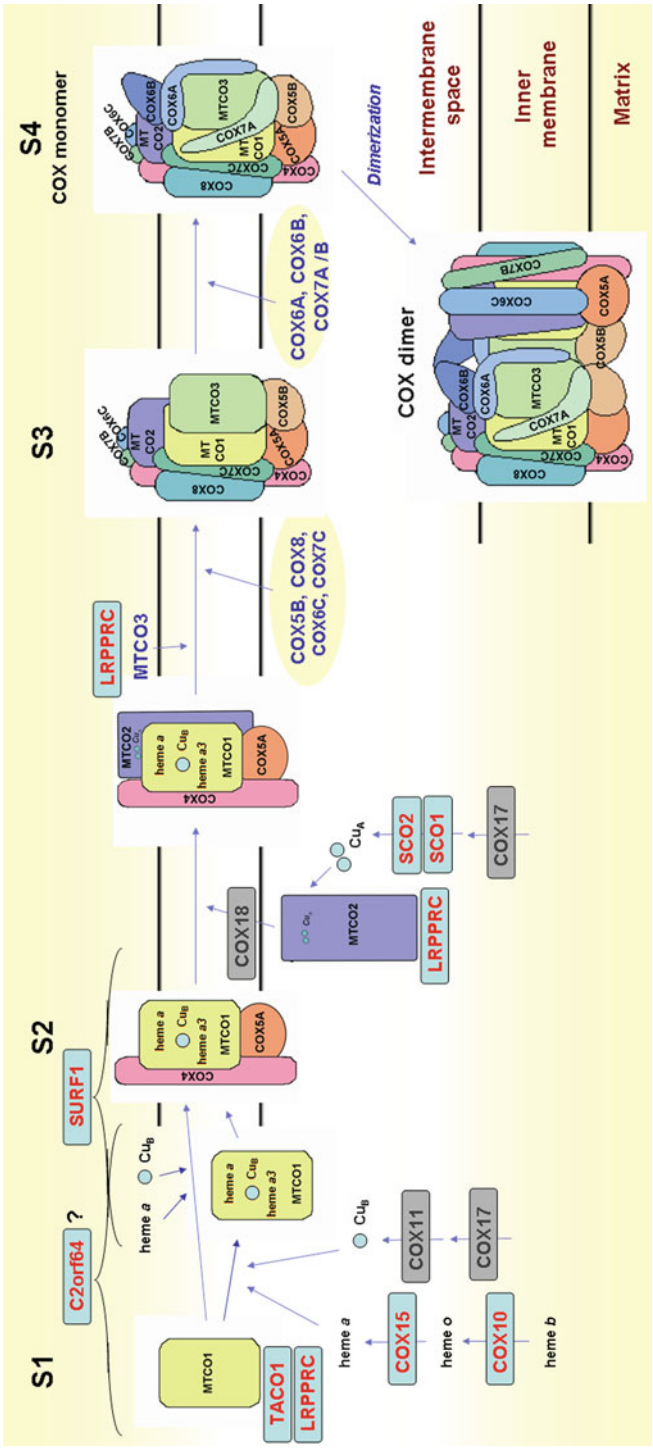

Fig. 4.3 Schematic outline of CIV assembly. Assembly factors are *boxed*; in *red* are those associated with human disease, whereas the others are in *grey*. See text for details

More than 20 nuclear encoded accessory proteins are involved in COX assembly of *S. cerevisiae*, most of which have known human orthologs (Fontanesi et al. 2006). Ancillary gene products are required for incorporation of hemes *a, a3* and copper atoms (CuA, B) into catalytic COX subunits and for maintaining the nascent intermediates in an assembly-competent state (Barrientos et al. 2009; Mick et al. 2007; Stiburek et al. 2006).

Similar to CI, CIII and CV, CIV contains mtDNA encoded subunits, i.e., MTCO1, MTCO2, MTCO3 that are synthesized on mitochondrial ribosomes. In *S. cerevisiae* the insertion into the mitochondrial inner membrane of these in situ translated subunits requires a specific integrase, i.e., OXA1 (standing for oxidase-cytochrome *c* assembly 1), which is located as a homo-oligomeric complex in the inner mitochondrial membrane (Bonnefoy et al. 1994). The function of OXA1 and their OXA1-like orthologs is probably not specific for CIV; for instance, OXA1 depletion in *Neurospora crassa* results in combined reduction of CI and CIV (Nargang et al. 2002). Likewise, knockdown of OXA1L in human cells impairs the biogenesis of CV and CI, whereas CIV seems unaffected (Stiburek et al. 2007). No mutation in human *OXA1L* has been reported in patients with cIV or combined MRC deficiency (Coenen et al. 2005).

We will hereafter focus on those CIV assembly factors that are involved in human disease.

4.5.2.1 SURF1 (MIM 185620)

Although the precise function of SURF1 remains to be elucidated, studies on the yeast ortholog Shy1, and in human mutant cells and tissues, indicate a role for SURF1 in the formation of the early subcomplexes of COX. The mature SURF1 protein is a 30 kDa hydrophobic polypeptide with two transmembrane domains at the N and C termini, which anchor the protein to the mitochondrial inner membrane (Tiranti et al. 1998).

Mutations in *SURF1* are the most common cause of LS associated with COX deficiency. This association is highly specific, and is partly explained by the observation that almost all the SURF1 mutations reported to date cause the complete absence of the protein. Very few missense mutations have been detected (Pecina et al. 2004), sometimes in association with less severe phenotypes (Piekutowska-Abramczuk et al. 2009). *SURF1* mutations determine marked reduction in the amount of fully assembled COX and accumulation of early assembly intermediates S1 and S2 (Tiranti et al. 1999; Stiburek et al. 2005). Although the mechanistic function is still unclear, these results indicate a role for the SURF1 protein in the early stages of COX assembly, most likely before the incorporation of MTCO2 into the S2 assembly intermediate (Nijtmans et al. 1998). In SURF1 null human samples (Tiranti et al. 1998), as well as in yeast strains lacking Shy1 (Mashkevich et al. 1997), fully assembled, functionally active CIV is found in residual amounts, suggesting partial functional redundancy.

Recent studies in bacteria have indicated that two prokaryotic Surf1 putative orthologs (Surf1c and Surf1q) are heme *a* binding proteins, suggesting a role for Surf1 in heme *a* insertion into MTCO1 (Bundschuh et al. 2009).

4.5.2.2 SCO1 (MIM603644) and SCO2 (MIM 604272)

In yeast, two functionally and evolutionarily related COX assembly genes, *SCO1* and *SCO2* (for synthesis of cytochrome oxidase), enable MTCO1 and MTCO2 to be incorporated into nascent CIV by promoting the insertion of Cu^{++} atoms in the catalytic sites CuB and CuA of either subunit. This process requires additional factors, partly conserved in yeast and mammals, including COX17 (MIM 604813) and COX11 (MIM 603648), and is carried out at the very early steps of COX assembly, being necessary to promote the incorporation and to stabilize the assembly of the first protein subunits of CIV, including MTCO1 and MTCO2. In SCO1- and SCO2-deficient cells, S1 and S2 subassemblies accumulate, whereas fully assembled COX is markedly reduced (Williams et al. 2004; Leary et al. 2004; Stiburek et al. 2005); the absence of MTCO2 in the subcomplexes indicates that the formation of the MTCO2-bound CuA center is necessary for the addition of MTCO2 to the S2 intermediate.

Mutations in the human ortholog of *SCO2* were initially found in infants with fatal cardioencephalomyopathy and COX deficiency (Papadopoulou et al. 1999). More than 10 mutations have been reported; however, one particular mutant allele, E140K, is present in almost all affected individuals, either in combination with a second mutant allele, or in homozygosity. The latter genotype is associated with a relatively milder phenotype and delayed disease onset (Joost et al. 2010). Heart hypertrophy in *SCO2*-mutant patients is usually severe, whereas brain involvement may vary, from LS-like to spinal-muscular atrophy-like presentations (Pronicki et al. 2010).

Mutations in *SCO1* have been found in a single large family with multiple cases of neonatal hepatopathy, severe ketoacidosis and isolated, severe COX deficiency in liver and muscle (Valnot et al. 2000). Another *SCO1* case presented with hypertrophic cardiomyopathy, encephalopathy and hepatomegaly with fatal outcome (Stiburek et al. 2009).

