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REVIEW

Eukaryotic complex I: functional diversity and experimental systems to unravel the assembly process

Claire Remacle · M. Rosario Barbieri · Pierre Cardol · Patrice P. Hamel

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Abstract With more than 40 subunits, one FMN co-factor and eight FeS clusters, complex I or NADH:ubiquinone oxidoreductase is the largest multimeric respiratory enzyme in the mitochondria. In this review, we focus on the diversity of eukaryotic complex I. We describe the additional activities that have been reported to be associated with mitochondrial complex I and discuss their physiological significance. The recent identification of complex I-like enzymes in the hydrogenosome, a mitochondria-derived organelle is also discussed here. Complex I assembly in the mitochondrial inner membrane is an intricate process that requires the cooperation of the nuclear and mitochondrial genomes. The most prevalent forms of mitochondrial dysfunction in humans are deficiencies in complex I and remarkably, the molecular basis for 60% of complex I-linked defects is currently unknown. This suggests that mutations in yet-to-be-discovered assembly genes should exist. We review the different experimental systems for the study of complex I assembly. To our knowledge, in none of them, large screenings of complex I mutants have been performed. We propose that the unicellular green alga *Chlamydomonas reinhardtii* is a promising system for such a study. Complex I mutants can be easily scored on a phenotypical basis and a large number of transformants generated by insertional mutagenesis can be screened, which opens the possibility to find new genes involved in the assembly of the enzyme. Moreover, mitochondrial transformation, a recent technological advance, is now available, allowing the manipulation of all five complex I mitochondrial genes in this organism.

Keywords Mitochondria · Human disease · Complex I · Assembly factors · Mitochondrial transformation · Model systems · Chlamydomonas

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C. Remacle (✉) · P. Cardol
Genetics of Microorganisms Laboratory,
Department of Life Sciences,
University of Liège, 4000 Liège, Belgium
e-mail: c.remacle@ulg.ac.be

M. R. Barbieri · P. P. Hamel (✉)
Department of Plant Cellular and Molecular Biology
and Department of Molecular and Cellular Biochemistry,
The Ohio State University, 500 Aronoff Laboratory,
318 W. 12th Ave., Columbus, OH 43210, USA
e-mail: hamel.16@osu.edu

Introduction

With over 40 subunits and a molecular mass of about 900–1,000 kDa, NADH:ubiquinone oxidoreductase (complex I; EC 1.6.5.3) is the multimeric respiratory enzyme that transfers electrons from NADH to ubiquinone concomitantly with the translocation of protons across the mitochondrial inner membrane. The redox reaction can be summarized as follows: $NADH + Q + 5H^+_{matrix} \rightarrow NAD^+ + QH_2 + 4H^+_{intermembrane\ space}$. Therefore, complex I together with complexes III and IV contributes to generate a proton gradient used to synthesize ATP in the mitochondrial matrix by complex V, the ATP synthase (see Fig. 1 for a schematic representation of the respiratory chain in plant mitochondria).

Bacterial complex I

A simpler form of the proton-pumping NADH:ubiquinone oxidoreductase exists in several bacteria. Bacterial

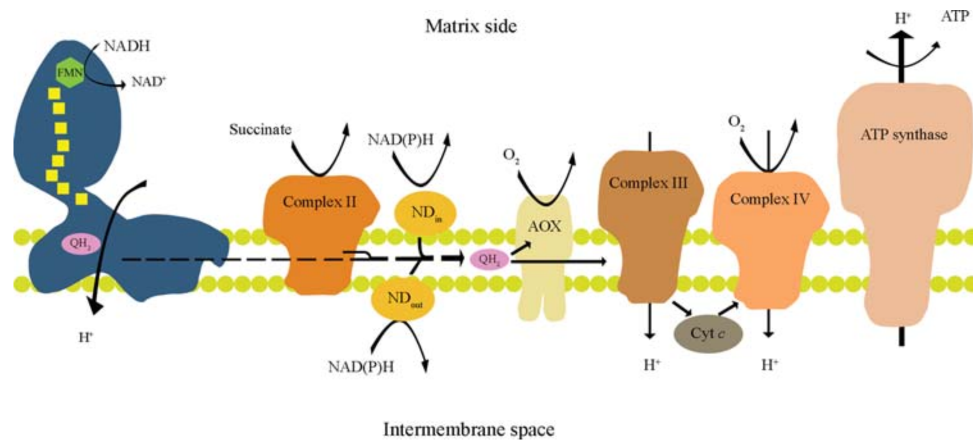

Fig. 1 Schematic organization of the electron transfer chain in the plant mitochondrial inner membrane. The NADH:ubiquinone oxido-reductase complex (complex I) is highlighted on the *left*. The *yellow squares* represent the eight FeS clusters. Complex I, complex II (succinate: ubiquinone oxido-reductase) and type II dehydrogenases ND_{in} and ND_{ex} contribute to the reduction of ubiquinone (QH_2) pool. Oxygen reduction by the QH_2 pool can occur through: (a) alternative oxidase (*AOX*) or (b) sequentially by complex III (ubiquinol:cyto-chrome *c* oxidoreductase), cytochrome *c* and complex IV (cytochrome *c* oxidase). Of all these membrane complexes, only complexes I, III and IV participate in the generation of the proton gradient across the membrane. This proton gradient is used for the synthesis of ATP by the ATP synthase (complex V). Note that type II dehydrogenases and AOX activities do not occur in mammalian mitochondria

complex I exhibits a molecular mass of about 550 kDa and is composed of a basic core of 14 structural subunits, whose homologues are also part of mitochondrial complex I in all eukaryotes known so far (Table 1). In bacteria, the 14 genes are organized in a single operon -*nuo* or *nqo,*[1] depending on the species (Yagi and Matsuno-Yagi 2003) and encode the hydrophilic and hydrophobic subunits of the enzyme (Friedrich 1998). Recently, an additional subunit (Nqo15) was shown to co-purify with the enzyme core in the bacteria *Thermus thermophilus*. This subunit is not encoded in the operon and seems to be only present in the closest relatives of *T. thermophilus* (Hinchliffe et al. 2006). In the bacterial operon, seven genes encode peripheral hydrophilic proteins (NuoB-G and NuoI, see Table 1), including all subunits harboring binding motifs for NADH, FMN and eight to nine iron–sulfur clusters.[2] In addition, two of these subunits (NuoB and NuoD) are also involved in quinone binding (Schuler et al. 1999; Prieur et al. 2001; Schuler and Casida 2001; Tocilescu et al. 2007). The other seven genes code for hydrophobic membrane proteins (NuoA, NuoH and NuoJ-N) (Table 1). These subunits do not carry typical cofactor-binding motifs and consequently no redox groups have been detected. Three of these subunits (NuoH, L, M) are believed to be involved in quinone binding, whereas NuoA, J, K, L, M and N are postulated to carry the proton translocation activity (Friedrich and Bottcher 2004; Kao et al. 2004, 2005). Low-resolution microscopic studies showed that bacterial complex I subunits are organized in a L-shaped structure, with a hydrophilic arm protruding into the cytosol and a hydrophobic domain located at the cytoplasmic membrane (Guenebaut et al. 1998; Peng et al. 2003). The crystal structure of the hydrophilic domain of complex I from *T. thermophilus,* accounting for more than half of the molecular mass of the entire complex, has been solved recently, revealing that this subcomplex contains all the prosthetic centers (one FMN molecule and nine iron–sulfur clusters) (Hinchliffe and Sazanov 2005; Sazanov and Hinchliffe 2006).

Eukaryotic complex I

Over the course of evolution a number of non-core subunits have been added to the complex I core enzyme in eukaryotes, increasing its molecular mass to 900–1,000 kDa. All eukaryotic complex I have a dual genetic origin: seven to nine subunits[3] (referred to as ND subunits) are usually encoded in the mitochondrial genome, while the remaining subunits are nuclear gene products. The use of several fractionation methods combined with mass spectrometry analysis has enabled determination of the subunit composition of mitochondrial complex I from

[1] *nqo* for NADH:quinone oxido-reductase and *nuo* for NADH:ubiquinone oxido-reductase.

[2] Complex I typically contains eight FeS clusters (referred to as N1a, N3, N1b, N4, N5, N6a, N6b, N2). An additional FeS cluster (N7) was found in four bacterial species including *Escherichia coli* and *T. thermophilus* but is not evolutionary conserved (Ohnishi 1998; Sazanov 2007).

[3] Seven ND subunits are encoded in the mammalian mitochondrial genome and nine ND subunits are encoded in the mitochondria of vascular plants. The mitochondrial genomes of the green alga *Chlamydomonas reinhardtii* and the protist *Reclinomonas americana* encode for 5 and 12 ND subunits, respectively.

Table 1 Nomenclature of the 14 complex I core subunits

Bacteria	Mammals	Vascular plants	*C. reinhardtii*
NuoA	Nd3[b]	ND3[b]	Nuo3
NuoB	PSST	PSST	Nuo10
NuoC[a]	30 kDa	ND9[b]	Nuo9
NuoD[a]	49 kDa	ND7[b]	Nuo7
NuoE	24 kDa	28.3 kDa	Nuo5
NuoF	51 kDa	53.5 kDa	Nuo6
NuoG	75 kDa	81.5 kDa	NuoS1
NuoH	Nd1[b]	ND1[b]	ND1[b]
NuoI	TYKY	TYKY	Nuo8
NuoJ	Nd6[b]	ND6[b]	ND6[b]
NuoK	Nd4L[b]	ND4L[b]	Nuo4L
NuoL	Nd5[b]	ND5[b]	ND5[b]
NuoM	Nd4[b]	ND4[b]	ND4[b]
NuoN	Nd2[b]	ND2[b]	ND2[b]

For vascular plants, the nomenclature is the one used for *Arabidopsis thaliana* (Heazlewood et al. 2003). In the case of mammals, the nomenclature for bovine complex I was chosen (Hirst et al. 2003; Fearnley et al. 2007)

[a] NuoC and NuoD are fused in a single subunit in *E. coli*

[b] Mitochondrially encoded

different eukaryotes. Complex I comprises 45 subunits in bovine heart (Hirst et al. 2003; Fearnley et al. 2007), at least 42 subunits in the vascular plants *Arabidopsis thaliana* and *Oryza sativa* (Heazlewood et al. 2003; Meyer et al. 2008), and at least 39 and 37 subunits in the fungi *Neurospora crassa* (Videira and Duarte 2002) and *Yarrowia lipolytica*, respectively (Abdrakhmanova et al. 2004). In the green alga *Chlamydomonas reinhardtii*, complex I has an apparent molecular mass of 950–1,000 kDa and is comprised of 42 subunits ranging in size from 7 to 77 kDa (van Lis et al. 2003; Cardol et al. 2004). All mitochondrial complex I share 33 subunits in common, including the 14 core subunits (Cardol et al. 2005). The remaining subunits are either specific to one, two or several lineages (Videira and Duarte 2002; Cardol et al. 2005).

The role of the non-core subunits is not fully understood. It is generally assumed that they are required for assembly of the complex and/or regulation of its activity (Vogel et al. 2004). A novel role for non-core subunits as regulatory molecules outside the mitochondrial compartment is now emerging. One example is GRIM-19 (Gene associated with Retinoic-IFN-induced Mortality 19), a nuclear located protein that was originally identified as a critical regulatory protein for interferon-beta and for retinoic acid induced cell death (Angell et al. 2000). GRIM-19 binds specifically to STAT3 (signal transducer and activator 3), a transcriptional factor and inhibits its transcriptional activity (Zhang et al. 2003). Because STAT3 promotes the transcription of genes involved in cell proliferation or encoding anti-apoptosis factors, GRIM-19 mediated inhibition of STAT3 activity is viewed as a way to activate the cell death program. Unexpectedly, GRIM-19 also localizes to the mitochondria and was shown to be a *bona fide* subunit of human complex I (Fearnley et al. 2001; Murray et al. 2003; Zhang et al. 2003; Lu and Cao 2008). GRIM-19 knock-out leads to complex I assembly disruption and embryonic lethality in mice. Functional dissection of GRIM-19 revealed that the subunit is required for the assembly of complex I but is also involved in the control of the electron transfer activity of the enzyme (Lu and Cao 2008). This dual function of GRIM-19 in the assembly process and regulation of the enzymatic activity was mapped to distinct domains in the subunit. An additional function of GRIM-19 in the control of the mitochondrial transmembrane potential ($\Delta\Psi m$) was also uncovered (Lu and Cao 2008). This role is unique to GRIM-19 as other subunits such as NDUFA9 (MWFE) and NDUFS3 (30 kDa) did not appear to regulate the $\Delta\Psi m$. This finding is relevant in the context of the apoptotic response because variations in $\Delta\Psi m$ have been associated with the cell death program. The molecular mechanism of GRIM-19 mediated control of $\Delta\Psi m$ is currently unknown.

Mutations or loss of expression in *GRIM-19* were found in certain types of cancer, suggesting a role for this protein in tumorigenesis (Maximo et al. 2008). Because GRIM-19 controls cell growth via its interaction with STAT3, it is conceivable that tumorigenesis occurs via loss of GRIM-19/STAT3 interaction.

