Chapter 11
Complexes I in the Green Lineage

Claire Remacle, Patrice Hamel, Véronique Larosa, Nitya Subrahmanian, and Pierre Cardol

Abstract In land plants and green algae, mitochondria and chloroplasts were acquired sequentially through primary endosymbiotic events with a α-proteobacterium and a cyanobacterium, respectively. The inner membrane of the mitochondria harbors the enzyme complexes of the respiratory chain, the largest of them being the rotenone-sensitive NADH:ubiquinone oxidoreductase or complex I. In the thylakoid membrane of the chloroplast, besides the photosynthetic machinery, a light-independent respiratory-chain inherited from cyanobacteria drives electrons from NAD(P)H to oxygen. In most plants and algae, it comprises a homolog of bacterial complex I (NAD(P)H:plastoquinone (PQ) oxidoreductase) and a PQ oxidase (PTOX). This chapter will be thus dedicated to similarities and peculiarities of plant mitochondrial complex I compared to the well studied enzyme in mammals and fungi, as well as to the structure and role of a complex I homolog in chloroplast.

Keywords Alga • Carbonic anhydrase • Chloroplast • Higher plant • Mutant

C. Remacle (✉) • V. Larosa • P. Cardol
Laboratory of Genetics of Microorganisms, Institute of Botany B22,
University of Liège, B-4000 Liège, Belgium
e-mail: c.remacle@ulg.ac.be

P. Hamel (✉) • N. Subrahmanian
Department of Molecular Genetics and Department of Molecular
and Cellular Biochemistry, 500 Aronoff Laboratory,
318 W 12th Ave., Columbus, OH 43210, USA

Plant Cellular and Molecular Biology Graduate Program, The Ohio State University,
Columbus, OH, USA
e-mail: hamel.16@osu.edu

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11.1 The Mitochondrial Complex I in Plants

11.1.1 Subunit Composition (Table 11.1)

The subunit composition of mitochondrial complex I has been intensively studied in the land plant model *Arabidopsis thaliana* and in the Chlorophycean green alga *Chlamydomonas reinhardtii*. In *Chlamydomonas*, the mitochondrial respiratory-chain complexes were separated by blue native (BN) gel electrophoresis from purified mitochondria (van Lis et al. 2003; Cardol et al. 2004). With an apparent molecular mass between 950 and 1,000 kD, complex I was then resolved into its constitutive subunits in a second dimensional SDS-gel and 30 components ranging from 7 to 77 kD could be subsequently identified by mass spectrometry analyses (Cardol et al. 2004). By searching for putative homologs of fungal (*e.g.* Videira and Duarte 2002) and mammalian (Carroll et al. 2002; Hirst et al. 2003) complex I subunits, the *Chlamydomonas* enzyme was thought to comprise at least 42 proteins. In the land plants *Vicia faba* (broad bean), *Solanum tuberosum* (potato), and *Triticum aestivum* (wheat), pioneer work in the 1990s has shown that the complex I enzyme comprised up to 30 subunits ranging from 6 to 75 kD but only few subunits were identified (Leterme and Boutry 1993; Herz et al. 1994; Combettes and Grienenberger 1999). A few years later, a similar approach to the one described for *Chlamydomonas* led to the identification of at least 39 subunits in *Arabidopsis* and in *Oryza sativa* (rice), ten of which appeared to be specific to land plants (Heazlewood et al. 2003). More recently, new attempts to characterize complex I subunit composition in *Arabidopsis* have been undertaken. A third dimensional gel electrophoresis (BN/SDS/SDS) approach developed to study complex I in the fungus *Yarrowia lipolytica* (Abdrakhmanova et al. 2004) was applied to *Arabidopsis* and enabled the subsequent identification of 42 different subunits (Meyer et al. 2008). Finally, a three step purification procedure allowed the recovery of a complex I fraction containing 49 subunits, among which one third were described as unique to plants (Klodmann et al. 2010). This procedure included (i) a mitochondrial membrane isolation step, (ii) the separation of enzymes complexes by sucrose gradient centrifugation and (iii) a cytochrome *c* affinity chromatography to remove complex III or supercomplexes. Compared to previous protocols published for the isolation of complex I from various plants that included a chromatography step (*e.g.* Leterme and Boutry 1993; Combettes and Grienenberger 1999), this procedure avoids high salt conditions and is expected, according to the authors, to preserve the integrity of complex I. Most novel subunits identified in plant complex I are small hydrophobic proteins that are probably part of the membrane domain (Klodmann et al. 2010). Some of them are highly divergent orthologs of small hydrophobic supernumerary subunits found in fungi or mammals (Gabaldon et al. 2005; Carroll et al. 2006; Morgner et al. 2008; Bridges et al. 2010; Cardol 2011). One should note that over the years the number of subunits that were identified as conserved between mammals, fungi and plants increased: 27 in 2003 (Heazlewood et al. 2003), 32–33 in 2005 (Cardol et al. 2004; Gabaldon et al. 2005), 34–37 in 2009/2010 (Huynen et al. 2009; Klodmann et al. 2010), and 41 in 2011 (Cardol 2011). This gradual discovery was fueled by the progressive