4.5.2.3 COX10 (MIM 602125) and COX15 (MIM 603646)

COX10 and COX15 are enzymes involved in the terminal steps of the biosynthesis of hemes *a* and *a3*. *COX10* encodes the heme *a*:farnesyltransferase, which catalyzes the conversion of protoheme (heme *b*) to heme *o*. COX15 converts heme *o* into heme *a* by hydroxylation, with the help of a monooxygenase still uncharacterized in humans. While complete loss of COX10 or COX15 activities is incompatible with extra-uterine life, rare missense mutations have been found in both, resulting in partial COX deficiency. Since heme *a* is incorporated during early COX assembly,

mutations in either COX10 or COX15 lead to the arrest and degradation of the complex, with virtually no accumulation of assembly intermediates (Antonicka et al. 2003).

Mutations in *COX10* are associated with a spectrum of conditions including LS; encephalopathy with proximal renal tubulopathy; sensorineural deafness, metabolic acidosis, hypotonia and hypertrophic cardiomyopathy. Likewise, mutations of *COX15* can cause fatal infantile hypertrophic cardiomyopathy, as well as rapidly progressive or protracted LS.

4.5.2.4 COX11 (MIM 602125), COX17 (MIM 603646) and COX19 (MIM 610429)

Studies carried out in yeast have led to the identification of additional genes necessary for the maturation and insertion of the copper prosthetic groups in nascent COX.

The Cox11 protein is a constituent of the inner mitochondrial membrane and, like Cox10 and Cox15, is likely involved in the biosynthesis of heme *a*. Mutations in the *S. cerevisiae* cox11 gene result in a 'petite' phenotype, associated with isolated COX defect.

The Cox17 protein contains a cysteine-rich motif and two additional cysteine residues that are also conserved in copper-binding metallothioneins. Yeast cox17 is a cytosolic protein involved in copper recruitment to mitochondria. Cox17 null mutations cause CIV assembly failure and Cox17 KO mice are embryonic lethal, exhibiting severe COX deficiency. Yeast Cox17 is likely to act downstream from Cox23, a factor that has not been identified in mammals.

Yeast, and human, Cox19 is a cytosolic protein, with a minor fraction being located in the intermembrane space of mitochondria. Its exact function is still unknown, but its structure is similar to that of Cox17, suggesting a role in copper translocation to mitochondria.

Both *COX17* and *COX19* are candidates for CIV defects, but no mutation in either gene has been reported in humans.

4.5.2.5 LRPPRC (MIM 607544) and TACO1 (MIM 612958)

LRPPRC (or LPR130) is a leucine-rich protein of the pentatricopeptide repeat family; its role is still unclear, but it seems to regulate the expression of mtDNA genes (Sasarman et al. 2010), including RNA stability, maturation and processing (Schmitz-Linneweber and Small 2008). While missense LRPPRC mutations are associated with isolated COX deficiency, further decrease of LRPPRC levels determines profound reduction of all mitochondrial transcripts associated with generalized defect of all mtDNA-dependent OXPHOS complexes.

A single LRPPRC mutation, A354V, causes the French-Canadian type of Leigh syndrome (LSFC, MIM 220111), which is restricted to the Charlevoix

Seguenay-Lac Saint-Jean region in Quebec, Canada. The clinical presentation is characterized by mild psychomotor regression and lactic acidosis, with severe COX deficiency in brain and liver. A single patient has been reported to carry an exon deletion, in compound heterozygosity with the A354V (Mootha et al. 2003).

TACO1 (standing for translation activator of CO1; initially named *CCD44*) is a mitochondrial translational activator required for efficient translation of *MTCOI*. A homozygous frameshift mutation in this gene, c.472insC, has been found in patients from a single family, presenting with slowly progressive LS, caused by impaired MTCO1 protein synthesis (Weraarpachai et al. 2009). No other TACO1 mutations have been found to date, suggesting that integrity of this factor is needed for extra-uterine life (Seeger et al. 2010).

4.5.2.6 C2ORF64

PET191, the yeast ortholog of huma C2ORF64, is a COX assembly factor, albeit its mechanistic role is still unknown. A homozygous mutation in *C2ORF64* was recently described in two siblings affected by fatal neonatal cardiomyopathy. 2D-BNGE analysis revealed the accumulation of a small assembly intermediate containing subunit MTCO1 but not MTCO2, COX4, or COX5b, indicating that C2ORF64 is involved in a very early step of COX assembly (Huigsloot et al. 2011).

4.6 Complex V

Complex V (ATP synthase, E.C. 3.6.3.14) dissipates the proton electrochemical gradient generated by the respiratory chain to produce ATP. It comprises an integral membrane cylindrical rotor-like structure, the F0 particle, and a peripheral matrix-facing F1 particle, the catalytic ATP synthase domain (Boyer 1997). F1 has a bulb-like shape, formed by six "cloves". F0 and F1 are physically connected to each other by two additional structures: a centrally located, asymmetrical stalk and an externally tethered stator. F0 is a rotor harboring a proton channel. Following the electrochemical gradient, protons flow through the channel guided by the stator, impressing a rotary motion to F0, which is transmitted to the catalytic head (F1) by the centrally projecting stalk. The sequential tilt of the cloves, impressed by the asymmetrical stalk during each F1 rotation cycle, drives the condensation of ADP and Pi to form three ATP molecules for each cycle (Devenish et al. 2008). All five subunits of F1 (α, β, γ, δ, ε), and most of the F0 subunits (b c, d, e, f, g, OSCP and F6) are nuclear encoded (Collinson et al. 1996). Only two proteins (MTATP6 and 8) are encoded by mtDNA (Boyer 1993). Both MTATP6 and ATP8 are part of the F0, and connect the latter to the stator (Fig. 4.4). Dimeric and higher oligomeric forms of ATP synthase (Arnold et al. 1998; Paumard et al. 2002) seem critical to maintain the shape of mitochondria by promoting the formation of the inner membrane cristae.

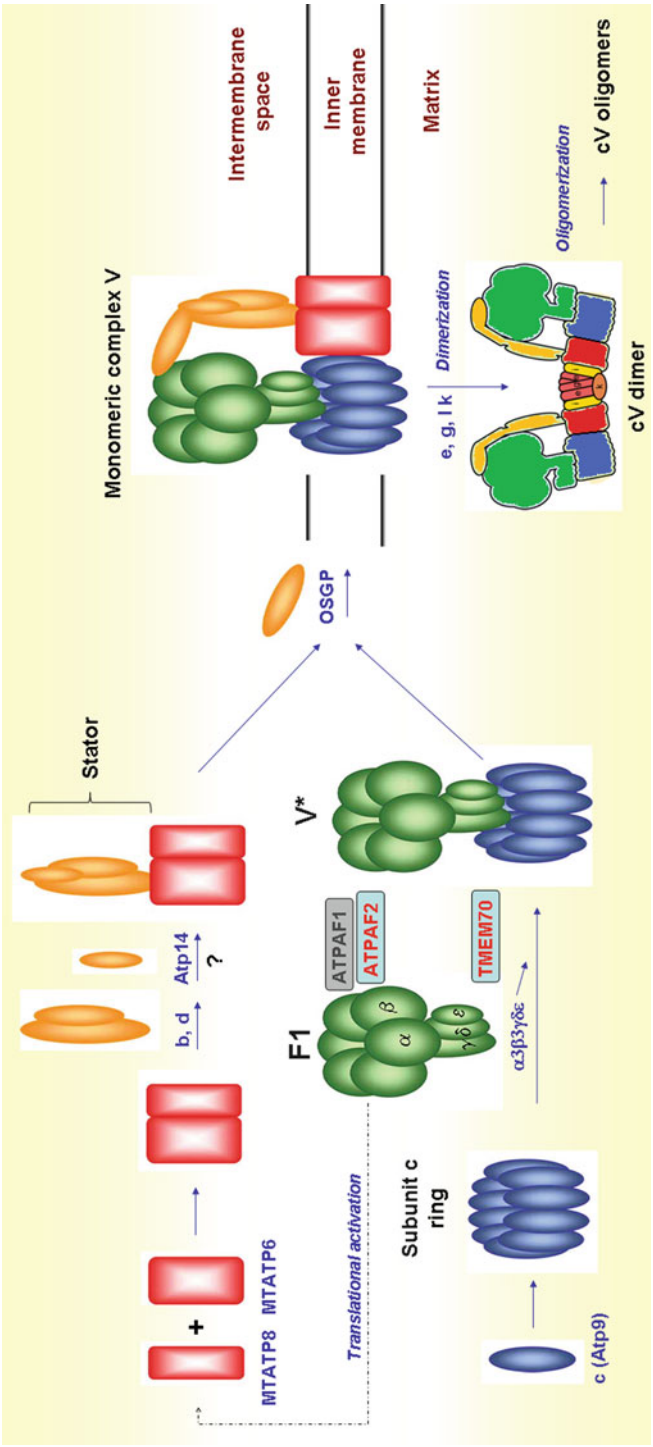

Fig. 4.4 Schematic outline of CV assembly. Assembly factors are *boxed*; in *red* are those associated with human disease, whereas the others are in *grey*. The existence of human orthologs of yeast CV assembly factors follows the model by Rak et al. (2011). See text for details