The dual function of GRIM-19 as a cell death regulatory molecule and a complex I subunit suggests that complex I might be a key component in the control of programmed cell death by the mitochondria. This control might be exerted via a partition of GRIM-19 between the nucleus and the mitochondrial compartment.

Recently, RNA binding activity was evidenced for human GRIM-19 (Reeves et al. 2007). The target RNA is a non-coding transcript produced by the human cytomegalovirus upon infection of human cells. This interaction was interpreted as a strategy for the virus to suppress the cell death response by preventing the interaction of GRIM-19 with its STAT3 target and hence blocking GRIM-19 dependent apoptosis (Reeves et al. 2007). Because apoptosis is a normal cellular response in case of viral infection, suppression of the apoptotic response via a GRIM-19/viral RNA interaction is a means to ensure efficient proliferation of the virus in the host cell. That GRIM-19 is a target for successful viral infection is further suggested by the finding that viral proteins also interact with GRIM-19 and thereby prevent its signaling in the apoptotic response (Seo et al. 2002). A GRIM-19 homolog has been found in complex I of vascular plants (Heazlewood et al. 2003), fungi (Abdrakhmanova et al. 2004) and green alga (Cardol et al.

2004), suggesting a role for this component in complex I assembly or activity. It is not known if the regulatory function of GRIM-19 outside the mitochondria is also conserved in these organisms.

Eukaryotic complex I topology

Microscopic studies revealed that, similar to bacteria, eukaryotic complex I also displays a characteristic L-shaped structure (Hofhaus et al. 1991; Guenebaut et al. 1997; Grigorieff 1998; Djafarzadeh et al. 2000), with a hydrophilic arm exposed to the matrix and a hydrophobic arm embedded in the mitochondrial inner membrane. Substantial information about the complex I subunit topology is now also available. Using a chaotropic agent, Galante and Hatefi were able to fractionate the bovine complex I into three parts: the FMN containing part (FP), the iron–sulfur cluster containing part (IP) and a membrane hydrophobic part (HP) (Galante and Hatefi 1978) (Fig. 2). Additional fractionation analyses of the bovine complex by Walker's group (Finel et al. 1992), using the detergent *N,N*-dimethyldodecylamine *N*-oxide (LDAO), resulted in two fragments, Iα (containing FP and IP) and Iβ (equivalent to HP). In a separate study, the bovine complex could be fractionated into three main subcomplexes: the Iλ hydrophilic part, the Iβ hydrophobic part and the Iγ connecting module (Sazanov et al. 2000; Sazanov and Walker 2000) (Fig. 2). More importantly, the subunit composition of these fractions was established by mass spectrometric analyses (Carroll et al. 2002, 2003; Hirst et al. 2003).

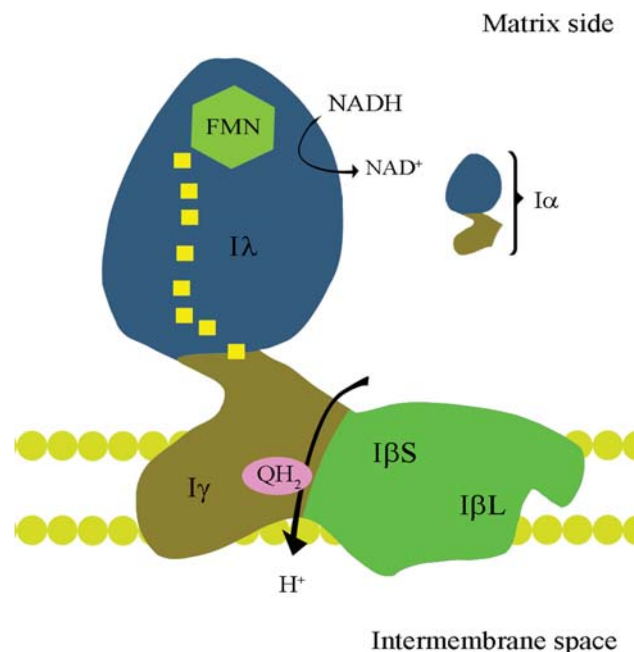

Fig. 2 Modular organization of complex I. The peripheral arm protruding into the matrix side of the mitochondrial inner membrane is formed by subcomplex Iλ, which contains the FeS clusters (*yellow squares*) and a flavoprotein fraction. The membrane arm contains two membrane modules: Iγ and Iβ. A larger subcomplex Iα, composed of both Iλ and Iγ subcomplexes, has been described from mutant and fractionation analyses of complex I (see text for details)

Some subunits of complex I have been found to be phosphorylated. The cAMP-dependent phosphorylation of the 18 and 42 kDa subunits was shown in complex I isolated from bovine heart mitochondria (Sardanelli et al. 1995). The site of phosphorylation of the 42-kDa subunit (NDUFA10) was further identified by mass spectrometry (Schulenberg et al. 2003; Schilling et al. 2005; Palmisano et al. 2007), although the phosphorylation of this protein has not been confirmed by others (Chen et al. 2004). The 18-kDa subunit was proposed to be AQDQ (NDUFS4) by protein sequencing and phosphorylation of this protein was found in complex I from mammalian cell cultures (Papa et al. 1996, 2002; Scacco et al. 2000; Papa 2002). This finding is now disputed since Chen et al. reported that the 18-kDa subunit was not AQDQ but ESSS (NDUFB11) (Chen et al. 2004). In addition to ESSS, phosphorylation of MWFE (NDUFA1), B14.5a (NDUFA7), B14.5b (NDUFC2) and B16.6 (GRIM-19/NDUFA13) has also been reported (Chen et al. 2004; Palmisano et al. 2007; Pocsfalvi et al. 2007). Interestingly, the Apoptosis Inducing Factor AIF (see further) has also been found to be phosphorylated upon its association with complex I (Palmisano et al. 2007). In many cases, it appears that the phosphorylation is a dynamic process as both phosphorylated and unphosphorylated forms of the protein are detected. The physiological significance of this post-translational modification is not well understood and one hypothesis is that phosphorylation regulates the activity of complex I (Palmisano et al. 2007). Indeed, cAMP treatment promotes NADH-ubiquinone oxidoreductase activity via phosphorylation of complex I and this event prevents the production of reactive oxygen species (Papa et al. 2002; Bellomo et al. 2006; Piccoli et al. 2006). A recent study showed that the import of AQDQ/NDUFS4 in the mitochondria is regulated by phosphorylation of the mitochondrial targeting sequence (De Rasmo et al. 2008). Phosphorylation is not a process limited to complex I as some subunits of complex IV and V are also able to undergo phosphorylation. In the case of complex V, phosphorylation was demonstrated to regulate the dimerization of the enzyme in yeast (Reinders et al. 2007).

Diversity of eukaryotic complex I

Complex I associated functions

In addition to the classical NADH:ubiquinone oxidoreductase reaction performed by all mitochondrial complex I

(see above), additional enzymatic activities have been found or proposed to be associated with complex I from plants, bovine and fungi. The physiological significance of these complex I-associated activities is not fully understood.

In plants, complex I displays some unique features, including the presence of plant-specific non-core subunits (Heazlewood et al. 2003; Meyer et al. 2008).The most striking example is a group of subunits, three in *C. reinhardtii* and five in *A. thaliana*, (Heazlewood et al. 2003; Cardol et al. 2004; Parisi et al. 2004; Perales et al. 2004; Sunderhaus et al. 2006) that are structurally related to γ -carbonic anhydrases (CA), a family of zinc-containing enzymes that catalyze the reversible interconversion of CO_2 and HCO_3 (Smith and Ferry 2000). Interestingly, the bacterial γ -CA prototype from *Methanosarcina thermophila* is also active when Fe^{2+} is substituted for Zn^{2+} in the holoenzyme (Tripp et al. 2004). Preliminary results suggest that these carbonic anhydrase-like subunits (CAL) in complex I have also the capacity to bind Fe (Parisi et al. 2004). The significance of this finding is unclear because recombinant CALs failed to display carbonic anhydrase activity (Perales et al. 2004). It is possible that CALs are active only when integrated with complex I. However, at present there is also no direct evidence for such an activity in complex I from plants (Braun and Zabaleta 2007). Interestingly, in cyanobacteria, CA activity was found to be associated with NAD(P)H dehydrogenases (Maeda et al. 2002). It has been postulated that the CO_2 hydration process by cyanobacterial CA is coupled directly to electron flow and proton translocation within the NAD(P)H dehydrogenases (Maeda et al. 2002). A similar function in the conversion of mitochondrial CO_2 into bicarbonate can still be envisioned for complex I-integrated CALs as many metabolic reactions in the plant mitochondrial matrix lead to the release of CO_2 (e.g. photorespiration, decarboxylation of citric acid intermediates in the Krebs cycle). Electronic microscopy analysis of complex I from *A. thaliana*, maize and the green alga Polytomella, revealed that the CALs define a spherical extra domain, attached to the hydrophobic arm and protuding in the mitochondrial matrix (Sunderhaus et al. 2006; Peters et al. 2008). This CAL domain is probably important for the stability of complex I based on the finding that loss of Arabidopsis CAL2 function (one of the five CALs present in complex I) results in drastically reduced levels of complex I (Perales et al. 2005).

The last enzyme of ascorbate biosynthesis, namely the L-galactono-1,4-lactone dehydrogenase (GalLDH), is physically associated to complex I in *Arabidopsis* (Heazlewood et al. 2003). Further work shows that ascorbate biosynthesis is sensitive to the classical inhibitor of complex I, rotenone, suggesting that GalLDH activity is regulated by electron transport through complex I (Millar et al. 2003). In BN-PAGE experiments GalLDH localizes on a minor high mobility form of complex I, suggesting that there is only a small fraction of complex I that associates with GalLDH (Millar et al. 2003). One possibility is that the GalLDH activity is modulated by the redox status of complex I in Arabidopsis (Millar et al. 2003).

In the fungus *Y. lipolytica*, complex I displays rhodanese activity (thiosulfate:cyanide sulfurtransferase activity) because one of the subunits is a rhodanese homologue. The rhodanese enzyme is suggested to be involved in FeS cluster biosynthesis in eukaryotes based on its mitochondrial localization and ability to participate in the transfer reaction of sulfur atoms (Mueller 2006; Cipollone et al. 2007). In a deletion strain of *Y. lipolytica* that lacks this subunit, the rhodanese activity of complex I is lost but complex I does not present any functional defect neither in the FeS clusters nor in its assembly (Abdrakhmanova et al. 2005). It is conceivable that the rhodanese activity is required in instances where inactive FeS clusters need to be regenerated in the complex but this awaits to be proven experimentally. Such a function has also been postulated for Nqo15, an additional subunit recently identified in complex I from *T. thermophilus* (cf. above), with structural similarity to the mitochondrial Fe chaperone frataxin (Hinchliffe et al. 2006; Sazanov and Hinchliffe 2006). A role for frataxin as an Fe source in the repair of FeS cluster in aconitase is already established (Bulteau et al. 2004).

In bovine and *N. crassa* complex I, a subunit homologous to an acyl carrier protein (ACP) has been found in the purified enzyme (Sackmann et al. 1991; Carroll et al. 2003). Mitochondrial ACPs are involved in the synthesis of type II fatty acids (Byers and Gong 2007) but also in the making of lipoic acid (Rebeille et al. 2007), an important co-factor of several multienzyme complexes (e.g. pyruvate dehydrogenase). In *N. crassa*, the complex I-linked ACP functions in de novo fatty acids synthesis (Zensen et al. 1992) and loss of ACP function leads to a selective impairment of complex I assembly and disturbance of phospholipid contents in the mitochondrial membranes (Schneider et al. 1995). By similarity to complex I composition in mammals and *N. crassa*, one ACP subunit has been assigned to complex I in Chlamydomonas (Cardol et al. 2004), Arabidopsis and rice (Heazlewood et al. 2003), although mass spectrometry analysis has failed to identify such a protein in complex I preparations from these organisms. It has been demonstrated that complex I-associated ACP is not a *bona fide* complex I subunit in Arabidopsis but occurs predominantly as two soluble isoforms (mtACP1 and mtACP2) in the mitochondrial matrix (Meyer et al. 2007). A similar result was obtained when the localization of complex I-associated ACP was re-examined in bovine heart (Cronan et al. 2005). On this basis, a role

for ACP as a non-core complex I subunit was ruled out in the plant and mammalian enzyme. Recently, a combination of monoclonal antibody immunoprecipitation and TAP-tag purification followed by mass spectrometry analysis identified ACP (homolog of human NDUFAB1) along with two trans-2-enoyl-CoA reductase proteins (mENR1 and mENR2) as complex I subunits in *Trypanosoma brucei* (Panigrahi et al. 2008). ACP depletion by RNAi or gene knock-out lead to a defect in fatty acid and lipoic acid synthesis but the impact on complex I assembly has not been examined (Stephens et al. 2007). The role of mENR1 and mENR2 type II fatty acid synthesis is still hypothetical at this point but their presence along with ACP in complex I is intriguing. So far, a role for ACP in complex I assembly was only established in Neurospora and a possible scenario is that ACP is involved in the synthesis/delivery of myristic acid, a fatty acid attached to the ND5 subunit in the fungal enzyme (Plesofsky et al. 2000). This post-translational modification of ND5 has only been reported for the Neurospora enzyme.