Table 11.1 Protein components of plant mitochondrial complex I

	Arabidopsis thaliana	*Chlamydomonas reinhardtii*	Mammals[e]	Fungi[f]
	Bacterial core			
1	At5g11770[a,b,c]	AAQ63698[d]	NDUFS7/PSST	NUO19.3/NUKM
2	At1g16700, At1g79010[a,b,c]	AAQ63697[d]	NDUFS8/TYKY	NUO21.3c/NUIM
3	At4g02580[a,b,c]	AAQ63695[d]	NDUFV2/24 kD	NUO24/NUHM
4	AtMg00070[a,b,c]	AAQ55457[d]	NDUFS3/30 kD	NUO30.4 (31)/NUGM
5	AtMg00510[a,b,c]	AAQ63700[d]	NDUFS2/49 kD	NUO49/NUCM
6	At5g08530[a,b,c]	AAQ63696[d]	NDUFV1/51 kD	51/NUBM
7	At5g37510[a,b,c]	AAQ73136[d]	NDUFS1/75 kD	NUO78/NUAM
8	AtMg00516[a,b,c]	AAB93446	ND1	ND1/NU1M
9	AtMg00285[b,c]	AAB93444	ND2	ND2/NU2M
10	AtMg00990[c]	AAQ55461[d]	ND3	ND3/NU3M
11	AtMg00580[b,c]	AAB93441	ND4	ND4/NU4M
12	AtMg00650	AAO61142	ND4L	ND4L/NULM
13	AtMg00513[a,b,c]	AAB93442	ND5	ND5/NU5M
14	AtMg00270[b]	AAB93445	ND6	ND6/NU6M
	Conserved supernumerary			
15	At3g08610[a,b,c]	AAS48198	NDUFA1/MWFE	NUO9.8/NIMM
16	At5g47890[b,c]	AAQ63699[d]	NDUFA2/B8	NUO10.5/NI8M
17	At2g02510[b,c]	AAS48194[d]	NDUFB3/B12	NUO10.6/NB2M
18	At5g52840[a,b,c]	AAQ73139[d]	NDUFA5/B13	NUO29.9/NUFM
19	At3g03070[c]	AAQ64639[d]	NDUFS6/13 kD A	NUO18.4/NUMM
20	At3g12260[a,b]	AAQ84469[d]	NDUFA6/B14	NUO14.8/NB4M
21	At2g42210[b]	AAS58499[d]	NDUFA11/B14.7	NUO21.3b/NUJM
22	At3g57785, At2g42310[a,b,c]	AAS48192[d]	NDUFB11/ESSS	NUO11.7/NUWM
23	At3g62790, At2g47690[a,b,c]	AAQ98888	NDUFS5/PFFD	NUO11.5/NIPM
24	At2g31490[a,b,c]	AAS48193[d]	NDUFB4/B15	NUO6.6/NUVM

(continued)

Table 11.1 (continued)

	Arabidopsis thaliana	*Chlamydomonas reinhardtii*	Mammals[e]	Fungi[f]
25	At1g04630, At2g33220[a,b,c]	AAQ64637[d]	NDUFA13/B16.6	NUO14 (13.5)/NB6M
26	At3g03100[a,b]	AAQ64638[d]	NDUFA12/B17.2	NUO13.4/N7BM
27	At2g02050[a,b,c]	AAQ73135[d]	NDUFB7/B18	NB8M
28	At5g67590[a,b]	AAQ64640[d]	NDUFS4/AQDQ	NUO21/NUYM
29	At5g18800, At3g06310[b,c]	AAQ55460	NDUFA8/PGIV	NUO20.8/NUPM
30	At4g34700	AAQ73134	NDUFB9/B22	NI2M
31	At1g49140, At3g18410[a,b,c]	AAQ55459[d]	NDUFB10/PDSW	NUO12.3/NIDM
32	At2g20360[a,b,c]	AAQ55458[d]	NDUFA9/39 kD	NUO40/NUEM
33	At5g47570[b,c]	XP_001700273	NDUFB8/ASHI	NUO20.1/NIAM
34	At1g76200[b,c]	–	NDUFB2/AGGG	NCU01436[g]
35	At4g16450[a,b,c]	AAQ64641[d]	NDUFB1/MNLL	NUO20.9/NUXM
36	At4g20150 (NDU9)[a,b,c]	AAS58501[d]	NDUFC2/B14.5B	NUO10.4
37	At4g00585[b,c]	XP_001697243[h]	NDUFC1/KFYI	NCU08300[g]/NUUM
38	At2g46540[h]	XP_001692978[h]	NDUFA3/B9	NUO9.5/NI9M
39	AAM6246[h]	AAQ73138[h]	NDUFAB1/ACPM	SDAP
40	At5g08060[h]	XP_001703194[h]	NDUFA7/B14.5A	NCU08930[g]/NUZM
41	At3g29970[h]	–	NDUFA4/MLRQ	NCU02016[h]
42	At1g67785[b,c]	–	NDUFB5/SGDH	NUO17.8
43	AAG51141[h]	–	NDUFA10/42 kD	–
44	At3g47930[a,c] L-galactono-1·4-lactone dehydrogenase	EDP08950[h]	XP_001253523[h]	NCU03188[g]
	Plant specific			
45	γ-carbonic anhydrase At5g63510, At1g19580, At3g48680· At1g47260, At5g66510[a,b,c]	AAS48196, AAS48197, AAS48195[d]	–[g]	–[g]
46	At3g07480[h]	AAS58502[d] ferredoxin-like	–	–
47	At5g14105[c]	–	–	–