4.6.1 Human Diseases Associated with CV Deficiency

Maternally transmitted ATP synthase dysfunction can be caused by mutations in *MTATP6* or *MTATP8*, the two mtDNA genes encoding ATP6 and 8 polypeptides. Heteroplasmic missense mutations in MTATP6 (Tatuch and Robinson 1993, Schon et al. 2001) are associated with adult-onset NARP (neuropathy, ataxia and retinitis pigmentosa) or maternally inherited Leigh syndrome (MILS), the clinical severity being proportional to the heteroplasmic mutation load. A single patient with hypertrophic cardiomyopathy carried a nonsense mutation in MTATP8 (Jonckheere et al. 2008). Three disease-causing nuclear genes have been identified so far, two encoding assembly factors (ATPAF2, TMEM70), whereas the third (ATP5E) encodes the epsilon subunit of the F1 domain (Mayr et al. 2010). In other CV deficient cases the genetic cause is still unknown (Sperl et al. 2006).

4.6.2 Complex V Assembly Model and Factors

Experiments based on pulse–chase protein labeling and 2D-BNGE in bacterial, yeast and mammalian cells have delineated the current model for CV assembly. The process starts with the formation of the F1 catalytic core, carried out by specific assembly factors ATPAF1 and ATPAF2 in mammalian mitochondria (corresponding to Atp11 and Atp12 in yeast). Next, the initial F1 intermediate interacts with subunit c (Atp9 in yeast) and the other F0 subunits, forming an assembly intermediate named V*. The two mtDNA-encoded subunits, MTATP6 and MTATP8, are added during the last assembly stage, at least in mammalian cells (Nijtmans et al. 1995), since the V* intermediate and lower order subassemblies build up in MTATP6 or MTATP8 mutant mitochondria (Houstek et al. 2006). In yeast, two distinct assembly intermediates have been characterized, one formed by Atp6, Atp8, at least two stator subunits, and the Atp10 chaperone, the second by the F1 ATPase particle and the Atp9/subunit c ring. This recent result indicates that the assembly process is not a linear addition of single subunits one next to the other, but consists of at least two separate, coordinately regulated pathways eventually converging together at the end stage (Rak et al. 2011). This is in agreement with the notion that the F1 and F0 components seem to derive from functionally unrelated ancestral proteins (Mulkidjanian et al. 2007) that follow independent assembly pathways (Schatz 1968; Tzagoloff 1969). The very final steps in mammalian CV biogenesis include the formation of dimers, coincidental with the addition of subunits e and g (Schagger and Pfeiffer 2000), and the formation of higher order oligomers (V1–V4) (Krause et al. 2005).

4.6.2.1 ATPAF2 (MIM 608918)

Among the several factors known for CV assembly in yeast, only orthologs of ATP11/ATPAF1 and ATP12/ATPAF2 are known in mammals (Wang et al. 2001;

De Meirleir et al. 2004 These proteins are chaperones interacting with subunits β and α of F1, essential for the assembly of the α + β heterooligomer (Ackerman and Tzagoloff 1990, Wang et al. 2000).

To date, only one case of complex V deficiency has been referred to a homozygous missense ATPAF2 mutation associated with degenerative encephalopathy, connatal lactic acidosis and methyl-glutaconic aciduria (De Meirleir et al. 2004). The amount of fully assembled CV was low, but no subassembly intermediates were detected, suggesting that ATPAF2 acts very early during CV assembly (Houstek et al. 1999).

4.6.2.2 TMEM70 (MIM 612418)

Mutations in TMEM70 were found in patients, mostly of Gipsy origin, with neonatal encephalocardiomyopathy and isolated CV deficiency (Cizkova et al. 2008). This is the most frequent cause of ATP synthase deficiency (Honzik et al. 2010; Spiegel et al. 2011). The prevalent homozygous mutation, an A-to-G transition in intron 2 of the *TMEM70* gene, resulting in aberrant splicing and loss of the mRNA transcript, is associated with highly variable clinical severity.

2D-BNGE analysis of samples from TMEM70-mutant patients shows the presence of traces of free F1 ATPase, and small amounts of CV holocomplex, but no F0-F1 subassemblies (Houstek et al. 2009). Following transfection of wild-type *TMEM70* into mutant fibroblasts, CV holoenzyme amount and activity reverse to normal, whereas unassembled F1 disappears, indicating that TMEM70 may be involved in the assembly of F1 itself or in the F1 interaction with some of the F0 subunits.

Ultrastructural studies in TMEM70 mutant mitochondria show loss of invaginations of cristae and formation of concentric membrane rings. These morphological alterations could affect the integrity of mitochondrial nucleoids and hence mtDNA replication and expression (Cameron et al. 2011a), which could explain the variable reduction of other OXPHOS activities in some TMEM70 mutant cells.

Similar to other OXPHOS complexes, and by analogy with yeast, additional assembly factors are likely to concur to the biogenesis of mammalian CV, e.g., FMC1 for F1, and ATP10, ATP23 for F0 assembly. Other factors, such as NCA1-3, NAM1, AEP1-3 ATP22, and ATP25, are involved in mRNA stability, translation, and processing of yeast CV genes (Houstek et al. 2009). Last but not least, assembly of CV dimers and oligomers, which are critical for the formation of tubular cristae (Wagner et al. 2010), is likely to require *ad hoc* chaperones and stabilizing proteins.

4.7 Fe–S Cluster Biosynthesis

Fe–S clusters are essential prosthetic groups for CI, CII and CIII, and for several other mitochondrial and non-mitochondrial enzymes. The biosynthesis of Fe–S clusters and their incorporation into proteins is a complex process that requires

numerous factors (Lill and Kispal 2000), including scaffold proteins (i.e., ISCU, mNFU1), as well as cysteine desulfurases (ISCS), iron donors and chaperones (i.e., frataxin) (Li et al. 2009). More than 20 proteins involved in Fe–S cluster biogenesis have been identified in yeast (Lill and Mühlenhoff 2008). In humans, Fe–S clusters are synthesized and assembled into proteins in a series of complex biochemical reactions organized in two parallel pathways (Rouault and Tong 2008). In the first, ISCU is the main scaffold protein, whereas the second has NFU1. The pathways take place in the mitochondrial matrix; a fraction of the Fe–S pool is then exported from mitochondria to supply Fe–S dependent enzymes in the cytosol and nucleus.

Mutations of Fe–S related genes are linked to human disease characterized by multiple MRC deficiency.

4.7.1 ISCU (MIM 611911)

ISCU mutations (MIM 255125) cause hereditary myopathy with lactic acidosis, sometimes complicated by cardiomyopathy or episodes of myoglobinuria. The activities of aconitase and CII, both containing Fe–S clusters, are severely impaired in ISCU mutant patients (Mochel et al. 2008; Kollberg et al. 2009), with less severe reduction of CI and CIII activities as well. Two ISCU isoforms, cytosolic and mitochondrial, are produced by alternative splicing from the same gene transcript (Tong and Rouault 2006; Li et al. 2006), both being active in homeostatic regulation of iron uptake, intracellular iron distribution and mitochondrial iron utilization to form Fe–S clusters.

4.7.2 Frataxin (MIM 606829)

Frataxin interacts with ISCU in the same homeostatic pathway, acting as a mitochondrial iron chaperone that stores and supplies iron in a bioavailable form for the mitochondrial biosynthesis of Fe–S clusters and heme moieties. While the complete absence of frataxin leads to early embryonic lethality, mutations that reduce the amount of frataxin, including the most common one, a $(GAA)_n$ expansion within the first intron of *FXN,* the frataxin-encoding gene, cause Friedreich ataxia (FRDA, MIM 229300), an autosomal recessive, progressive disorder that combines spinocerebellar ataxia and cardiomyopathy (Campuzano et al. 1996). Mutant cells with low frataxin display defective activity of the Fe–S cluster-containing subunits of MRC CI, II and III. A physical interaction of frataxin with SDHA and SDHB subunits of CII has been shown in both human and yeast cell lines (Gonzalez-Cabo et al. 2005).