It is possible that some of the enzymatic activities are associated to complex I because the corresponding proteins use the complex as a tether to the inner membrane. A precedent for this in plant mitochondria is exemplified by the core I and core II subunits of respiratory complex III that are also the β and α subunits of the mitochondrial processing peptidase (MPP), respectively (Braun and Schmitz 1995). In other organisms, the MPP activity is soluble and the β and α subunits are distinct from the core I and core II proteins of complex III. The latter do not show the consensus motifs for the α and β subunits of MPP.

Non-canonical complex I in eukaryotes

Complex I-like subunits and complex I activity are both present in the hydrogenosome (Dyall et al. 2004; Hrdy et al. 2004; Boxma et al. 2005), a mitochondria-derived organelle that has lost its oxidative phosphorylation capacity (and hence respiratory enzymes) but still produces ATP and molecular hydrogen through fermentation of metabolic intermediates from the cytosol (Dyall and Johnson 2000). In the parasites *Trichomonas vaginalis* and *Nyctotherus ovalis,* both the 51- (NuoF) and 24-kDa (NuoE) hydrophilic subunits of the peripheral arm of complex I are present in the hydrogenosomal membranes (Dyall et al. 2004; Hrdy et al. 2004; Boxma et al. 2005). The presence of additional complex I subunits in the *N. ovalis* hydrogenosome has been predicted via the identification of cDNAs derived from genes present in the nucleus and also in the highly reduced hydrogenosomal genome. These are the 75-kDa subunit (NuoG), the 49-kDa subunit (NuoD) and the hydrophobic subunits ND2 (NuoN), ND4L (NuoK) and ND5 (NuoL) of the membrane domain (Boxma et al. 2005).

A survey of the nuclear genome of *Trichomonas* failed to detect any conserved subunits of the bacterial complex other than the 51- and 24-kDa subunits (Dyall et al. 2004 and this review). It thus appears that the *T. vaginalis* hydrogenosome lacks a multimeric complex I but instead, it possesses a NADH-dehydrogenase module, distinct from the monomeric type II NADH dehydrogenase found in some fungi (Melo et al. 2004).

In *T. vaginalis*, ferredoxin functions as the electron acceptor for the NADH dehydrogenase module while in *N. ovalis*, quinone is reduced by the activity of complex I in the hydrogenosomal membranes (Hrdy et al. 2004; Hrdy et al. 2005). In *T. vaginalis*, a function for the NADH dehydrogenase module in central hydrogenosomal carbohydrate metabolism was postulated based on its physical interaction with the NAD-dependent malic enzyme and pyruvate-ferredoxin oxido-reductase (Dyall et al. 2004). In such a model, electrons derived from the oxidative decarboxylation of malate (by malic enzyme) are transferred to NAD^+ and further to a terminal Fe-type hydrogenase by the NADH-dehydrogenase module (Horner et al. 2000). The NADH dehydrogenase module in *T. vaginalis* is reminiscent of the *Paracoccus denitrificans* subcomplex composed of the 51-kDa (NuoF) and 24-kDa (NuoE) complex I subunits that was shown to exhibit NADH dehydrogenase activity (Yano et al. 1996).

The presence of a complex I multimeric enzyme in the hydrogenosome of *N. ovalis* is intriguing and it is so far the only example of a mitochondrial-like complex I in hydrogenosomes. It is possible that the enzyme has a function in addition to the one proposed in carbohydrate metabolism for the NADH dehydrogenase module in *T. vaginalis*. One attractive hypothesis is that its activity is needed to maintain a proton gradient across the hydrogenosomal membrane. Such a membrane gradient is required for the import of nuclear-encoded proteins into the hydrogenosome in the absence of a respiratory chain (Bradley et al. 1997). Interestingly, mitochondria of the trypanosomatid *Phytomonas serpens* are similar to the *T. vaginalis* hydrogenosome, in the sense that they lack a respiratory chain but have retained a multimeric complex I with seemingly unique characteristics (González-Halphen and Maslov 2004; Cermakova et al. 2007). Again, the function of this complex I is not known, but a role in maintaining the membrane potential is suspected (Nawathean and Maslov 2000; Cermakova et al. 2007). Recent proteomic data reveal that the complex I subunit composition from trypanosomatid *T. brucei* is unique, suggesting that the enzyme has diverged from typical mitochondrial complex I (Panigrahi et al. 2008).

Complex I and human diseases

Oxidative phosphorylation disorders occur in 1 out of 7,600–10,000 live births in humans, of which complex I deficiency is frequently the cause (Kirby et al. 1999; Triepels et al. 2001; Skladal et al. 2003). Complex I defects cause a wide range of clinical disorders, ranging from lethal neonatal disease to adult onset neurodegenerative disorders. The most common phenotypes are Leber Hereditary Optic Neuropathy (LHON) and Leigh syndrome. Cardio- and encephalo-myopathies as well as some forms of Parkinson disease are also associated with complex I deficiencies (Pitkanen et al. 1996; Loeffen et al. 2000). Mutations causing complex I-linked disease in humans have been described in 11 of the 38 nuclear-encoded subunits of complex I (http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id = 252010), (Fernandez-Moreira et al. 2007) and in the seven mitochondrially encoded subunits (http://www.mitomap.org) (Ruiz-Pesini et al. 2007). Out of the nuclear disease-linked mutations, 7 map to one of the 14 core complex I subunits that are conserved in the bacterial enzyme while 4 of them affect non-core subunits. The fact that 60% of patients with complex I defects carry no mutations in the structural genes suggests that mutations in yet-to-be discovered assembly factors are important causes of disease (Smeitink et al. 2001).

Complex I assembly

Our understanding of the biogenesis of complex I still remains incomplete and is derived mainly from studies in the fungus *N. crassa* (Schulte 2001). In the last 4 years, human complex I assembly has been investigated in great detail and recently, a comprehensive review summarizing our understanding of this process has been published (Vogel et al. 2007c). Here, we will only give a brief survey of our current knowledge on this topic and refer the reader to the Vogel et al. review for a more detailed description.

Proposed models for complex I assembly

In Neurospora, the peripheral arm can be formed in the absence of mitochondrially encoded subunits (Tuschen et al. 1990). The independent assembly of the membrane and the peripheral arms of the complex was also demonstrated in Neurospora disruption mutants (Duarte et al. 1995). Analysis of complex I mutants revealed that the membrane arm appeared to be formed from two subcomplexes, referred to as the large and small intermediates. The large and small sub-complexes of the membrane arm are then joined with the peripheral arm to form the holoenzyme (Schulte et al. 1994; Schulte 2001). Interestingly, biochemical investigation of the subcomplex composition revealed that two novel, non-subunit proteins, named CIA30 and CIA84, are bound to the large membrane arm assembly intermediate. Disruption of either of the two genes encoding the CIA proteins led to a specific block of complex I assembly and thus, CIA30 and CIA84 are regarded as complex I assembly proteins (Kuffner et al. 1998).

In Chlamydomonas, two subcomplexes containing complex I subunits and harboring NADH dehydrogenase activity are found in addition to the whole complex I. The first subcomplex, a 200-kDa soluble fragment containing the 75 (NuoG) and 49-kDa (NuoD) subunits has been detected in both wild type and all complex I mutants examined to date (mutations in ND1, 3, 4, 4L, 5 and 6) (Cardol et al. 2002; 2006; 2008). The second, a 700-kDa subcomplex partially anchored to the membrane, accumulates in mutants that have lost either ND4 or ND5 (Duby and Matagne 1999; Cardol et al. 2002, 2008; Remacle et al. 2006). It is proposed that the 700-kDa fragment is formed by the addition of hydrophilic and hydrophobic subunits to the 200-kDa fragment. The 700-kDa fragment is then firmly anchored to the membrane by attachment of the 250-kDa hydrophobic module (not yet detected) (Cardol et al. 2006). This very simple and partial scheme of assembly resembles the one proposed in humans in the sense that a soluble fragment would be formed and anchored to the membrane through the recruitment of both hydrophilic and hydrophobic subunits (Vogel et al. 2007c).

In human cells, several assembly models have been proposed, first on the basis of the analysis of assembly intermediates found in complex I patients (Antonicka et al. 2003; Ugalde et al. 2004a), and then by tracing assembly intermediates in wild-type cell lines by pulse-chase techniques (Ugalde et al. 2004b; Lazarou et al. 2007) or by monitoring the fate of a nuclear-encoded tagged subunit (Vogel et al. 2007a). The most recent consensus model stipulates that an early assembly intermediate is anchored to the membrane prior to its extension with additional membrane and peripheral subunits (Vogel et al. 2007a). Consistently with this model, three different assembly intermediates of 100–150 kDa (hydrophilic), 400–500 kDa (hydrophilic + hydrophobic) and 850 kDa have been detected.

The fact that complex I is then associated with complex III and complex IV into supercomplexes or respirasomes (Schagger 2002) implies that there are additional levels of control for the assembly of this multimeric enzyme. The role of these supercomplexes is not clear but may involve substrate channeling as well as complex stabilization (Schagger et al. 2004).

The involvement of complex I specific assembly factors?

The involvement of dedicated factors for complex I assembly is expected because it is now well recognized that

the assembly of multimeric enzymes is assisted by components that are not part of the mature enzyme but nevertheless essential in promoting its assembly into an active form. Assembly of a functional complex I requires the coordination of the nuclear and mitochondrial genomes and is likely to be an intricate process. For example, the assembly of human complex IV, another multimeric respiratory enzyme of dual genetic origin is believed to be under the control of at least 14 assembly factors (for reviews Barrientos et al. 2002; Fontanesi et al. 2006; Zee and Glerum 2006). Most of the human complex IV assembly factors control co-factor metabolism (Cu or heme) or the insertion of mitochondrially encoded structural subunits in the inner membrane (Zee and Glerum 2006). Interestingly, molecular lesions in 6 of the 12 complex IV assembly genes are the direct cause of severe pathologies in humans (Zee and Glerum 2006). Based on our understanding of the complex IV assembly process, the operation of complex I assembly factors/chaperones is expected. Because complex I does not contain heme or metal co-factors, putative complex I assembly factors might function in the delivery of the multiple FeS clusters to their subunits or in the insertion/stability of hydrophobic subunits. Note that it is now clear that the biogenesis of photosynthetic membranes require specific factors that control the insertion of hydrophobic subunits or the delivery of FeS clusters in photosynthetic complexes (Lezhneva et al. 2004; Touraine et al. 2004; Gohre et al. 2006; Ossenbuhl et al. 2006; Ma et al. 2007).

Complex I chaperones

To date, out of the two chaperones found in *N. crassa*, CIA30 and CIA84, we have only identified homologues of CIA30 by bioinformatics analyses of other eukaryotic genomes (Cardol et al. 2005). The CIA84 assembly factor seems to be restricted to fungi, although other groups have reported the identification of a putative orthologue in mammals and in the green lineage (Gabaldon et al. 2005). Note that we could not detect homologues of the CIA30 and CIA84 assembly factors in the nuclear genome of *T. vaginalis*, consistent with the fact that this parasite does not assemble a multimeric complex I, but a NADH dehydrogenase module composed of only two subunits in the hydrogenosome (see above).

The role of CIA30 (NDUFAF1) in complex I assembly has been demonstrated via RNAi knock-downs in human cell lines and the detection of a missense mutation in the corresponding gene in a patient with complex I-linked cardioencephalomyopathy (Vogel et al. 2005; Dunning et al. 2007). The association of human CIA30/NDUFAF1 with modules of 400–500 kDa containing the mitochondrially encoded complex I subunits led to the proposal that the chaperone operates in the early steps of complex I assembly (Dunning et al. 2007).

Other identified assembly factors in humans are Ecsit, a protein essential for inflammatory response and embryonic development that interacts with CIA30/NDUFAF1 (Vogel et al. 2007b), the apoptosis-inducing factor (AIF) (Vahsen et al. 2004) and the chaperone B17.2L (Ogilvie et al. 2005). Ecsit is a signaling molecule that partitions between the cytoplasm and the mitochondria where only a small fraction of the protein is detected. In the mitochondria, Ecsit was found to be associated with CIA30/NDUFAF1 chaperone in high molecular weight complexes. It is highly possible that other complex I chaperones are present in the high molecular weight complexes that contain Ecsit and CIA30/NDUFAF1. A role of Ecsit in the assembly/stability of mitochondrial complex I is inferred from the finding that Ecsit knock-down results in a significant decrease in complex I accumulation and activity. The placement of Ecsit in a signaling pathway is intriguing and suggests that complex I might regulate the inflammatory response/embryonic development.

Another signaling molecule involved in complex I assembly is the AIF that is housed in the mitochondrial intermembrane space but undergoes relocation to the nucleus to perform chromatin condensation during apoptosis. Loss of AIF in mice results in a specific defect in complex I assembly/stability but the role of AIF in complex I biogenesis/maintenance remains unclear (Vahsen et al. 2004; Joza et al. 2005).