4.7.3 NFU1 (MIM 608100)

A homozygous missense mutation in the scaffold protein NFU1 has been found in affected members from one family, presenting with weakness, lethargy, and severe lactic acidosis. Biochemically, combined deficiency and low amount of the Fe–S MRC complexes were associated with defects of 2-oxoacid dehydrogenases, including pyruvate and 2-oxoglutarate dehydrogenase complexes (PDHc, OGDHc) (Cameron et al. 2011b). Although PDHc and OGDHc do not harbor Fe–S clusters, both covalently bind a lipoate moiety that is the product of a Fe–S cluster enzyme, lipoate synthase. Notably, and different from ISCU patients, the amount and activity of mitochondrial aconitase were normal. The mutation seems to affect the splicing process leading to no immunodetectable mitochondrial NFU1 protein.

4.7.4 BOLA3 (MIM 613183)

BolA family members are putative reductases interacting with glutaredoxins (Huynen et al. 2005). The proposed role for BOLA3 is to interact with glutaredoxin 5, which is involved, along with several chaperones, in the insertion of [2Fe–2S] and [4Fe–4S] clusters into apoproteins.

A homozygous single-base insertion in *BOLA3*, predicting the formation of a premature stop codon, was identified in a single subject presenting with cardiomyopathy and epileptic encephalopathy. As for NFU1 mutation, also in *BOLA3* mutant cells the activity and amount of Fe–S containing MRC complexes were reduced, particularly CI (Cameron et al. 2011b).

4.7.5 GRX5 (MIM 609588)

Several cochaperones bind to ISCU for the incorporation of [2Fe–2S] clusters into recipient apoproteins, whereas GLRX5 is a specific chaperone for the incorporation of [4Fe–4S] species.

A functional defect in the human glutaredoxin GRX5 was found in a patient with microcytic anemia and iron overload (Camaschella et al. 2007). Mutant cells showed low levels of aconitase, whereas MRC deficiency was not reported. However, the yeast Δgrx5 strain displayed respiratory deficiency, due to the absence of Rip1, the iron–sulfur Rieske protein, that is part of CIII (Bellí et al. 2004) or to the reduction of SDH activity (Kim et al. 2010). The mutant yeast strain also showed a significant decrease in the amount of mtDNA, suggesting a role for grx5 in supporting mitochondrial genome stability (Kim et al. 2010).

4.7.6 ABCB7 (MIM 300135)

The ATP-binding cassette member 7 (ABCB7) is an iron mitochondrial exporter, which controls the supply of Fe–S clusters to cytosolic Fe–S dependent proteins. Mutations in this gene have been found in families with X-linked sideroblastic anemia with ataxia syndrome (XLSA/A; MIM301310). The human ABC7 protein is localized in the inner mitochondrial membrane and belongs to the ATP-binding cassette transporter superfamily. Its yeast ortholog, atm1p, plays a central role in the maturation of cytosolic Fe–S cluster-containing proteins. As expected, deletion of Abcb7 in mouse impairs the activity of cytosolic but not mitochondrial Fe–S enzymes (Pondarré et al. 2006). The presence of cerebellar ataxia in XLSA/A indicates that, as evidenced also in FRDA, spino-cerebellar pathways and cells are exquisitely dependent on Fe–S bioavailability.

4.8 Respiratory Chain Supercomplexes

Integration of the redox reactions occurring in each complex increases the efficiency of the electron flow to molecular oxygen, reducing the electron leak from the MRC. The physical counterpart of this phenomenon is supercomplexes, i.e., supra-molecular structures composed of individual MRC complexes assembled in variable stoichiometric ratios.

The existence of supercomplexes dates back to a "*solid-state*" MRC model consisting of orderly sequences of complex I–complex IV (Chance and Williams 1955), based on the isolation of two or more complexes in consistently defined stoichiometric ratios (Fowler and Richardson 1963; Blair 1967). The "*solid-state*" model was later replaced by a "*fluid*" model, according to which MRC complexes are independently embedded in the lipid bilayer of the inner mitochondrial membrane and electron transfer depends on random collisions between complexes and the mobile carriers, CoQ and cyt *c*. The "*fluid*" model was based on the demonstration that respiratory complexes can be purified individually, retaining their enzymatic activity (Hackenbrock et al. 1986), and by lack of ultrastructural and in vitro evidence of supercomplexes (Capaldi 1982). However, BNGE analysis has later demonstrated the existence of larger sized protein structures (Krause et al. 2004; Schagger 2002; Schagger and Pfeiffer 2000), suggesting that individual MRC complexes exist in dynamic equilibrium with different types of supercomplexes, also referred to as respirasomes (Schagger and Pfeiffer 2000; Acín-Perez et al. 2008; Wittig and Schagger 2009). Kinetic measurements using metabolic flow control analysis have provided further evidence on the existence of functionally relevant, specific supercomplexes (e.g., CI+CIII) (Bianchi et al. 2004). This "*plasticity*" model integrates the two previous ones (Acin-Perez et al. 2008) and includes the following features: (1) supercomplexes are formed by single MRC complexes; (2) are functionally active entities, some of which contain CoQ and cyt *c* ; (3) they can respire, i.e., they

can transfer electrons from NADH to O_2; (4) are dynamic structures of variable stoichiometry that (5) optimize electron transfer efficiency to the different energy needs of cells (Wittig et al. 2006; Acín-Pérez et al. 2008; Wittig and Schägger 2009). Finally, a fraction of supercomplexes is associated with CV dimers to bend and fold the inner membrane and form the mitochondrial cristae (Gilkerson et al. 2003; Rabl et al. 2009; Zick et al. 2009).

Although results may vary, depending on concentration and type of detergent, in mitochondria from different species virtually all CI seem to be organized into a supercomplex composed of a CI monomer, a CIII dimer, and up to four COX complexes ($I_1III_2IV_{0-4}$) (Schagger and Pfeiffer 2000). In digitonin-solubilized bovine heart mitochondria, a $CI_1III_2IV_1$ supercomplex has been detected by BNGE, and its three-dimensional structure resolved by electron microscopy (Schäfer et al. 2007). The association into supercomplexes is essential for the stability of CI. In fact, when either complex CIII or CIV fail to get assembled (Acin-Perez et al. 2004; Diaz et al. 2006), leading to impaired formation of the supercomplex, mitochondria display CI deficiency (Lamantea et al. 2002; Saada et al. 2012). Conversely, absence of CI affects the formation of supercomplexes but does not usually impair the activity of the other complexes (Acin-Perez et al. 2008), although rare mutations in CI subunits, for instance NDUFS4 (Ugalde et al. 2004), can be associated with combined CI and CIII deficiency.

The molecular mechanisms and regulation of supercomplex assembly are still unknown. The process is likely to require specific factors, none of which has been identified so far. However, the CI chaperone NDUFAF2 is present in both a 830-kDa CI assembly intermediate and a structure composed of this intermediate bound to a CIII dimer, before completion of CI assembly (Lazarou et al. 2007). This observation supports the hypothesis that supercomplex assembly occurs in conjunction with the formation of individual complexes, in agreement with the experimental evidence showing that critical levels of CIII and CIV are required for supercomplexes to form and to warrant stability and integrity of CI. Together, these results suggest that assembly factors specific to individual complexes may be involved in the formation of supercomplexes. In addition, other components could play a role in supercomplex assembly, for instance cardiolipin. Defects in cardiolipin synthesis and remodeling due to mutations in the X-linked gene tafazzin, encoding an acyltransferase, cause Barth syndrome, a disease characterized by mitochondrial myopathy, cardiomyopathy, growth retardation, and leukopenia (Barth et al. 1999).

Recently, supercomplexes have been proposed as the building blocks for the formation of much larger supramolecular structures, such as respiratory "strings" (Wittig et al. 2006); "core fragments" of respiratory strings have been isolated as respiratory supercomplex multimers, of apparent mass of 35–45 MDa. Likewise, "ATP synthasomes" are supercomplexes formed by adenine nucleotide and inorganic phosphate carriers bound to CV (Ko et al. 2003).

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References

Acín-Pérez R, Bayona-Bafaluy MP, Fernández-Silva P et al (2004) Respiratory complex III is required to maintain complex I in mammalian mitochondria. Mol Cell 13:805–815

Acín-Pérez R, Fernández-Silva P, Peleato ML, Pérez-Martos A, Enriquez JA (2008) Respiratory active mitochondrial supercomplexes. Mol Cell 32:529–539

Ackerman SH, Tzagoloff A (1990) Identification of two nuclear genes (ATP11, ATP12) required for assembly of the yeast F1-ATPase. Proc Natl Acad Sci USA 87:4986–4990

Andersson SG, Zomorodipour A, Andersson JO et al (1998) The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. Nature 396:133–140

Antonicka H, Leary SC, Guercin GH 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

Arnold S, Kadenbach B (1997) Cell respiration is controlled by ATP, an allosteric inhibitor of cytochrome-c oxidase. Eur J Biochem 249:350–354

Arnold I, Pfeiffer K, Neupert W, Stuart RA, Schägger H (1998) Yeast mitochondrial F1F0-ATP synthase exists as a dimer: identification of three dimer-specific subunits. EMBO J 17:7170–7178

Barghuti F, Elian K, Gomori JM, Shaag A, Edvardson S, Saada A, Elpeleg O (2008) The unique neuroradiology of complex I deficiency due to NDUFA12L defect. Mol Genet Metab 94:78–82

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

Barth PG, Wanders RJ, Vreken P, Janssen EA, Lam J, Baas F (1999) X-linked cardioskeletal myopathy and neutropenia (Barth syndrome) (MIM 302060). J Inherit Metab Dis 22:555–567

Bayley JP, Devilee P (2010) Warburg tumours and the mechanisms of mitochondrial tumour suppressor genes. Barking up the right tree? Curr Opin Genet Dev 20:324–329

Bayley JP, Kunst HP, Cascon A et al (2010) SDHAF2 mutations in familial and sporadic paraganglioma and phaeochromocytoma. Lancet Oncol 11:366–372

Bellí G, Molina MM, García-Martínez J, Pérez-Ortín JE, Herrero E (2004) *Saccharomyces cerevisiae* glutaredoxin 5-deficient cells subjected to continuous oxidizing conditions are affected in the expression of specific sets of genes. J Biol Chem 279:12386–12395

Bernard DG, Gabilly ST, Dujardin G, Merchant S, Hamel PP (2003) Overlapping specificities of the mitochondrial cytochrome c and c1 heme lyases. J Biol Chem 278:49732–49742

Bianchi C, Genova ML, Parenti Castelli G, Lenaz G (2004) The mitochondrial respiratory chain is partially organized in a supercomplex assembly: kinetic evidence using flux control analysis. J Biol Chem 279:36562–36569

Birchmeier W, Kohler CE, Schatz G (1976) Interaction of integral and peripheral membrane proteins: affinity labeling of yeast cytochrome oxidase by modified yeast cytochrome c. Proc Natl Acad Sci USA 73:4334–4338

Blair PV (1967) Preparation and properties of repeating units of electron transfer. Methods Enzymol 10:208–212

Blatch GL, Lassle M (1999) The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. Bioessays 21:932–939

Bonnefoy N, Kermorgant M, Groudinsky O, Minet M, Slonimski PP, Dujardin G (1994) Cloning of a human gene involved in cytochrome oxidase assembly by functional complementation of an oxa1- mutation in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 91:11978–11982

Boyer PD (1993) The binding change mechanism for ATP synthase–some probabilities and possibilities. Biochim Biophys Acta 1140:215–250

Boyer PD (1997) The ATP synthase—a splendid molecular machine. Annu Rev Biochem 66:717–749

Brandt U, Yu L, Yu CA, Trumpower BL (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

Bugiani M, Invernizzi F, Alberio S et al (2004) Clinical and molecular findings in children with complex I deficiency. Biochim Biophys Acta 1659:136–147

Bundschuh FA, Hannappel A, Anderka O et al (2009) Surf1, associated with Leigh syndrome in humans, is a heme-binding protein in bacterial oxidase biogenesis. J Biol Chem 284:25735–25741

Bych K, Kerscher S, Netz DJA, Pierik AJ, Zwicker K, Huynen MA, Lill R, Brandt U, Balk J (2008) Ind1 is requie for effective complex I assembly. EMBO J 27:1736–1746

Calvo SE, Tucker EJ, Compton AG et al (2010) High-throughput, pooled sequencing identifies mutations in NUBPL and FOXRED1 in human complex I deficiency. Nat Genet 42:851–858

Camaschella C, Campanella A, DeFalco L et al (2007) The human counterpart of zebrafish shiraz shows sideroblastic-like microcytic anemia and iron overload. Blood 110:1353–1358

Cameron JM, Levandovskiy V, Mackay N et al (2011a) Complex V TMEM70 deficiency results in mitochondrial nucleoid disorganization. Mitochondrion 11:191–199

Cameron JM, Janer A, Levandovskiy V et al (2011b) Mutations in iron-sulfur cluster scaffold genes NFU1 and BOLA3 cause a fatal deficiency of multiple respiratory chain and 2-oxoacid dehydrogenase enzymes. Am J Hum Genet 89:486–495

Campuzano V, Montermini L, Moltò MD et al (1996) Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science 271:1423–1427

Capaldi RA (1982) Arrangement of proteins in the mitochondrial inner membrane. Biochim Biophys Acta 694:291–306

Carrol J, Fearnley IM, Skehel JM et al (2006) Bovine complex I is a complex of 45 different subunits. J Biol Chem 281:32724–32727

Chance B, Williams GR (1955) A method for the localization of sites for oxidative phosphorylation. Nature 176:250–254

Cizkova A, Stranecky V, Mayr JA et al (2008) TMEM70 mutations cause isolated ATP synthase deficiency and neonatal mitochondrial encephalocardiomyopathy. Nat Genet 40:1288–1290

Coenen MJ, Smeitink JA, Smeets R, Trijbels FJ, van den Heuvel LP (2005) Mutation detection in four candidate genes (OXA1L, MRS2L, YME1L and MIPEP) for combined deficiencies in the oxidative phosphorylation system. J Inherit Metab Dis 28:1091–1097

Collinson IR, Skehel JM, Fearnley IM, Runswick MJ, Walker JE (1996) The F1F0-ATPase complex from bovine heart mitochondria: the molar ratio of the subunits in the stalk region linking the F1 and F0 domains. Biochemistry 35:12640–12646

Cree LM, Samuels DC, Chinnery PF (2009) The inheritance of pathogenic mitochondrial DNA mutations. Biochim Biophys Acta 1792:1097–1102

Cruciat CM, Hell K, Fölsch H, Neupert W, Stuart RA (1999) Bcs1p, an AAA-family member, is a chaperone for the assembly of the cytochrome bc(1) complex. EMBO J 18:5226–5233

Darley-Usmar VM, Georgevich G, Capaldi RA (1984) Reaction of thionitrobenzoate-modified yeast cytochrome c with monomeric and dimeric forms of beef heart cytochrome c oxidase. FEBS Lett 166:131–135

De Lonlay P, Valnot I, Barrientos A 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

De Meirleir L, Seneca S, Lissens W et al (2004) Respiratory chain complex V deficiency due to a mutation in the assembly gene ATP12. J Med Genet 41:120–124

Dell'agnello C, Leo S, Agostino A, Szabadkai G et al (2007) Increased longevity and refractoriness to Ca(2+)-dependent neurodegeneration in Surf1 knockout mice. Hum Mol Genet 16:431–444

Devenish RJ, Prescott M, Rodgers AJ (2008) The structure and function of mitochondrial F1F0-ATP synthases. Int Rev Cell Mol Biol 267:1–58

Diaz F, Fukui H, Garcia S, Moraes CT (2006) Cytochrome *c* oxidase is required for the assembly/stability of respiratory complex I in mouse fibroblasts. Mol Cell Biol 26:4872–4881

Dibrov E, Fu S, Lemire BD (1998) The *Saccharomyces cerevisiae* TCM62 gene encodes a chaperone necessary for the assembly of the mitochondrial succinate dehydrogenase (complex II). J Biol Chem 273:32042