B17.2L is a paralogue of the B17.2 subunit which, like AIF and Ecsit, is only found in mammals (see Table 2). Mutations in B17.2L are responsible for encephalomyopathy in patients (Ogilvie et al. 2005). Similarly to CIA30, B17.2L is not attached to the holoenzyme. Instead, it is associated with a 830-kDa subcomplex in several patients with complex I assembly defects (Ogilvie et al. 2005; Lazarou et al. 2007; Vogel et al. 2007b). The 830-kDa subcomplex contains subunits from both peripheral and membrane arms (Ogilvie et al. 2005), suggesting that B17.2L, unlike CIA30/NDUFAF1, plays a role in the late assembly of complex I. Interestingly, the B17.2L encoding gene is under the transcriptional control of c-myc, a mediator of cell proliferation. High expression levels of the B17.2L gene were noted in some tumors and reduced levels appear to inhibit tumorigenesis in certain carcinoma cell lines (Tsuneoka et al. 2005).

Recently, a missense mutation in the *C6ORF66* gene was shown to be responsible for a strong reduction of complex I assembly in muscles of patients presenting infantile mitochondrial encephalomyopathy (Saada et al. 2008). The corresponding protein is found in mitochondria but is not part of the final complex I enzyme, suggesting that C6ORF66 is a novel complex I assembly factor.

Table 2 Genetic model systems to analyze complex I assembly

Organism	Phenotypical screening of complex I mutants	Labeling of mitochondria-encoded subunits	Mitochondrial mutants		Nuclear mutants		Assembly factors			
			Random	SD[a]	KO[b] KD[c]	SD[a]	CIA30	CIA84	B17.2L	C6ORF66
N. crassa	−	+	+	−	+	+	+	+	−	−
Y. lipolytica	−	ND	−	−	+	+	+	+	−	−
C. reinhardtii	+	ND	+	+	+	−	+	−	−	−
Vascular plants	−	ND	+	−	+	−	+	−	−	−
C. elegans	−	ND	−	−	+	+	+	−	−	+
Mammalian cell lines	−	+	+	−	+	+	+	−	+	+

ND non-available data

[a] Site directed mutagenesis

[b] Knock-out (DNA insertion or point mutation)

[c] Knock-down (RNA silencing)

Orthologues are found in invertebrates such as the nematode *Caenorhabditis elegans* but appear to be absent in fungi and plants (Table 2 and Saada et al. 2008). Previously, this protein has been demonstrated to promote breast cell cancer invasiveness by inducing the excretion of the extracellular-matrix-degrading enzyme MMP-9 (Karp et al. 2007). Like GRIM-19 and B17.2L, C6ORF66 is yet an additional link between complex I and cancer. Further experimental investigation is required to dissect the role of these proteins in the tumorigenesis process.

It is conceivable that some complex I assembly factors are not only complex I-specific, but also control the assembly of other respiratory complexes in the mitochondrial inner membrane. This view is further supported by the fact that the loss of assembly of one complex in human mitochondria affects the assembly/stability of additional respiratory enzymes. This interdependence of respiratory complexes in mammalian mitochondria was reported in several instances. In humans and mice, a defect in complex IV impacts the assembly of complex I (Diaz et al. 2006; Li et al. 2007), suggesting that the assembly of the two enzymes is coordinated. One possible explanation to account for the dual deficiency is that complex I and complex IV share a common assembly factor. An example of such a factor is the Oxa1 protein, which is believed to facilitate the integration of proteins from the mitochondrial matrix into the inner membrane (for review Kiefer and Kuhn 2007). Interestingly, in the fungi *N. crassa* and *Podospora anserina,* a defect in Oxa1p results in an impaired assembly of complexes I and IV (Nargang et al. 2002; Sellem et al. 2005) whereas knock-down of human Oxa1p impairs the biogenesis of mitochondrial complex I and ATP synthase with no consequence on complex IV (Stiburek et al. 2007). In mammalian cells, the fact that assembled complex III is required to stabilize complex I speaks also to a structural dependence between complex I and III (Acin-Perez et al. 2004).

Model systems to study complex I assembly

In addition to human cell cultures, which still remain the focus of attention for the study of complex I deficiencies (see for example Hofhaus and Attardi 1993; Hofhaus et al. 1996; Vogel et al. 2005; Malfatti et al. 2007; Vogel et al. 2007d), research on mitochondrial complex I has also been conducted in non-human model systems, including fungi such as *Y. lipolytica* (Kerscher et al. 2001a, 2002, 2004) and *N. crassa* (for review Schulte 2001; Videira and Duarte 2002; Janssen et al. 2006), animals such as *C. elegans* (Grad and Lemire 2004; Grad et al. 2005), chinese hamster cell lines (Scheffler et al. 2004; Yadava and Scheffler 2004), mice (Qi et al. 2003, 2004) and vascular plants (Pla et al. 1995; Karpova and Newton 1999; Brangeon et al. 2000; Lee et al. 2002; Perales et al. 2005; Nakagawa and Sakurai 2006; de Longevialle et al. 2007).

In *Y. lipolytica* the absence of complex I is lethal, but complex I mutants can be rescued by targeting to the internal face of the mitochondrial inner membrane, a monomeric NADH dehydrogenase (Ndh2) located at the external face of the same membrane (Kerscher et al. 1999, 2001b). In contrast to *Y. lipolytica*, complex I in *N. crassa* is dispensable during vegetative growth but essential for the sexual phase of the fungus life cycle (Duarte et al. 1998; Duarte and Videira 2000). In both fungi, inactivation of the structural genes provided insights into the function of all nuclear-encoded complex I core subunits and a few of the non-core subunits (for review Kerscher et al. 2001a, 2002, 2004; Schulte 2001; Videira and Duarte 2002; Janssen et al. 2006). In the case of *Y. lipolytica*, functional

dissection of complex I subunits was only possible when Ndh2 was simultaneously targeted to the internal face of the mitochondrial inner membrane as a metabolic by-pass for the loss of complex I. Engineering of pathogenic mutations in nuclear-encoded subunits was also achieved in both fungal models, and detailed investigations enabled an assessment of the impact of disease-associated mutations on the activity/assembly of complex I (Ahlers et al. 2000; Kerscher et al. 2004; Duarte et al. 2005).

Another model of study for complex I deficiency is *C. elegans*, an experimental system that offers the unique possibility of monitoring the consequences of mitochondrial dysfunction on a multicellular organism (Tsang et al. 2001). *C. elegans* transgenic lines harboring missense mutations in the 51 kDa subunit of complex I could be reconstructed (Tsang et al. 2001). Such complex I deficient lines manifest typical characteristics of human mitochondrial disease, including decreased rates of respiration and lactic acidosis. Interestingly, they were also responsive to traditional pharmacological treatments that are used to provide relief to patients with complex I defects (Grad and Lemire 2004). Recent work illustrated the potential of metabolic engineering as a way to correct the defect in oxidative phosphorylation due to complex I mutations in *C. elegans* (Grad et al. 2005; DeCorby et al. 2007).

The fact that a mutation in a complex I subunit in *Drosophila melanogaster* results in a reduced fitness phenotype suggests that the fruit fly is also a promising model system to unravel the molecular basis of complex I-linked diseases (B. Graham, personal communication).

Chinese hamster cell lines derived from fibroblasts were used successfully to isolate several respiratory deficient mutants that could be further classified in complementation groups. Three complementation groups appeared to be defective in complex I activity and for two of these groups the affected genes encode the non-core complex I subunits MWFE and ESSS (Scheffler et al. 2004). The gene corresponding to the third group is currently unknown and hypothesized to encode an essential assembly factor. The isolation of null mutants in the MWFE and ESSS non-core subunits has allowed a detailed characterization of the role of these two non-core subunits in the activity and assembly of the enzyme (Scheffler et al. 2004; Yadava and Scheffler 2004).

In the visual system of mice, ribozyme-mediated suppression of the gene encoding the Ndufa1 (MWFE) complex I subunit induces damages to the optic nerve and retina (Qi et al. 2004). This degenerative phenotype is correlated with the increase in reactive oxygen species caused by a complex I defect. Interestingly, delivery of the human gene encoding superoxide dismutase in the affected organs partially rescues the degeneration (Qi et al. 2003). The recapitulation, in mice, of the hallmarks of human complex I-linked LHON suggests that the murine model could be useful in testing pharmacological or genetic therapies for complex I-related optic neuropathy.

In vascular plants, the consequence of complex I defects has been documented in three experimental models, *A. thaliana*, *Nicotiana sylvestris* and *Zea mays*. Interestingly, disruption of complex I function is not lethal in plants. However, the physiological impacts on reproductive and vegetative organs due to the complete knock-out of complex I activity are very distinct depending on the plant under study. It is unclear if this reflects a difference in the metabolic importance of complex I for a given plant species or if loss of different complex I subunits in each mutant accounts for the observed phenotype (Pla et al. 1995; Karpova and Newton 1999; Brangeon et al. 2000; Lee et al. 2002; Perales et al. 2005; Nakagawa and Sakurai 2006; de Longevialle et al. 2007).

Note that the technology to manipulate the mitochondrial ND genes is not yet available for any of the model systems described above. In addition, if molecular genetics methods to inactivate complex I genes and recreate complex I mutations are available, a genetic screen for complex I mutants, although feasible, would seem labor-intensive. To our knowledge, such a screening has never been reported in any of the model systems. Table 2 summarizes the features of the experimental systems described above. The lack of a suitable organism in which complex I deficiencies can be specifically screened and deciphered at the molecular level has restricted the discovery of new genes involved in complex I assembly. In this context, the development of an alternative, genetically tractable model system for dissecting the molecular basis of complex I biogenesis is highly desirable.

Chlamydomonas reinhardtii, an experimental system to dissect complex I biogenesis

Chlamydomonas reinhardtii, a fresh water, biflagellated green alga appears to be a very promising system to address the question of complex I biogenesis. With the release of a fully annotated genome (Merchant et al. 2007), the availability of a large EST database (Grossman et al. 2003; Jain et al. 2007), and the successful application of RNAi technology (Schroda 2006) in addition to classical and molecular genetic approaches (Harris 2001), Chlamydomonas has become a popular experimental system. Often viewed as a "plant-like" organism because it was first used in the dissection of many fundamental aspects of photosynthesis (Harris 2001), Chlamydomonas is in fact close to mammalians in many aspects of its biology that are unique and unrelated to photosynthesis (Grossman et al. 2007; Merchant et al. 2007). For instance, decade old studies on the assembly of flagella in Chlamydomonas

have now turned this unicellular eukaryote into the best system to investigate the molecular basis of ciliary disease in humans (for review Blacque et al. 2008). Chlamydomonas is also an ideal organism to study mitochondrial biogenesis because the respiratory and photosynthetic membrane systems are separate and can be investigated independently (Harris 2001). Indeed, Chlamydomonas respiratory mutants can be obtained and are viable if maintained under phototrophic conditions (where they rely on photosynthesis in the chloroplast). Unlike complex I mutants in *N. crassa*, complex I mutants in Chlamydomonas are not affected for sexual reproduction (Remacle et al. 2001a). Mutants deficient for complex I can be easily scored on the basis of their impaired growth in the dark (Remacle et al. 2001a). Indeed, contrary to complex III or complex IV mutants that do not grow in the dark because they lack two phosphorylation sites,[4] complex I mutants are still able to grow in these conditions. However, their growth is significantly slower than wild type because they only retain two of the three phosphorylation sites that are operational when electron transfer proceeds through the respiratory chain (Figs. 1, 3). Complex I deficient mutants are also affected for optimal growth in mixotrophic conditions where there is a contribution of photosynthesis and respiration, i.e. in the light in presence of a reduced carbon source such as acetate (Fig. 3). This defect is even more pronounced if mitochondrial respiration is affected by the addition of complex III inhibitors, such as antimycin A and myxothiazol, that block the cytochrome pathway of respiration (Figs. 1, 3). In this situation, complex I mutants only respire via non-phosphorylating enzymes (Fig. 1). This phenotypic trait was used to screen complex I deficient mutants obtained after treatment of Chlamydomonas cells with acriflavine, a mutagenic agent that binds more specifically to mitochondrial DNA. The isolated mutants carry molecular lesions in the mitochondrial ND genes. They are homoplasmic and directly amenable to biochemical studies (Remacle et al. 2001a, b; Cardol et al. 2002). In the absence of a multicellular eukaryotic system for identifying novel assembly factors, the green alga *C. reinhardtii* thus represents a valuable, alternative model of experimental investigation. The outcome of these studies is likely to be extended to the human system because (a) complex I assembly intermediates are detected in the mitochondrial *nd* mutants and they display some similarities with those found in human cell lines, and (b) the subunit composition of complex I in Chlamydomonas is now known and is similar to the human purified holoenzyme (Cardol et al. 2004). In order to identify factors that control the assembly of complex I, we chose a mutagenesis approach to isolate

[4] Hence the terminology of *dark-minus* or *dark-dier* phenotype for complex III or complex IV mutants.