DiMauro S, Davidzon G (2005) Mitochondrial DNA and disease. Ann Med 37:222–232
Distelmaier F, Koopman WJ, van den Heuvel LP et al (2009) Mitochondrial complex I deficiency: from organelle dysfunction to clinical disease. Brain 132:833–842
Dunning CJ, McKenzie M, Sugiana C et al (2007) Human CIA30 is involved in the early assembly of mitochondrial complex I and mutations in its gene cause disease. EMBO J 1126:3227–3237
Efremov RG, Baradaran R, Sazanov LA (2010) The architecture of respiratory complex I. Nature 465:441–445
Ernster L, Dallner G (1995) Biochemical, physiological and medical aspects of ubiquinone function. Biochim Biophys Acta 1271:195–204
Fassone E, Duncan AJ, Taanman JW 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
Feichtinger RG, Zimmermann F, Mayr JA et al (2010) Low aerobic mitochondrial energy metabolism in poorly- or undifferentiated neuroblastoma. BMC Cancer 10:149
Fernandez-Vizarra E, Bugiani M, Goffrini P et al (2007) Impaired complex III assembly associated with BCS1L gene mutations in isolated mitochondrial encephalopathy. Hum Mol Genet 16:1241–1252
Fernandez-Vizarra E, Tiranti V, 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
Fontanesi F, Soto IC, Horn D, Barrientos A (2006) Assembly of mitochondrial cytochrome c-oxidase, a complicated and highly regulated cellular process. Am J Physiol Cell Physiol 291:C1129–C1147
Fowler LR, Richardson SH (1963) Studies on the electron transfer system. On the mechanism of reconstitution of the mitochondrial electron transfer system. J Biol Chem 238:456–463
Gerards M, Sluiter W, van den Bosch BJ et al (2010) Defective complex I assembly due to C20orf7 mutations as a new cause of Leigh syndrome. J Med Genet 47:507–512
Gerards M, van den Bosch BJ, Danhauser K et al (2011) Riboflavin-responsive oxidative phosphorylation complex I deficiency caused by defective ACAD9: new function for an old gene. Brain 134:210–219
Ghezzi D, Zeviani M (2011) Mitochondrial disorders: nuclear gene mutations. In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.
Ghezzi D, Goffrini P, Uziel G et al (2009) SDHAF1, encoding a LYR complex-II specific assembly factor, is mutated in SDH-defective infantile leukoencephalopathy. Nat Genet 41:654–656
Ghezzi D, Arzuffi P, Zordan M et al (2011) Mutations in TTC19 cause mitochondrial complex III deficiency and neurological impairment in humans and flies. Nat Genet 43:259–263
Gilkerson RW, Selker JM, Capaldi RA (2003) The cristal membrane of mitochondria is the principal site of oxidative phosphorylation. FEBS Lett 546:355–358
González-Cabo P, Vázquez-Manrique RP, García-Gimeno MA, Sanz P, Palau F (2005) Frataxin interacts functionally with mitochondrial electron transport chain proteins. Hum Mol Genet 14:2091–2098
Grossman LI, Lomax MI (1997) Nuclear genes for cytochrome c oxidase. Biochim Biophys Acta 1352:174–192
Haack TB, Danhauser K, Haberberger B et al (2010) Exome sequencing identifies ACAD9 mutations as a cause of complex I deficiency. Nat Genet 42:1131–1134
Hackenbrock CR, Chazotte B, Gupte SS (1986) The random collision model and a critical assessment of diffusion and collision in mitochondrial electron transport. J Bioenerg Biomembr 18:331–368
Hägerhäll C (1997) Succinate: quinone oxidoreductases. Variations on a conserved theme. Biochim Biophys Acta 1320:107–141
Hägerhäll C, Hederstedt L (1997) A structural model for the membrane-integral domain of succinate: quinone oxidoreductases. FEBS Lett 389:25

Hao HX, Khalimonchuk O, Schraders M et al (2009) SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. Science 28:1139–1142

Hederstedt L (2002) Succinate:quinone oxidoreductase in the bacteria *Paracoccus denitrificans* and *Bacillus subtilis*. Biochim Biophys Acta 1553:74–83

Hinson JT, Fantin VR, Schonberger J et al (2007) Missense mutations in the BCS1L gene as a cause of the Bjornstad syndrome. N Engl J Med 356:809–819

Honzík T, Tesarová M, Mayr JA et al (2010) Mitochondrial encephalocardio-myopathy with early neonatal onset due to TMEM70 mutation. J Arch Dis Child 95:296–301

Houstek J, Klement P, Floryk D et al (1999) A novel deficiency of mitochondrial ATPase of nuclear origin. Hum Mol Genet 8:1967–1974

Houstek J, Pickova A, Vojtiskova A, Mracek T, Pecina P, Jesina P (2006) Mitochondrial diseases and genetic defects of ATP synthase. Biochim Biophys Acta 1757:1400–1405

Houstek J, Kmoch S, Zeman J (2009) TMEM70 protein – a novel ancillary factor of mammalian ATP synthase. Biochim Biophys Acta 1787:529–532

Huigsloot M, Nijtmans LG, Szklarczyk R et al (2011) A mutation in C2orf64 causes impaired cytochrome c oxidase assembly and mitochondrial cardiomyopathy. Am J Hum Genet 88:488–493

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

Huynen MA, Spronk CA, Gabaldón T, Snel B (2005) Combining data from genomes, Y2H and 3D structure indicates that BolA is a reductase interacting with a glutaredoxin. FEBS Lett 579:591–596

Iwata S, Lee JW, Okada K et al (1998) Complete structure of the 11-subunit bovine mitochondrial cytochrome bc1 complex. Science 281:64–71

Janssen RJ, Nijtmans LG, van den Heuvel LP, Smeitink JA (2006) Mitochondrial complex I: structure, function and pathology. J Inherit Metab Dis 29:499–515

Jiang X, Wang X (2004) Cytochrome c-mediated apoptosis. Annu Rev Biochem 73:87–106

Jonckheere A, Hogeveen M, Nijtmans L et al (2008) A novel mitochondrial ATP8 (MT-ATP8) gene mutation in a patient with apical hypertrophic cardiomyopathy and neuropathy. J Med Genet 45:129–133

Joost K, Rodenburg R, Piirsoo A et al (2010) A novel mutation in the SCO2 gene in a neonate with early-onset cardioencephalomyopathy. Pediatr Neurol 42:227–230

Kim KD, Chung WH, Kim HJ, Lee KC, Roe JH (2010) Monothiol glutaredoxin Grx5 interacts with Fe-S scaffold proteins Isa1 and Isa2 and supports Fe-S assembly and DNA integrity in mitochondria of fission yeast. Biochem Biophys Res Commun 392:467–472

Ko YH, Delannoy M, Hullihen J, Chiu W, Pedersen PL (2003) Mitochondrial ATP synthasome. Cristae-enriched membranes and a multiwell detergent screening assay yield dispersed single complexes containing the ATP synthase and carriers for Pi and ADP/ATP. J Biol Chem 278:12305–12309

Kollberg G, Tulinius M, Melberg A et al (2009) Clinical manifestation and a new ISCU mutation in iron-sulphur cluster deficiency myopathy. Brain 132:2170–2179

Koopman WJ, Nijtmans LG, Dieteren CE et al (2010) Mammalian mitochondrial complex I: biogenesis, regulation and reactive oxygen species generation. Antioxid Redox Signal 12:1431–1470

Krause F, Scheckhuber CQ, Werner A, Rexroth S, Reifschneider NH, Dencher NA, Osiewacz HD (2004) Supramolecular organization of cytochrome c oxidaseand alternative oxidase-dependent respiratory chains in the filamentous fungus Podospora anserine. J Biol Chem 279:26453–26461

Krause F, Reifschneider NH, Goto S, Dencher NA (2005) Active oligomeric ATP synthases in mammalian mitochondria. Biochem Biophys Res Commun 329:583–590

Kuffner R, Rohr A, Schmiede A, Krull C, Schulte U (1998) Involvement of two novel chaperones in the assembly of mitochondrial NADH: ubiquinone oxidoreductase (complex I). J Mol Biol 283:409–417

Lamantea E, Carrara F, Mariotti C, Morandi L, Tiranti V, Zeviani M (2002) A novel nonsense mutation (Q352X) in the mitochondrial cytochrome b gene associated with a combined deficiency of complexes I and III. Neuromuscul Disord 12:49–52

Lancaster CR, Kroger A, Auer M, Michel H (1999) Structure of fumarate reductase from Wolinella succinogenes at 2.2 A resolution. Nature 402:377–385

Lazarou M, McKenzie M, Ohtake A, Thorburn DR, Ryan MT (2007) Analysis of the assembly profiles for mitochondrial- and nuclear-DNA-encoded subunits into complex I. Mol Cell Biol 27:4228–4237