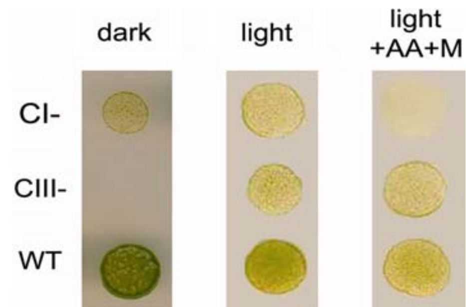

Fig. 3 Growth of respiratory deficient mutants. Chlamydomonas wild-type cells (*WT*) and respiratory mutants deficient for complex I (CI-) or complex III (CIII-) were spotted on a medium containing acetate as a carbon source in the dark, in the light and in the light with respiratory inhibitors of complex III (M, 7.5 μM myxothiazol; AA, 1 μM antimycin A) and incubated for 3 days (in the light) and 14 days (in the dark) at 25°C

loss-of-function nuclear mutations that result in complex I deficiency. This choice is justified because complex I deficient mutants are viable (if maintained phototrophically) and it is expected that inactivation of the genes encoding complex I specific assembly factors will also result in a viable phenotype.

Candidate complex I deficient strains were first screened on the basis of their slow growth phenotype in the dark. Then, mutants unable to assemble an active complex I were visualized by an in-gel colorimetric assay that reveals the NADH dehydrogenase activity of complex I in the presence of NADH and nitroblue tetrazolium. This simple technique is rapid and most appropriate to screen many mutants for impaired assembly of complex I (Cardol et al. 2006). Among 10,000 transformants analyzed, two non-allelic *amc* mutants (*amc1* and *amc2* for *a*ssembly of *m*itochondrial *c*omplex I) have been already isolated and shown to accumulate a subcomplex that retains NADH dehydrogenase activity (unpublished). While it is conceivable that the *amc* mutations inactivate a complex I structural gene, it is also possible that they map to a novel complex I assembly gene. A saturating mutant screen for complex I deficient strains is also a high priority, as this will increase the probability to recover mutations in unrecognized complex I assembly genes.

Recently, biolistic transformation of the mitochondrial genome of Chlamydomonas has been employed to reconstruct mutations in the mitochondrial ND genes (Remacle et al. 2006). Mitochondrial transformation is a new methodological development of considerable importance because many complex I linked diseases are associated with mutations in the mitochondrial ND genes. Due to the inherent difficulties associated with gene replacement in the mitochondrial genome, mitochondrial ND mutations could so far only be reconstructed in prokaryotes (Lunardi

et al. 1998; Zickermann et al. 1998; Pätsi et al. 2008). The real impact of mitochondrial ND mutations on complex I assembly and activity in patients is often difficult to assess due to the frequent heteroplasmic state of the mutations. Moreover, the occurrence of secondary mutations that are not the primary cause of the respiratory defect (Crimi et al. 2002) as well as the presence of sequence polymorphisms in the mitochondrial ND genes (Batandier et al. 2000; Cittadella et al. 2001) often make it difficult to establish a direct correlation between the clinical disease symptoms and the mitochondrial ND "mutation". The "cybrid" technology is now available and remains an invaluable tool in assessing the contribution of mitochondrial mutations to defects in the respiratory chain. Cybrids are cytoplasmic hybrid cells, created by introducing mtDNAs of interest into cells depleted of endogenous mtDNAs (Trounce and Pinkert 2007).

With a biolistic transformation system in hand, the mitochondrial genome of Chlamydomonas can now be virtually manipulated at will and is amenable to reverse genetics, site-directed mutagenesis and molecular tagging of all five mitochondrially encoded ND subunits of complex I (Table 1). However, the selective conditions used for reconstruction of the ND mutations via biolistic bombardment require a two month incubation period in the dark (Remacle et al. 2006). This constitutes an obvious limitation and the development of a more rapid selection for the generation of mitochondrial transformants is therefore a high priority. One possibility is to devise a selection for mitochondrial transformation events that is independent of mitochondrial respiration, yet linked to the mitochondrial function. Such a selection is already at use in *Saccharomyces cerevisiae* and relies on the fact that a null allele in a nuclear gene encoding a mitochondrially located protein can be rescued by expression of the protein from the same gene relocated to the mitochondrial genome (Steele et al. 1996).

Concluding remarks

At present, there is no ideal system to investigate the molecular mechanisms of complex I assembly due to the fact that this multimeric enzyme is of dual genetic origin with only a few subunits encoded in the mitochondrial genome while the majority is encoded in the nucleus. Chlamydomonas seems to be ideally suited to address the question of complex I assembly because (a) it is now amenable to the manipulation of both nuclear and mitochondrial genomes and (b) mutants impaired for mitochondrial function can be specifically screened for complex I defects (Fig. 4). We anticipate that the systematic screening of complex I deficient mutants will lead to the discovery of novel genes that control the assembly of the enzyme. This will be of significant impact on the medical field, as the molecular bases of many complex I deficiencies in humans are still not deciphered.

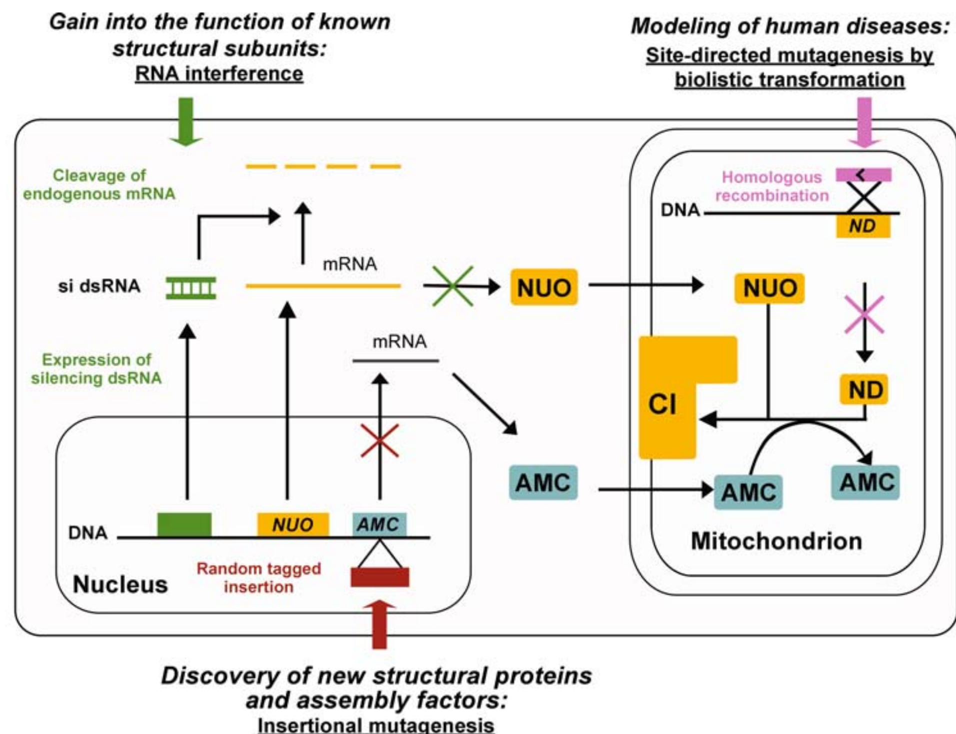

Fig. 4 Isolating complex I deficient mutants in Chlamydomonas. Known complex I nuclear genes (*NUO*) can be specifically inactivated by RNAi and novel complex I assembly genes (*AMC*) can be identified by insertional mutagenesis. Mitochondrial genes (*ND*) can be inactivated by random mutagenesis or manipulated by site-directed mutagenesis

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References

Abdrakhmanova A, Dobrynin K, Zwicker K, Kerscher S, Brandt U (2005) Functional sulfurtransferase is associated with mitochondrial complex I from *Yarrowia lipolytica*, but is not required for assembly of its iron–sulfur clusters. FEBS Lett 579:6781–6785

Abdrakhmanova A, Zickermann V, Bostina M, Radermacher M, Schagger H, Kerscher S, Brandt U (2004) Subunit composition of mitochondrial complex I from the yeast *Yarrowia lipolytica*. Biochim Biophys Acta 1658:148–156

Acin-Perez R, Bayona-Bafaluy MP, Fernandez-Silva P, Moreno-Loshuertos R, Perez-Martos A, Bruno C, Moraes CT, Enriquez JA (2004) Respiratory complex III is required to maintain complex I in mammalian mitochondria. Mol Cell 13:805–815

Ahlers PM, Garofano A, Kerscher SJ, Brandt U (2000) Application of the obligate aerobic yeast *Yarrowia lipolytica* as a eucaryotic model to analyse Leigh syndrome mutations in the complex I core subunits PSST and TYKY. Biochim Biophys Acta 1459:258–265

Angell JE, Lindner DJ, Shapiro PS, Hofmann ER, Kalvakolanu DV (2000) Identification of GRIM-19, a novel cell death-regulatory gene induced by the interferon-beta and retinoic acid combination, using a genetic approach. J Biol Chem 275:33416–33426

Antonicka H, Ogilvie I, Taivassalo T, Anitori RP, Haller RG, Vissing J, Kennaway NG, Shoubridge EA (2003) Identification and characterization of a common set of complex I assembly intermediates in mitochondria from patients with complex I deficiency. J Biol Chem 278:43081–43088

Barrientos A, Barros MH, Valnot I, Rotig A, Rustin P, Tzagoloff A (2002) Cytochrome oxidase in health and disease. Gene 286:53–63

Batandier C, Picard A, Tessier N, Lunardi J (2000) Identification of a novel T398A mutation in the ND5 subunit of the mitochondrial complex I and of three novel mtDNA polymorphisms in two patients presenting ocular symptoms. Hum Mutat 16:532

Bellomo F, Piccoli C, Cocco T, Scacco S, Papa F, Gaballo A, Boffoli D, Signorile A, D'Aprile A, Scrima R, Sardanelli AM, Capitanio N, Papa S (2006) Regulation by the cAMP cascade of oxygen free radical balance in mammalian cells. Antioxid Redox Signal 8:495–502

Blacque OE, Cevik S, Kaplan OI (2008) Intraflagellar transport: from molecular characterisation to mechanism. Front Biosci 13:2633–2652

Boxma B, de Graaf RM, van der Staay GW, van Alen TA, Ricard G, Gabaldon T, van Hoek AH, Moon-van der Staay SY, Koopman WJ, van Hellemond JJ, Tielens AG, Friedrich T, Veenhuis M, Huynen MA, Hackstein JH (2005) An anaerobic mitochondrion that produces hydrogen. Nature 434:74–79

Bradley PJ, Lahti CJ, Plumper E, Johnson PJ (1997) Targeting and translocation of proteins into the hydrogenosome of the protist Trichomonas: similarities with mitochondrial protein import. EMBO J 16:3484–3493

Brangeon J, Sabar M, Gutierres S, Combettes B, Bove J, Gendy C, Chetrit P, des Francs-Small CC, Pla M, Vedel F, De Paepe R (2000) Defective splicing of the first nad4 intron is associated with lack of several complex I subunits in the *Nicotiana sylvestris* NMS1 nuclear mutant. Plant J 21:269–280

Braun HP, Schmitz UK (1995) The bifunctional cytochrome c reductase/processing peptidase complex from plant mitochondria. J Bioenerg Biomembr 27:423–436

Braun HP, Zabaleta E (2007) Carbonic anhydrase subunits of the mitochondrial NADH dehydrogenase complex (complex I) in plants. Physiol Plant 129:114–122

Bulteau A-L, O'Neill HA, Kennedy MC, Ikeda-Saito M, Isaya G, Szweda LI (2004) Frataxin acts as an iron chaperone protein to modulate mitochondrial aconitase activity. Science 305:242–245

Byers DM, Gong H (2007) Acyl carrier protein: structure-function relationships in a conserved multifunctional protein family. Biochem Cell Biol 85:649–662

Cardol P, Boutaffala L, Memmi S, Devreese B, Matagne RF, Remacle C (2008) In Chlamydomonas, the loss of ND5 subunit prevents the assembly of whole mitochondrial complex I and leads to the formation of a low abundant 700 kDa subcomplex. Biochimica et Biophysica Acta (BBA)—Bioenergetics 1777:388–396

Cardol P, Gonzalez-Halphen D, Reyes-Prieto A, Baurain D, Matagne RF, Remacle C (2005) The mitochondrial oxidative phosphorylation proteome of *Chlamydomonas reinhardtii* deduced from the Genome Sequencing Project. Plant Physiol 137:447–459

Cardol P, Lapaille M, Minet P, Franck F, Matagne RF, Remacle C (2006) ND3 and ND4L subunits of mitochondrial complex I, both nucleus encoded in *Chlamydomonas reinhardtii*, are required for activity and assembly of the enzyme. Eukaryot Cell 5:1460–1467

Cardol P, Matagne RF, Remacle C (2002) Impact of mutations affecting ND mitochondria-encoded subunits on the activity and assembly of complex I in Chlamydomonas. Implication for the structural organization of the enzyme. J Mol Biol 319:1211–1221

Cardol P, Vanrobaeys F, Devreese B, Van Beeumen J, Matagne RF, Remacle C (2004) Higher plant-like subunit composition of mitochondrial complex I from *Chlamydomonas reinhardtii*: 31 conserved components among eukaryotes. Biochim Biophys Acta 1658:212–224

Carroll J, Fearnley IM, Shannon RJ, Hirst J, Walker JE (2003) Analysis of the subunit composition of complex I from bovine heart mitochondria. Mol Cell Proteomics 2:117–126

Carroll J, Shannon RJ, Fearnley IM, Walker JE, Hirst J (2002) Definition of the nuclear encoded protein composition of bovine heart mitochondrial complex I. Identification of two new subunits. J Biol Chem 277:50311–50317