Lazarou M, Smith SM, Thorburn DR, Ryan MT, McKenzie M (2009) Assembly of nuclear DNA-encoded subunits into mitochondrial complex IV, and their preferential integration into supercomplex forms in patient mitochondria. FEBS J 276:6701–6713

Leary SC, Kaufman BA, Pellecchia G et al (2004) Human SCO1 and SCO2 have independent, cooperative functions in copper delivery to cytochrome c oxidase. Hum Mol Genet 13:1839–1848

Leary SC, Cobine PA, Kaufman BA 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

Lee SJ, Yamashita E, Abe T et al (2001) Intermonomer interactions in dimer of bovine heart cytochrome c oxidase. Acta Crystallogr D: Biol Crystallogr 57:941–947

Lenaz G, Genova ML (2010) Structure and organization of mitochondrial respiratory complexes: a new understanding of an old subject. Antioxid Redox Signal 12:961–1008

Lenaz G, Baracca A, Carelli V, D'Aurelio M, Sgarbi G, Solaini G (2004) Bioenergetics of mitochondrial diseases associated with mtDNA mutations. Biochim Biophys Acta 1658:89–94

Levéen P, Kotarsky H, Mörgelin M, Karikoski R, Elmér E, Fellman V (2011) The GRACILE mutation introduced into Bcs1l causes postnatal complex III deficiency: a viable mouse model for mitochondrial hepatopathy. Hepatology 53:437–447

Li K, Tong WH, Hughes RM, Rouault TA (2006) Roles of the mammalian cytosolic cysteine desulfurase, ISCS, and scaffold protein, ISCU, in iron-sulfur cluster assembly. J Biol Chem 281:12344–12351

Li H, Gakh O, Smith DY, Isaya G (2009) Oligomeric yeast frataxin drives assembly of core machinery for mitochondrial iron-sulfur cluster synthesis. J Biol Chem 284:21971–21980

Lill R, Kispal G (2000) Maturation of cellular Fe-S proteins: an essential function of mitochondria. Trends Biochem Sci 25:352–356

Lill R, Mühlenhoff U (2008) Maturation of iron-sulfur proteins in eukaryotes: mechanisms, connected processes, and diseases. Annu Rev Biochem 77:669–700

Loeffen JL, Smeitink JA, Trijbels JM et al (2000) Isolated complex I deficiency in children: clinical, biochemical and genetic aspects. Hum Mutat 15:123–134

Mancuso C, Scapagini G, Currò D et al (2007) Mitochondrial dysfunction, free radical generation and cellular stress response in neurodegenerative disorders. Front Biosci 12:1107–1123

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

Massa V, Fernandez-Vizarra E, Alshahwan S 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

Mayr JA, Havlícková V, Zimmermann F et al (2010) Mitochondrial ATP synthase deficiency due to a mutation in the ATP5E gene for the F1 epsilon subunit. Hum Mol Genet 19:3430–3439

McFarland R, Turnbull DM (2009) Batteries not included: diagnosis and management of mitochondrial disease. J Intern Med 265:210–228

McKenzie M, Ryan MT (2010) Assembly factors of human mitochondrial complex I and their defects in disease. IUBMB Life 62:497–502

Mick DU, Wagner K, van der Laan M et al (2007) Shy1 couples Cox1 translational regulation to cytochrome c oxidase assembly. EMBO J 26:4347–4358

Mochel F, Knight MA, Tong WH et al (2008) Splice mutation in the iron-sulfur cluster scaffold protein ISCU causes myopathy with exercise intolerance. Am J Hum Genet 82:652–660
Mootha VK, Lepage P, Miller K (2003) Identification of a gene causing human cytochrome c oxidase deficiency by integrative genomics. Proc Natl Acad Sci USA 100:605–610
Mulkidjanian AY, Makarova KS, Galperin MY, Koonin EV (2007) Inventing the dynamo machine: the evolution of the F-type and V-type ATPases. Nat Rev Microbiol 5:892–899
Munnich A, Rustin P (2001) Clinical spectrum and diagnosis of mitochondrial disorders. Am J Med Genet 106:4–17
Nakamura K, Yamaki M, Sarada M et al (1996) Two hydrophobic subunits are essential for the heme b ligation and functional assembly of complex II (succinate-ubiquinone oxidoreductase) from *Escherichia coli*. J Biol Chem 271:521–527
Nargang FE, Preuss M, Neupert W, Herrmann JM (2002) The Oxa1 protein forms a homooligomeric complex and is an essential part of the mitochondrial export translocase in *Neurospora crassa*. J Biol Chem 277:12846–12853
Nijtmans LG, Klement P, Houstek J, van den Bogert C (1995) Assembly of mitochondrial ATP synthase in cultured human cells: implications for mitochondrial diseases. Biochim Biophys Acta 1272:190–198
Nijtmans LG, Taanman JW, Muijsers AO, Speijer D, Van den Bogert C (1998) Assembly of cytochrome-c oxidase in cultured human cells. Eur J Biochem 254:389–394
Nobrega FG, Nobrega MP, Tzagoloff A (1992) BCS1, a novel gene required for the expression of functional Rieske iron–sulfur protein in *Saccharomyces cerevisiae*. EMBO J 11:3821–3829
Nouws J, Nijtmans L, Houten SM et al (2010) Acyl-CoA dehydrogenase 9 is required for the biogenesis of oxidative phosphorylation complex I. Cell Metab 12:283–294
Ogilvie I, Kennaway NG, Shoubridge EA (2005) A molecular chaperone for mitochondrial complex I assembly is mutated in a progressive encephalopathy. J Clin Invest 115:2784–2792
Pagliarini DJ, Calvo SE, Chang B et al (2008) A mitochondrial protein compendium elucidates complex I disease biology. Cell 134:112–123
Papadopoulou LC, Sue CM, Davidson MM et al (1999) Fatal infantile cardioencephalomyopathy with COX deficiency and mutations in SCO2, a COX assembly gene. Nat Genet 23:333–337
Paumard P, Vaillier J, Coulary B et al (2002) The ATP synthase is involved in generating mitochondrial cristae morphology. EMBO J 21:221–230
Pecina P, Houstkova H, Hansıkova H, Zeman J, Houstek J (2004) Genetic defects of cytochrome c oxidase assembly. Physiol Res 53:S213–S223
Piekutowska-Abramczuk D, Magner M, Popowska E et al (2009) SURF1 missense mutations promote a mild Leigh phenotype. Clin Genet 76:195–204
Pondarré C, Antiochos BB, Campagna DR et al (2006) The mitochondrial ATP-binding cassette transporter Abcb7 is essential in mice and participates in cytosolic iron-sulfur cluster biogenesis. Hum Mol Genet 15:953–964
Pronicki M, Kowalski P, Piekutowska-Abramczuk D et al (2010) A homozygous mutation in the SCO2 gene causes a spinal muscular atrophy like presentation with stridor and respiratory insufficiency. Eur J Paediatr Neurol 14:253–260
Rabl R, Soubannier V, Scholz R et al (2009) Formation of cristae and crista junctions in mitochondria depends on antagonism between Fcj1 and Su e/g. J Cell Biol 185:1047–1063
Rak M, Gokova S, Tzagoloff A (2011) Modular assembly of yeast mitochondrial ATP synthase. EMBO J 30:920–930
Remacle C, Barbieri MR, Cardol P, Hamel PP (2008) Eukaryotic complex I: functional diversity and experimental systems to unravel the assembly process. Mol Genet Genomics 280: 93–110
Robinson KM, Lemire BD (1996) Covalent attachment of FAD to the yeast succinate dehydrogenase flavoprotein requires import into mitochondria, presequence removal, and folding. J Biol Chem 271:4055–4060
Rouault TA, Tong WH (2008) Iron-sulfur cluster biogenesis and human disease. Trends Genet 24:398–407

Saada A, Edvardson S, Rapoport M et al (2008) C6ORF66 is an assembly factor of mitochondrial complex I. Am J Hum Genet 82:32–38

Saada A, Vogel RO, Hoefs SJ 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

Saada A, Edvardson S, Shaag A et al (2012) Combined OXPHOS complex I and IV defect, due to mutated complex I assembly factor C20ORF7. J Inherit Metab Dis 35(1):125–131

Sasarman F, Brunel-Guitton C, Antonicka H et al (2010) LRPPRC and SLIRP interact in a ribonucleoprotein complex that regulates posttranscriptional gene expression in mitochondria. Mol Biol Cell 21:1315–1323

Schaefer AM, Taylor RW, Turnbull DM, Chinnery PF (2004) The epidemiology of mitochondrial disorders: past, present and future. Biochim Biophys Acta 1659:115–120