Cermakova P, Zk Verner, Man P, Lukes J, Horvath A (2007) Characterization of the NADH:ubiquinone oxidoreductase (complex I) in the trypanosomatid *Phytomonas serpens* (Kinetoplastida). FEBS J 274:3150–3158

Chen R, Fearnley IM, Peak-Chew SY, Walker JE (2004) The phosphorylation of subunits of complex I from bovine heart mitochondria. J Biol Chem 279:26036–26045

Cipollone R, Ascenzi P, Visca P (2007) Common themes and variations in the rhodanese superfamily. IUBMB Life 59:51–59

Cittadella R, Andreoli V, Manna I, Oliveri RL, Quattrone A (2001) A new human mtDNA polymorphism: MTND6: 14562 (C- > T). Hum Mutat 17:238

Crimi M, Sciacco M, Galbiati S, Bordoni A, Malferrari G, Del Bo R, Biunno I, Bresolin N, Comi GP (2002) A collection of 33 novel human mtDNA homoplasmic variants. Hum Mutat 20:409

Cronan JE, Fearnley IM, Walker JE (2005) Mammalian mitochondria contain a soluble acyl carrier protein. FEBS Lett 579:4892–4896

de Longevialle AF, Meyer EH, Andres C, Taylor NL, Lurin C, Millar AH, Small ID (2007) The pentatricopeptide repeat gene OTP43 is required for trans-splicing of the mitochondrial nad1 intron 1 in *Arabidopsis thaliana*. Plant Cell 19:3256–3265

De Rasmo D, Panelli D, Sardanelli AM, Papa S (2008) cAMP-dependent protein kinase regulates the mitochondrial import of the nuclear encoded NDUFS4 subunit of complex I. Cell Signal 20:989–997

DeCorby A, Gaskova D, Sayles LC, Lemire BD (2007) Expression of Ndi1p, an alternative NADH:ubiquinone oxidoreductase, increases mitochondrial membrane potential in a *C. elegans* model of mitochondrial disease. Biochimica et Biophysica Acta (BBA)—Bioenergetics 1767:1157–1163

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

Djafarzadeh R, Kerscher S, Zwicker K, Radermacher M, Lindahl M, Schagger H, Brandt U (2000) Biophysical and structural characterization of proton-translocating NADH-dehydrogenase (complex I) from the strictly aerobic yeast *Yarrowia lipolytica*. Biochim Biophys Acta 1459:230–238

Duarte M, Mota N, Pinto L, Videira A (1998) Inactivation of the gene coding for the 30.4-kDa subunit of respiratory chain NADH dehydrogenase: is the enzyme essential for Neurospora? Mol Gen Genet 257:368–375

Duarte M, Schulte U, Ushakova AV, Videira A (2005) Neurospora strains harboring mitochondrial disease-associated mutations in iron–sulfur subunits of complex I. Genetics 171:91–99

Duarte M, Sousa R, Videira A (1995) Inactivation of genes encoding subunits of the peripheral and membrane arms of neurospora mitochondrial complex I and effects on enzyme assembly. Genetics 139:1211–1221

Duarte M, Videira A (2000) Respiratory chain complex I is essential for sexual development in neurospora and binding of iron–sulfur clusters are required for enzyme assembly. Genetics 156:607–615

Duby F, Matagne RF (1999) Alteration of dark respiration and reduction of phototrophic growth in a mitochondrial DNA deletion mutant of Chlamydomonas lacking cob, nd4, and the 3′ end of nd5. Plant Cell 11:115–125

Dunning CJ, McKenzie M, Sugiana C, Lazarou M, Silke J, Connelly A, Fletcher JM, Kirby DM, Thorburn DR, Ryan MT (2007) Human CIA30 is involved in the early assembly of mitochondrial complex I and mutations in its gene cause disease. EMBO J 26:3227–3237

Dyall SD, Johnson PJ (2000) Origins of hydrogenosomes and mitochondria: evolution and organelle biogenesis. Curr Opin Microbiol 3:404–411

Dyall SD, Yan W, Delgadillo-Correa MG, Lunceford A, Loo JA, Clarke CF, Johnson PJ (2004) Non-mitochondrial complex I proteins in a hydrogenosomal oxidoreductase complex. Nature 431:1103–1107

Fearnley IM, Carroll J, Shannon RJ, Runswick MJ, Walker JE, Hirst J (2001) GRIM-19, a cell death regulatory gene product, is a subunit of bovine mitochondrial NADH:ubiquinone oxidoreductase (complex I). J Biol Chem 276:38345–38348

Fearnley IM, Carroll J, Walker JE (2007) Proteomic analysis of the subunit composition of complex I (NADH:ubiquinone oxidoreductase) from bovine heart mitochondria. Methods Mol Biol 357:103–125

Fernandez-Moreira D, Ugalde C, Smeets R, Rodenburg RJ, Lopez-Laso E, Ruiz-Falco ML, Briones P, Martin MA, Smeitink JA, Arenas J (2007) X-linked NDUFA1 gene mutations associated with mitochondrial encephalomyopathy. Ann Neurol 61:73–83

Finel M, Skehel JM, Albracht SP, Fearnley IM, Walker JE (1992) Resolution of NADH:ubiquinone oxidoreductase from bovine heart mitochondria into two subcomplexes, one of which contains the redox centers of the enzyme. Biochemistry 31:11425–11434

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

Friedrich T (1998) The NADH:ubiquinone oxidoreductase (complex I) from *Escherichia coli*. Biochim Biophys Acta 1364:134–146

Friedrich T, Bottcher B (2004) The gross structure of the respiratory complex I: a Lego System. Biochim Biophys Acta 1608:1–9

Gabaldon T, Rainey D, Huynen MA (2005) Tracing the evolution of a large protein complex in the eukaryotes, NADH:ubiquinone oxidoreductase (Complex I). J Mol Biol 348:857–870

Galante YM, Hatefi Y (1978) Resolution of complex I and isolation of NADH dehydrogenase and an iron–sulfur protein. Methods Enzymol 53:15–21

Gohre V, Ossenbuhl F, Crevecoeur M, Eichacker LA, Rochaix J-D (2006) One of Two Alb3 Proteins Is Essential for the Assembly of the Photosystems and for Cell Survival in Chlamydomonas. Plant Cell 18:1454–1466

González-Halphen D, Maslov D (2004) NADH-ubiquinone oxidoreductase activity in the kinetoplasts of the plant trypanosomatid *Phytomonas serpens*. Parasitol Res 92:341–346

Grad LI, Lemire BD (2004) Mitochondrial complex I mutations in *Caenorhabditis elegans* produce cytochrome c oxidase deficiency, oxidative stress and vitamin-responsive lactic acidosis. Hum Mol Genet 13:303–314

Grad LI, Sayles LC, Lemire BD (2005) Introduction of an additional pathway for lactate oxidation in the treatment of lactic acidosis and mitochondrial dysfunction in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 102:18367–18372

Grigorieff N (1998) Three-dimensional structure of bovine NADH:ubiquinone oxidoreductase (complex I) at 22 A in ice. J Mol Biol 277:1033–1046

Grossman AR, Croft M, Gladyshev VN, Merchant SS, Posewitz MC, Prochnik S, Spalding MH (2007) Novel metabolism in Chlamydomonas through the lens of genomics. Curr Opin Plant Biol 10:190–198

Grossman AR, Harris EE, Hauser C, Lefebvre PA, Martinez D, Rokhsar D, Shrager J, Silflow CD, Stern D, Vallon O, Zhang Z (2003) *Chlamydomonas reinhardtii* at the crossroads of genomics. Eukaryot Cell 2:1137–1150

Guenebaut V, Schlitt A, Weiss H, Leonard K, Friedrich T (1998) Consistent structure between bacterial and mitochondrial NADH:ubiquinone oxidoreductase (complex I). J Mol Biol 276:105–112

Guenebaut V, Vincentelli R, Mills D, Weiss H, Leonard KR (1997) Three-dimensional structure of NADH-dehydrogenase from *Neurospora crassa* by electron microscopy and conical tilt reconstruction. J Mol Biol 265:409–418

Harris EH (2001) *CHLAMYDOMONAS* AS A MODEL ORGANISM. Annu Rev Plant Physiol Plant Mol Biol 52:363–406

Heazlewood JL, Howell KA, Millar AH (2003) Mitochondrial complex I from Arabidopsis and rice: orthologs of mammalian and fungal components coupled with plant-specific subunits. Biochim Biophys Acta 1604:159–169

Hinchliffe P, Carroll J, Sazanov LA (2006) Identification of a novel subunit of respiratory complex I from *Thermus thermophilus*. Biochemistry 45:4413–4420

Hinchliffe P, Sazanov LA (2005) Organization of iron–sulfur clusters in respiratory complex I. Science 309:771–774

Hirst J, Carroll J, Fearnley IM, Shannon RJ, Walker JE (2003) The nuclear encoded subunits of complex I from bovine heart mitochondria. Biochim Biophys Acta 1604:135–150

Hofhaus G, Attardi G (1993) Lack of assembly of mitochondrial DNA-encoded subunits of respiratory NADH dehydrogenase and loss of enzyme activity in a human cell mutant lacking the mitochondrial ND4 gene product. EMBO J 12:3043–3048

Hofhaus G, Johns DR, Hurko O, Attardi G, Chomyn A (1996) respiration and growth defects in transmitochondrial cell lines carrying the 11778 mutation associated with Leber's hereditary optic neuropathy. J Biol Chem 271:13155–13161

Hofhaus G, Weiss H, Leonard K (1991) Electron microscopic analysis of the peripheral and membrane parts of mitochondrial NADH dehydrogenase (complex I). J Mol Biol 221:1027–1043

Horner DS, Foster PG, Embley TM (2000) Iron Hydrogenases and the Evolution of Anaerobic Eukaryotes. Mol Biol Evol 17:1695–1709

Hrdy I, Cammack R, Stopka P, Kulda J, Tachezy J (2005) Alternative pathway of metronidazole activation in *Trichomonas vaginalis* hydrogenosomes. Antimicrob Agents Chemother 49:5033–5036

Hrdy I, Hirt RP, Dolezal P, Bardonova L, Foster PG, Tachezy J, Martin Embley T (2004) Trichomonas hydrogenosomes contain the NADH dehydrogenase module of mitochondrial complex I. Nature 432:618–622

Jain M, Shrager J, Harris EH, Halbrook R, Grossman AR, Hauser C, Vallon O (2007) EST assembly supported by a draft genome sequence: an analysis of the *Chlamydomonas reinhardtii* transcriptome. Nucleic Acids Res 35:2074–2083

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

Joza N, Oudit GY, Brown D, Benit P, Kassiri Z, Vahsen N, Benoit L, Patel MM, Nowikovsky K, Vassault A, Backx PH, Wada T, Kroemer G, Rustin P, Penninger JM (2005) Muscle-specific loss of apoptosis-inducing factor leads to mitochondrial dysfunction, skeletal muscle atrophy, and dilated cardiomyopathy. Mol Cell Biol 25:10261–10272

Kao MC, Di Bernardo S, Nakamaru-Ogiso E, Miyoshi H, Matsuno-Yagi A, Yagi T (2005) Characterization of the membrane domain subunit NuoJ (ND6) of the NADH-quinone oxidoreductase from *Escherichia coli* by chromosomal DNA manipulation. Biochemistry 44:3562–3571

Kao MC, Di Bernardo S, Perego M, Nakamaru-Ogiso E, Matsuno-Yagi A, Yagi T (2004) Functional roles of four conserved charged residues in the membrane domain subunit NuoA of the proton-translocating NADH-quinone oxidoreductase from *Escherichia coli*. J Biol Chem 279:32360–32366

Karp CM, Shukla MN, Buckley DJ, Buckley AR (2007) HRPAP20: a novel calmodulin-binding protein that increases breast cancer cell invasion. Oncogene 26:1780–1788

Karpova OV, Newton KJ (1999) A partially assembled complex I in NAD4-deficient mitochondria of maize. Plant J 17:511–521

Kerscher S, Drose S, Zwicker K, Zickermann V, Brandt U (2002) *Yarrowia lipolytica*, a yeast genetic system to study mitochondrial complex I. Biochim Biophys Acta 1555:83–91

Kerscher S, Grgic L, Garofano A, Brandt U (2004) Application of the yeast *Yarrowia lipolytica* as a model to analyse human pathogenic mutations in mitochondrial complex I (NADH:ubiquinone oxidoreductase). Biochim Biophys Acta 1659:197–205

Kerscher S, Kashani-Poor N, Zwicker K, Zickermann V, Brandt U (2001a) Exploring the catalytic core of complex I by *Yarrowia lipolytica* yeast genetics. J Bioenerg Biomembr 33:187–196

Kerscher SJ, Eschemann A, Okun PM, Brandt U (2001b) External alternative NADH:ubiquinone oxidoreductase redirected to the internal face of the mitochondrial inner membrane rescues complex I deficiency in *Yarrowia lipolytica*. J Cell Sci 114:3915–3921

Kerscher SJ, Okun JG, Brandt U (1999) A single external enzyme confers alternative NADH:ubiquinone oxidoreductase activity in *Yarrowia lipolytica*. J Cell Sci 112(Pt 14):2347–2354

Kiefer D, Kuhn A (2007) YidC as an essential and multifunctional component in membrane protein assembly. Int Rev Cytol 259:113–138

Kirby DM, Crawford M, Cleary MA, Dahl HH, Dennett X, Thorburn DR (1999) Respiratory chain complex I deficiency: an underdiagnosed energy generation disorder. Neurology 52:1255–1264

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

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

Lee B-h, Lee H, Xiong L, Zhu J-K (2002) A mitochondrial complex I defect impairs cold-regulated nuclear gene expression. Plant Cell 14:1235–1251

Lezhneva L, Amann K, Meurer J (2004) The universally conserved HCF101 protein is involved in assembly of [4Fe-4S]-cluster-containing complexes in *Arabidopsis thaliana* chloroplasts. Plant J 37:174–185

Li Y, D'Aurelio M, Deng J-H, Park J-S, Manfredi G, Hu P, Lu J, Bai Y (2007) an assembled complex IV maintains the stability and activity of complex I in mammalian mitochondria. J Biol Chem 282:17557–17562

Loeffen JL, Smeitink JA, Trijbels JM, Janssen AJ, Triepels RH, Sengers RC, van den Heuvel LP (2000) Isolated complex I deficiency in children: clinical, biochemical and genetic aspects. Hum Mutat 15:123–134

Lu H, Cao X (2008) GRIM-19 is essential for maintenance of mitochondrial membrane potential. Mol. Biol. Cell:E07-07-0683

Lunardi J, Darrouzet E, Dupuis A, Issartel JP (1998) The nuoM arg368his mutation in NADH:ubiquinone oxidoreductase from Rhodobacter capsulatus: a model for the human nd4–11778 mtDNA mutation associated with Leber's hereditary optic neuropathy. Biochim Biophys Acta 1407:114–124

Ma J, Peng L, Guo J, Lu Q, Lu C, Zhang L (2007) LPA2 is required for efficient assembly of photosystem II in *Arabidopsis thaliana*. Plant Cell 19:1980–1993

Maeda S-i, Badger MR, Price GD (2002) Novel gene products associated with NdhD3/D4-containing NDH-1 complexes are involved in photosynthetic CO_2 hydration in the cyanobacterium, Synechococcus sp. PCC7942. Mol Microbiol 43:425–435

Malfatti E, Bugiani M, Invernizzi F, de Souza CF-M, Farina L, Carrara F, Lamantea E, Antozzi C, Confalonieri P, Sanseverino MT, Giugliani R, Uziel G, Zeviani M (2007) Novel mutations of ND genes in complex I deficiency associated with mitochondrial encephalopathy. Brain:awm114

Maximo V, Lima J, Soares P, Silva A, Bento I, Sobrinho-Simoes M (2008) GRIM-19 in health and disease. Adv Anat Pathol 15:46–53

Melo AM, Bandeiras TM, Teixeira M (2004) New insights into type II NAD(P) H:quinone oxidoreductases. Microbiol Mol Biol Rev 68:603–616

Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB, Terry A, Salamov A, Fritz-Laylin LK, Marechal-Drouard L, Marshall WF, Qu LH, Nelson DR, Sanderfoot AA, Spalding MH, Kapitonov VV, Ren Q, Ferris P, Lindquist E, Shapiro H, Lucas SM, Grimwood J, Schmutz J, Cardol P, Cerutti H, Chanfreau G, Chen CL, Cognat V, Croft MT, Dent R,

Dutcher S, Fernandez E, Fukuzawa H, Gonzalez-Ballester D, Gonzalez-Halphen D, Hallmann A, Hanikenne M, Hippler M, Inwood W, Jabbari K, Kalanon M, Kuras R, Lefebvre PA, Lemaire SD, Lobanov AV, Lohr M, Manuell A, Meier I, Mets L, Mittag M, Mittelmeier T, Moroney JV, Moseley J, Napoli C, Nedelcu AM, Niyogi K, Novoselov SV, Paulsen IT, Pazour G, Purton S, Ral JP, Riano-Pachon DM, Riekhof W, Rymarquis L, Schroda M, Stern D, Umen J, Willows R, Wilson N, Zimmer SL, Allmer J, Balk J, Bisova K, Chen CJ, Elias M, Gendler K, Hauser C, Lamb MR, Ledford H, Long JC, Minagawa J, Page MD, Pan J, Pootakham W, Roje S, Rose A, Stahlberg E, Terauchi AM, Yang P, Ball S, Bowler C, Dieckmann CL, Gladyshev VN, Green P, Jorgensen R, Mayfield S, Mueller-Roeber B, Rajamani S, Sayre RT, Brokstein P, Dubchak I, Goodstein D, Hornick L, Huang YW, Jhaveri J, Luo Y, Martinez D, Ngau WC, Otillar B, Poliakov A, Porter A, Szajkowski L, Werner G, Zhou K, Grigoriev IV, Rokhsar DS, Grossman AR (2007) The Chlamydomonas genome reveals the evolution of key animal and plant functions. Science 318:245–250

Meyer EH, Heazlewood JL, Millar AH (2007) Mitochondrial acyl carrier proteins in *Arabidopsis thaliana* are predominantly soluble matrix proteins and none can be confirmed as subunits of respiratory Complex I. Plant Mol Biol 64:319–327

Meyer EH, Taylor NL, Millar AH (2008) Resolving and identifying protein components of plant mitochondrial respiratory complexes using three dimensions of gel electrophoresis. J Proteome Res 7:786–794

Millar AH, Mittova V, Kiddle G, Heazlewood JL, Bartoli CG, Theodoulou FL, Foyer CH (2003) Control of Ascorbate Synthesis by Respiration and Its Implications for Stress Responses. Plant Physiol 133:443–447

Mueller EG (2006) Trafficking in persulfides: delivering sulfur in biosynthetic pathways. Nat Chem Biol 2:185–194

Murray J, Zhang B, Taylor SW, Oglesbee D, Fahy E, Marusich MF, Ghosh SS, Capaldi RA (2003) The subunit composition of the human NADH dehydrogenase obtained by rapid one-step immunopurification. J Biol Chem 278:13619–13622

Nakagawa N, Sakurai N (2006) A mutation in At-nMat1a, which encodes a nuclear gene having high similarity to group II intron maturase, causes impaired splicing of mitochondrial NAD4 transcript and altered carbon metabolism in *Arabidopsis thaliana*. Plant Cell Physiol 47:772–783

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

Nawathean P, Maslov DA (2000) The absence of genes for cytochrome c oxidase and reductase subunits in maxicircle kinetoplast DNA of the respiration-deficient plant trypanosomatid *Phytomonas serpens*. Curr Genet 38:95–103

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

Ohnishi T (1998) Iron–sulfur clusters/semiquinones in Complex I. Biochimica et Biophysica Acta (BBA)—Bioenergetics 1364:186–206

Ossenbuhl F, Inaba-Sulpice M, Meurer J, Soll J, Eichacker LA (2006) The synechocystis sp PCC 6803 oxa1 homolog is essential for membrane integration of reaction center precursor protein pD1. Plant Cell 18:2236–2246

Palmisano G, Sardanelli AM, Signorile A, Papa S, Larsen MR (2007) The phosphorylation pattern of bovine heart complex I subunits. Proteomics 7:1575–1583

Panigrahi AK, Zikova A, Dalley RA, Acestor N, Ogata Y, Anupama A, Myler PJ, Stuart KD (2008) Mitochondrial complexes in *Trypanosoma brucei*: a novel complex and a unique oxidoreductase complex. Mol Cell Proteomics 7:534–545

Papa S (2002) The NDUFS4 nuclear gene of complex I of mitochondria and the cAMP cascade. Biochim Biophys Acta 1555:147–153

Papa S, Sardanelli AM, Cocco T, Speranza F, Scacco SC, Technikova-Dobrova Z (1996) The nuclear-encoded 18 kDa (IP) AQDQ subunit of bovine heart complex I is phosphorylated by the mitochondrial cAMP-dependent protein kinase. FEBS Lett 379:299–301

Papa S, Scacco S, Sardanelli AM, Petruzzella V, Vergari R, Signorile A, Technikova-Dobrova Z (2002) Complex I and the cAMP cascade in human physiopathology. Biosci Rep 22:3–16

Parisi G, Perales M, Fornasari MS, Colaneri A, Gonzalez-Schain N, Gomez-Casati D, Zimmermann S, Brennicke A, Araya A, Ferry JG, Echave J, Zabaleta E (2004) Gamma carbonic anhydrases in plant mitochondria. Plant Mol Biol 55:193–207

Pätsi J, Kervinen M, Finel M, Hassinen IE (2008) Leber hereditary optic neuropathy mutations in the ND6 subunit of mitochondrial complex I affect ubiquinone reduction kinetics in a bacterial model of the enzyme. Biochem J 409:129–137

Peng G, Fritzsch G, Zickermann V, Schagger H, Mentele R, Lottspeich F, Bostina M, Radermacher M, Huber R, Stetter KO, Michel H (2003) Isolation, characterization and electron microscopic single particle analysis of the NADH:ubiquinone oxidoreductase (complex I) from the hyperthermophilic eubacterium Aquifex aeolicus. Biochemistry 42:3032–3039

Perales M, Eubel H, Heinemeyer J, Colaneri A, Zabaleta E, Braun HP (2005) Disruption of a nuclear gene encoding a mitochondrial gamma carbonic anhydrase reduces complex I and supercomplex I + III2 levels and alters mitochondrial physiology in Arabidopsis. J Mol Biol 350:263–277

Perales M, Parisi G, Fornasari MS, Colaneri A, Villarreal F, Gonzalez-Schain N, Echave J, Gomez-Casati D, Braun HP, Araya A, Zabaleta E (2004) Gamma carbonic anhydrase like complex interact with plant mitochondrial complex I. Plant Mol Biol 56:947–957

Peters K, Dudkina NV, Jansch L, Braun HP, Boekema EJ (2008) A structural investigation of complex I and I + III(2) supercomplex from Zea mays at 11–13 A resolution: assignment of the carbonic anhydrase domain and evidence for structural heterogeneity within complex I. Biochim Biophys Acta 1777(1):84–93

Piccoli C, Scacco S, Bellomo F, Signorile A, Iuso A, Boffoli D, Scrima R, Capitanio N, Papa S (2006) cAMP controls oxygen metabolism in mammalian cells. FEBS Lett 580:4539–4543

Pitkanen S, Feigenbaum A, Laframboise R, Robinson BH (1996) NADH-coenzyme Q reductase (complex I) deficiency: heterogeneity in phenotype and biochemical findings. J Inherit Metab Dis 19:675–686

Pla M, Mathieu C, De Paepe R, Chetrit P, Vedel F (1995) Deletion of the last two exons of the mitochondrial nad7 gene results in lack of the NAD7 polypeptide in a *Nicotiana sylvestris* CMS mutant. Mol Gen Genet 248:79–88

Plesofsky N, Gardner N, Videira A, Brambl R (2000) NADH dehydrogenase in *Neurospora crassa* contains myristic acid covalently linked to the ND5 subunit peptide. Biochimica et Biophysica Acta (BBA)—Molecular Cell Research 1495:223–230

Pocsfalvi G, Cuccurullo M, Schlosser G, Scacco S, Papa S, Malorni A (2007) Phosphorylation of B14.5a subunit from bovine heart complex I identified by titanium dioxide selective enrichment and shotgun proteomics. Mol Cell Proteomics 6:231–237

Prieur I, Lunardi J, Dupuis A (2001) Evidence for a quinone binding site close to the interface between NUOD and NUOB subunits of Complex I. Biochim Biophys Acta 1504:173–178

Qi X, Lewin AS, Hauswirth WW, Guy J (2003) Suppression of complex I gene expression induces optic neuropathy. Ann Neurol 53:198–205

Qi X, Lewin AS, Sun L, Hauswirth WW, Guy J (2004) SOD2 gene transfer protects against optic neuropathy induced by deficiency of complex I. Ann Neurol 56:182–191

Rebeille F, Alban C, Bourguignon J, Ravanel S, Douce R (2007) The role of plant mitochondria in the biosynthesis of coenzymes. Photosynth Res 92:149–162

Reeves MB, Davies AA, McSharry BP, Wilkinson GW, Sinclair JH (2007) Complex I binding by a virally encoded RNA regulates mitochondria-induced cell death. Science 316:1345–1348

Reinders J, Wagner K, Zahedi RP, Stojanovski D, Eyrich B, van der Laan M, Rehling P, Sickmann A, Pfanner N, Meisinger C (2007) Profiling phosphoproteins of yeast mitochondria reveals a role of phosphorylation in assembly of the ATP synthase. Mol Cell Proteomics 6:1896–1906

Remacle C, Baurain D, Cardol P, Matagne RF (2001a) Mutants of *Chlamydomonas reinhardtii* deficient in mitochondrial complex I: characterization of two mutations affecting the nd1 coding sequence. Genetics 158:1051–1060

Remacle C, Cardol P, Coosemans N, Gaisne M, Bonnefoy N (2006) High-efficiency biolistic transformation of Chlamydomonas mitochondria can be used to insert mutations in complex I genes. Proc Natl Acad Sci USA 103:4771–4776