Schäfer E, Dencher NA, Vonck J, Parcej DN (2007) Three-dimensional structure of the respiratory chain supercomplex I1III2IV1 from bovine heart mitochondria. Biochemistry 46:12579–12585

Schägger H (2002) Respiratory chain supercomplexes of mitochondria and bacteria. Biochim Biophys Acta 1555:154–159

Schagger H, Pfeiffer K (2000) Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. EMBO J 19:1777–1783

Schagger H, von Jagow G (1991) Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form. Anal Biochem 199:223–231

Schatz G (1968) Impaired binding of mitochondrial adenosine triphosphatase in the cytoplasmic "petite" mutant of *Saccharomyces cerevisiae*. J Biol Chem 243:2192–2199

Scheffler IE, Yadava N, Potluri P (2004) Molecular genetics of complex I-deficient Chinese hamster cell lines. Biochim Biophys Acta 1659:160–171

Schmitz-Linneweber C, Small I (2008) Pentatricopeptide repeat proteins: a socket set for organelle gene expression. Trends Plant Sci 13:663–670

Schneider D, Pohl T, Walter J et al (2008) Assembly of the *Escherichia coli* NADH:ubiquinone oxidoreductase (complex I). Biochim Biophys Acta 1777:735–739

Schon EA, Santra S, Pallotti F, Girvin ME (2001) Pathogenesis of primary defects in mitochondrial ATP synthesis. Semin Cell Dev Biol 12:441–448

Seeger J, Schrank B, Pyle A et al (2010) Clinical and neuropathological findings in patients with TACO1 mutations. Neuromuscul Disord 20:720–724

Sheftel AD, Stehling O, Pierik AJ et al (2009) Human ind1, an iron-sulfur cluster assembly factor for respiratory complex I. Mol Cell Biol 29:6059–6073

Sperl W, Jesina P, Zeman J et al (2006) Deficiency of mitochondrial ATP synthase of nuclear genetic origin. Neuromuscul Disord 16:821–829

Spiegel R, Khayat M, Shalev SA et al (2011) TMEM70 mutations are a common cause of nuclear encoded ATP synthase assembly defect: further delineation of a new syndrome. J Med Genet 48:177–182

Stiburek L, Vesela K, Hansikova H et al (2005) Tissue-specific cytochrome c oxidase assembly defects due to mutations in SCO2 and SURF1. Biochem J 392:625–632

Stiburek L, Hansikova H, Tesarova M, Cerna L, Zeman J (2006) Biogenesis of eukaryotic cytochrome c oxidase. Physiol Res 55:S27–S41

Stiburek L, Fornuskova D, Wenchich L, Pejznochova M, Hansikova H, Zeman J (2007) Knockdown of human Oxa1l impairs the biogenesis of F1Fo-ATP synthase and NADH:ubiquinone oxidoreductase. J Mol Biol 374:506–516

Stiburek L, Vesela K, Hansikova H, Hulkova H, Zeman J (2009) Loss of function of Sco1 and its interaction with cytochrome c oxidase. Am J Physiol Cell Physiol 296:C1218–C1226

Sugiana C, Pagliarini DJ, McKenzie M et al (2008) Mutation of C20orf7 disrupts complex I assembly and causes lethal neonatal mitochondrial disease. Am J Hum Genet 83:468–478

Sun F, Huo X, Zhai Y et al (2005) Crystal structure of mitochondrial respiratory membrane protein complex II. Cell 121:1043–1057

Tatuch Y, Robinson BH (1993) The mitochondrial DNA mutation at 8993 associated with NARP slows the rate of ATP synthesis in isolated lymphoblast mitochondria. Biochem Biophys Res Commun 192:124–128

Thorburn DR, Sugiana C, Salemi R et al (2004) Biochemical and molecular diagnosis of mitochondrial respiratory chain disorders. Biochim Biophys Acta 1659:121–128
Tiranti V, Hoertnagel K, Carrozzo R et al (1998) Mutations of SURF-1 in Leigh disease associated with cytochrome c oxidase deficiency. Am J Hum Genet 63:1609–1621
Tiranti V, Galimberti C, Nijtmans L, Bovolenta S, Perini MP, Zeviani M (1999) Characterization of SURF-1 expression and Surf-1p function in normal and disease conditions. Hum Mol Genet 8:2533–2540
Tong WH, Rouault TA (2006) Functions of mitochondrial ISCU and cytosolic ISCU in mammalian iron-sulfur cluster biogenesis and iron homeostasis. Cell Metab 3:199–210
Tsukihara T, Aoyama H, Yamashita E et al (1996) The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 A. Science 272:1136–1144
Tzagoloff A (1969) Assembly of the mitochondrial membrane system. II. Synthesis of the mitochondrial adenosine triphosphatase, F1. J Biol Chem 244:5027–5033
Tzagoloff A, Jang J, Glerum M, Wu M (1996) FLX1 codes for a carrier protein involved in maintaining a proper balance of flavin nucleotides in yeast mitochondria. J Biol Chem 271:7392–7397
Ugalde C, Janssen RJ, van den Heuvel LP, Smeitink JA, Nijtmans LG (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
Valnot I, Osmond S, Gigarel N et al (2000) Mutations of the SCO1 gene in mitochondrial cytochrome c oxidase deficiency with neonatal-onset hepatic failure and encephalopathy. Am J Hum Genet 67:1104–1109
van Nederveen FH, Gaal J, Favier J et al (2009) An immunohistochemical procedure to detect patients with paraganglioma and phaeochromocytoma with germline SDHB, SDHC, or SDHD gene mutations: a retrospective and prospective analysis. Lancet Oncol 10:764–771
Visapää I, Fellman V, Vesa J et al (2002) GRACILE syndrome, a lethal metabolic disorder with iron overload, is caused by a point mutation in BCS1L. Am J Hum Genet 71:863–876
Vogel RO, Smeitink JA, Nijtmans LG (2007) Human mitochondrial complex I assembly: a dynamic and versatile process. Biochim Biophys Acta 1767:1215–1227
Wagner K, Perschil I, Fichter CD, van der Laan M (2010) Stepwise assembly of dimeric F(1)F(o)-ATP synthase in mitochondria involves the small F(o)-subunits k and i. Mol Biol Cell 21:1494–1504
Wang ZG, Sheluho D, Gatti DL, Ackerman SH (2000) The alpha-subunit of the mitochondrial F(1) ATPase interacts directly with the assembly factor Atp12p. EMBO J 19:1486–1493
Wang ZG, White PS, Ackerman SH (2001) Atp11p and Atp12p are assembly factors for the F(1)-ATPase in human mitochondria. J Biol Chem 276:30773–30778
Weraarpachai W, Antonicka H, Sasarman F 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
West AP, Brodsky IE, Rahner C et al (2011) TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. Nature 472:476–480
Williams SL, Valnot I, Rustin P, Taanman JW (2004) Cytochrome c oxidase subassemblies in fibroblast cultures from patients carrying mutations in COX10, SCO1, or SURF1. J Biol Chem 279:7462–7469
Wittig I, Schägger H (2009) Supramolecular organization of ATP synthase and respiratory chain in mitochondrial membranes. Biochim Biophys Acta 1787:672–680
Wittig I, Carrozzo R, Santorelli FM, Schägger H (2006) Supercomplexes and subcomplexes of mitochondrial oxidative phosphorylation. Biochim Biophys Acta 1757:1066–1072
Xu F, Ackerley C, Maj MC et al (2008) Disruption of a mitochondrial RNA-binding protein gene results in decreased cytochrome b expression and a marked reduction in ubiquinol-cytochrome *c* reductase activity in mouse heart mitochondria. Biochem J 416:15–26
Yankovskaya V, Horsefield R, Törnroth S et al (2003) Architecture of succinate dehydrogenase and reactive oxygen species generation. Science 299:700–704
Yoshikawa S, Shinzawa-Itoh K, Tsukihara T (1998) Crystal structure of bovine heart cytochrome c oxidase at 2.8 A resolution. J Bioenerg Biomembr 30:7–14

Zara V, Conte L, Trumpower BL (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

Zara V, Conte L, Trumpower BL (2009) Biogenesis of the yeast cytochrome bc(1) complex. Biochim Biophys Acta 1793:89–96

Zick M, Rabl R, Reichert AS (2009) Cristae formation-linking ultrastructure and function of mitochondria. Biochim Biophys Acta 1793:5–19