Remacle C, Duby F, Cardol P, Matagne RF (2001b) Mutations inactivating mitochondrial genes in *Chlamydomonas reinhardtii*. Biochem Soc Trans 29:442–446

Ruiz-Pesini E, Lott MT, Procaccio V, Poole JC, Brandon MC, Mishmar D, Yi C, Kreuziger J, Baldi P, Wallace DC (2007) An enhanced MITOMAP with a global mtDNA mutational phylogeny. Nucl Acids Res 35:D823–D828

Saada A, Edvardson S, Rapoport M, Shaag A, Amry K, Miller C, Lorberboum-Galski H, Elpeleg O (2008) C6ORF66 is an assembly factor of mitochondrial complex I. Am J Hum Genet 82:32–38

Sackmann U, Zensen R, Rohlen D, Jahnke U, Weiss H (1991) The acyl-carrier protein in *Neurospora crassa* mitochondria is a subunit of NADH:ubiquinone reductase (complex I). Eur J Biochem 200:463–469

Sardanelli AM, Technikova-Dobrova Z, Scacco SC, Speranza F, Papa S (1995) Characterization of proteins phosphorylated by the cAMP-dependent protein kinase of bovine heart mitochondria. FEBS Lett 377:470–474

Sazanov LA (2007) Respiratory complex I: mechanistic and structural insights provided by the crystal structure of the hydrophilic domain. Biochemistry 46:2275–2288

Sazanov LA, Hinchliffe P (2006) Structure of the hydrophilic domain of respiratory complex I from *Thermus thermophilus*. Science 311:1430–1436

Sazanov LA, Peak-Chew SY, Fearnley IM, Walker JE (2000) Resolution of the membrane domain of bovine complex I into subcomplexes: implications for the structural organization of the enzyme. Biochemistry 39:7229–7235

Sazanov LA, Walker JE (2000) Cryo-electron crystallography of two sub-complexes of bovine complex I reveals the relationship between the membrane and peripheral arms. J Mol Biol 302:455–464

Scacco S, Vergari R, Scarpulla RC, Technikova-Dobrova Z, Sardanelli A, Lambo R, Lorusso V, Papa S (2000) cAMP-dependent phosphorylation of the nuclear encoded 18-kDa (IP) subunit of respiratory complex I and activation of the complex in serum-starved mouse fibroblast cultures. J Biol Chem 275:17578–17582

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

Schagger H, de Coo R, Bauer MF, Hofmann S, Godinot C, Brandt U (2004) Significance of respirasomes for the assembly/stability of human respiratory chain complex I. J Biol Chem 279:36349–36353

Scheffler IE, Yadava N, Potluri P (2004) Molecular genetics of complex I-deficient Chinese hamster cell lines. Biochimica et Biophysica Acta (BBA)—Bioenergetics 1659:160–171

Schilling B, Aggeler R, Schulenberg B, Murray J, Row RH, Capaldi RA, Gibson BW (2005) Mass spectrometric identification of a novel phosphorylation site in subunit NDUFA10 of bovine mitochondrial complex I. FEBS Lett 579:2485–2490

Schneider R, Massow M, Lisowsky T, Weiss H (1995) Different respiratory-defective phenotypes of *Neurospora crassa* and *Saccharomyces cerevisiae* after inactivation of the gene encoding the mitochondrial acyl carrier protein. Curr Genet 29:10–17

Schroda M (2006) RNA silencing in Chlamydomonas: mechanisms and tools. Curr Genet 49:69–84

Schulenberg B, Aggeler R, Beechem JM, Capaldi RA, Patton WF (2003) Analysis of steady-state protein phosphorylation in mitochondria using a novel fluorescent phosphosensor dye. J Biol Chem 278:27251–27255

Schuler F, Casida JE (2001) Functional coupling of PSST and ND1 subunits in NADH:ubiquinone oxidoreductase established by photoaffinity labeling. Biochim Biophys Acta 1506:79–87

Schuler F, Yano T, Di Bernardo S, Yagi T, Yankovskaya V, Singer TP, Casida JE (1999) NADH-quinone oxidoreductase: PSST subunit couples electron transfer from iron–sulfur cluster N2 to quinone. Proc Natl Acad Sci USA 96:4149–4153

Schulte U (2001) Biogenesis of respiratory complex I. J Bioenerg Biomembr 33:205–212

Schulte U, Fecke W, Krull C, Nehls U, Schmiede A, Schneider R, Ohnishi T, Weiss H (1994) In vivo dissection of the mitochondrial respiratory NADH: ubiquinone oxidoreductase (complex I). Biochim Biophys Acta 1187:121–124

Sellem CH, Lemaire C, Lorin S, Dujardin G, Sainsard-Chanet A (2005) Interaction between the oxa1 and rmp1 genes modulates respiratory complex assembly and life span in *Podospora anserina*. Genetics 169:1379–1389

Seo T, Lee D, Shim YS, Angell JE, Chidambaram NV, Kalvakolanu DV, Choe J (2002) Viral interferon regulatory factor 1 of Kaposi's Sarcoma-associated herpesvirus interacts with a cell death regulator, GRIM19, and inhibits interferon/retinoic acid-induced cell death. J Virol 76:8797–8807

Skladal D, Halliday J, Thorburn DR (2003) Minimum birth prevalence of mitochondrial respiratory chain disorders in children. Brain 126:1905–1912

Smeitink J, Sengers R, Trijbels F, van den Heuvel L (2001) Human NADH:ubiquinone oxidoreductase. J Bioenerg Biomembr 33:259–266

Smith KS, Ferry JG (2000) Prokaryotic carbonic anhydrases. FEMS Microbiol Rev 24:335–366

Steele DF, Butler CA, Fox TD (1996) Expression of a recoded nuclear gene inserted into yeast mitochondrial DNA is limited by mRNA-specific translational activation. Proc Natl Acad Sci USA 93:5253–5257

Stephens JL, Lee SH, Paul KS, Englund PT (2007) Mitochondrial fatty acid synthesis in *Trypanosoma brucei*. J Biol Chem 282:4427–4436

Stiburek L, Fornuskova D, Wenchich L, Pejznochova M, Hansikova H, Zeman J (2007) Knockdown of human Oxa1 l impairs the biogenesis of F1Fo-ATP synthase and NADH:ubiquinone oxidoreductase. J Mol Biol 374:506–516

Sunderhaus S, Dudkina NV, Jansch L, Klodmann J, Heinemeyer J, Perales M, Zabaleta E, Boekema EJ, Braun HP (2006) Carbonic anhydrase subunits form a matrix-exposed domain attached to

the membrane arm of mitochondrial complex I in plants. J Biol Chem 281:6482–6488

Tocilescu MA, Fendel U, Zwicker K, Kerscher S, Brandt U (2007) Exploring the ubiquinone binding cavity of respiratory complex I. J Biol Chem 282:29514–29520

Touraine B, Boutin J-P, Marion-Poll A, Briat J-F, Peltier G, Lobreaux S (2004) Nfu2: a scaffold protein required for [4Fe-4S] and ferredoxin iron–sulphur cluster assembly in Arabidopsis chloroplasts. Plant J 40:101–111

Triepels RH, Van Den Heuvel LP, Trijbels JM, Smeitink JA (2001) Respiratory chain complex I deficiency. Am J Med Genet 106:37–45

Tripp BC, Bell CB 3rd, Cruz F, Krebs C, Ferry JG (2004) A role for iron in an ancient carbonic anhydrase. J Biol Chem 279:6683–6687

Trounce IA, Pinkert CA (2007) Cybrid models of mtDNA disease and transmission, from cells to mice. Curr Top Dev Biol 77:157–183

Tsang WY, Sayles LC, Grad LI, Pilgrim DB, Lemire BD (2001) Mitochondrial respiratory chain deficiency in *Caenorhabditis elegans* results in developmental arrest and increased life span. J Biol Chem 276:32240–32246

Tsuneoka M, Teye K, Arima N, Soejima M, Otera H, Ohashi K, Koga Y, Fujita H, Shirouzu K, Kimura H, Koda Y (2005) A novel Myc-target gene, mimitin, that is involved in cell proliferation of esophageal squamous cell carcinoma. J Biol Chem 280:19977–19985

Tuschen G, Sackmann U, Nehls U, Haiker H, Buse G, Weiss H (1990) Assembly of NADH: ubiquinone reductase (complex I) in Neurospora mitochondria. Independent pathways of nuclear-encoded and mitochondrially encoded subunits. J Mol Biol 213:845–857

Ugalde C, Janssen RJ, van den Heuvel LP, Smeitink JA, Nijtmans LG (2004a) Differences in assembly or stability of complex I and other mitochondrial OXPHOS complexes in inherited complex I deficiency. Hum Mol Genet 13:659–667

Ugalde C, Vogel R, Huijbens R, Van Den Heuvel B, Smeitink J, Nijtmans L (2004b) Human mitochondrial complex I assembles through the combination of evolutionary conserved modules: a framework to interpret complex I deficiencies. Hum Mol Genet 13:2461–2472

Vahsen N, Cande C, Briere JJ, Benit P, Joza N, Larochette N, Mastroberardino PG, Pequignot MO, Casares N, Lazar V, Feraud O, Debili N, Wissing S, Engelhardt S, Madeo F, Piacentini M, Penninger JM, Schagger H, Rustin P, Kroemer G (2004) AIF deficiency compromises oxidative phosphorylation. EMBO J 23:4679–4689

van Lis R, Atteia A, Mendoza-Hernandez G, Gonzalez-Halphen D (2003) Identification of novel mitochondrial protein components of *Chlamydomonas reinhardtii*. A proteomic approach. Plant Physiol 132:318–330

Videira A, Duarte M (2002) From NADH to ubiquinone in Neurospora mitochondria. Biochim Biophys Acta 1555:187–191

Vogel R, Nijtmans L, Ugalde C, van den Heuvel L, Smeitink J (2004) Complex I assembly: a puzzling problem. Curr Opin Neurol 17:179–186

Vogel RO, Dieteren CE, van den Heuvel LP, Willems PH, Smeitink JA, Koopman WJ, Nijtmans LG (2007a) Identification of mitochondrial complex I assembly intermediates by tracing tagged NDUFS3 demonstrates the entry point of mitochondrial subunits. J Biol Chem 282:7582–7590

Vogel RO, Janssen RJ, Ugalde C, Grovenstein M, Huijbens RJ, Visch HJ, van den Heuvel LP, Willems PH, Zeviani M, Smeitink JA, Nijtmans LG (2005) Human mitochondrial complex I assembly is mediated by NDUFAF1. FEBS J 272:5317–5326

Vogel RO, Janssen RJRJ, van den Brand MAM, Dieteren CEJ, Verkaart S, Koopman WJH, Willems PHGM, Pluk W, van den Heuvel LPWJ, Smeitink JAM, Nijtmans LGJ (2007b) Cytosolic signaling protein Ecsit also localizes to mitochondria where it interacts with chaperone NDUFAF1 and functions in complex I assembly. Genes Dev 21:615–624

Vogel RO, Smeitink JAM, Nijtmans LGJ (2007c) Human mitochondrial complex I assembly: a dynamic and versatile process. Biochimica et Biophysica Acta (BBA)—Bioenergetics 1767:1215–1227

Vogel RO, van den Brand MAM, Rodenburg RJ, van den Heuvel LPWJ, Tsuneoka M, Smeitink JAM, Nijtmans LGJ (2007d) Investigation of the complex I assembly chaperones B17.2L and NDUFAF1 in a cohort of CI deficient patients. Mol Genet Metab 91:176–182

Yadava N, Scheffler IE (2004) Import and orientation of the MWFE protein in mitochondrial NADH-ubiquinone oxidoreductase. Mitochondrion 4:1–12

Yagi T, Matsuno-Yagi A (2003) The proton-translocating NADH-quinone oxidoreductase in the respiratory chain: the secret unlocked. Biochemistry 42:2266–2274

Yano T, Sled VD, Ohnishi T, Yagi T (1996) Expression and characterization of the flavoprotein subcomplex composed of 50-kDa (NQO1) and 25-kDa (NQO2) subunits of the proton-translocating NADH-quinone oxidoreductase of *Paracoccus denitrificans*. J Biol Chem 271:5907–5913

Zee JM, Glerum DM (2006) Defects in cytochrome oxidase assembly in humans: lessons from yeast. Biochem Cell Biol 84:859–869

Zensen R, Husmann H, Schneider R, Peine T, Weiss H (1992) De novo synthesis and desaturation of fatty acids at the mitochondrial acyl-carrier protein, a subunit of NADH:ubiquinone oxidoreductase in *Neurospora crassa*. FEBS Lett 310:179–181

Zhang J, Yang J, Roy SK, Tininini S, Hu J, Bromberg JF, Poli V, Stark GR, Kalvakolanu DV (2003) The cell death regulator GRIM-19 is an inhibitor of signal transducer and activator of transcription 3. Proc Natl Acad Sci USA 100:9342–9347

Zickermann V, Barquera B, Wikstrom M, Finel M (1998) Analysis of the pathogenic human mitochondrial mutation ND1/3460, and mutations of strictly conserved residues in its vicinity, using the bacterium *Paracoccus denitrificans*. Biochemistry 37:11792–11796