48	At1g67350[b,c]	–	–	–
49	At1g68680[b]	–	–	
50	At2g27730[a,b,c]	–	–	
51	–	AAS58503[d]	–	
52	–	AAS58498[d]	–	

[a,b,c,d]Subunits identified by mass spectrometry as complex I components (Heazlewood et al. 2003; Meyer et al. 2008; Klodmann et al. 2010; Cardol et al. 2004)

[e]Accession numbers of mammalian sequences can be found in (*e.g.* Carroll et al. 2006; Hirst et al. 2003)

[f]Accession numbers for fungal sequences can be found for *Y. lipolytica* (Abdrakhmanova et al. 2004), *N. crassa* (Marques et al. 2005) and *Pichia pastoris* (Bridges et al. 2010)

[g]Although such proteins are absent from mammalian and fungal complex I, homologs are found in other eukaryotes lineages such as *Amoebozoa* (Gawryluk and Gray 2010), see text for further details

[h]Not identified by biochemical approaches as complex I component

acquisition of proteomic and genomic data in various organisms in combination to the availability of profile-to-sequence and profile-to-profile comparisons tools. At this stage, additional efforts are still needed to characterize the composition of mitochondrial complex I in distant species in order to highlight real lineage-specific or conserved subunits and decipher their role.

Nonetheless, all eukaryotic complex I enzymes investigated to date comprise approximately 25 non-core subunits, in addition to the 14/15 core subunits present in the bacterial enzyme. These additional proteins, the so-called supernumerary (or accessory) subunits, are presumed to participate in assembly, stability, or regulation, rather than in enzyme activity (*e.g.* Friedrich et al. 1998; Heazlewood et al. 2003; Marques et al. 2005; Abdrakhmanova et al. 2006; Bridges et al. 2010). Another role for non-core subunits as regulatory molecules outside the mitochondrial compartment is also emerging, as exemplified for the GRIM-19 subunit (Gene associated with Retinoic-IFN- induced mortality 19). This nucleus located protein, originally identified as a critical regulatory protein for interferon-beta and retinoic acid induced cell death (Angell et al. 2000), also localizes to mitochondrial human complex I. A GRIM19 homolog associated to complex I has been found in the green lineage (Heazlewood et al. 2003; Cardol et al. 2004) and in fungi (Abdrakhmanova et al. 2004; Bridges et al. 2010, reviewed in Remacle et al. 2008).

In *Arabidopsis*, five proteins structurally related to bacterial gamma-type carbonic anhydrases (γ-CA) have been assigned to the membrane arm of complex I (Parisi et al. 2004; Perales et al. 2004; Sunderhaus et al. 2006) while three were found in association with *Chlamydomonas* complex I (Cardol et al. 2004). Single particle electron microscopy analysis of complex I from *Polytomella* (a chloroplastless close relative of *Chlamydomonas*), *Arabidopsis*, *Zea mays* and *S. tuberosum* indicate that these γ-CA subunits could constitute a spherical domain attached to the central part of the membrane arm of complex I and exposed to the matrix (Fig. 11.1a) (Perales et al. 2005; Sunderhaus et al. 2006; Peters et al. 2008; Bultema et al. 2009). In *Arabidopsis*, γ-CA1 and γ-CA2 have also been shown to be important for complex I assembly and possibly involved in mitochondrial one-carbon metabolism (Perales et al. 2005). Overexpression of γ-CA2 in *Arabidopsis* leads to a male sterile phenotype (Villarreal et al. 2009). More recent experiments indicated that γ-CA2 trimers are capable of binding inorganic carbon (Martin et al. 2009). Since these subunits were believed to be plant-specific, it was postulated that this complex I domain might play a role in relationship to photosynthesis. However, two γ-CA were recently found in association with complex I in the amoeboid protozoon *Acanthamoeba castellanii* (Gawryluk and Gray 2010), which does not have a chloroplast and is considered to be a sister group to opisthokonts, a group of eukaryotes including metazoa and fungi (Keeling et al. 2005). γ-CA subunits are also encoded in the genomes of most eukaryotes (including the non-photosynthetic alga *Polytomella*), with the exception of opisthokonts (Gawryluk and Gray 2010). Altogether these findings suggest that this γ-CA module was lost during evolution of the opisthokont lineage and that it might play a more general role in complex I function in other eukaryotes (see Gawryluk and Gray 2010 for further discussion). In *Chlamydomonas*, a small subunit (AAS58502) was identified in complex I from

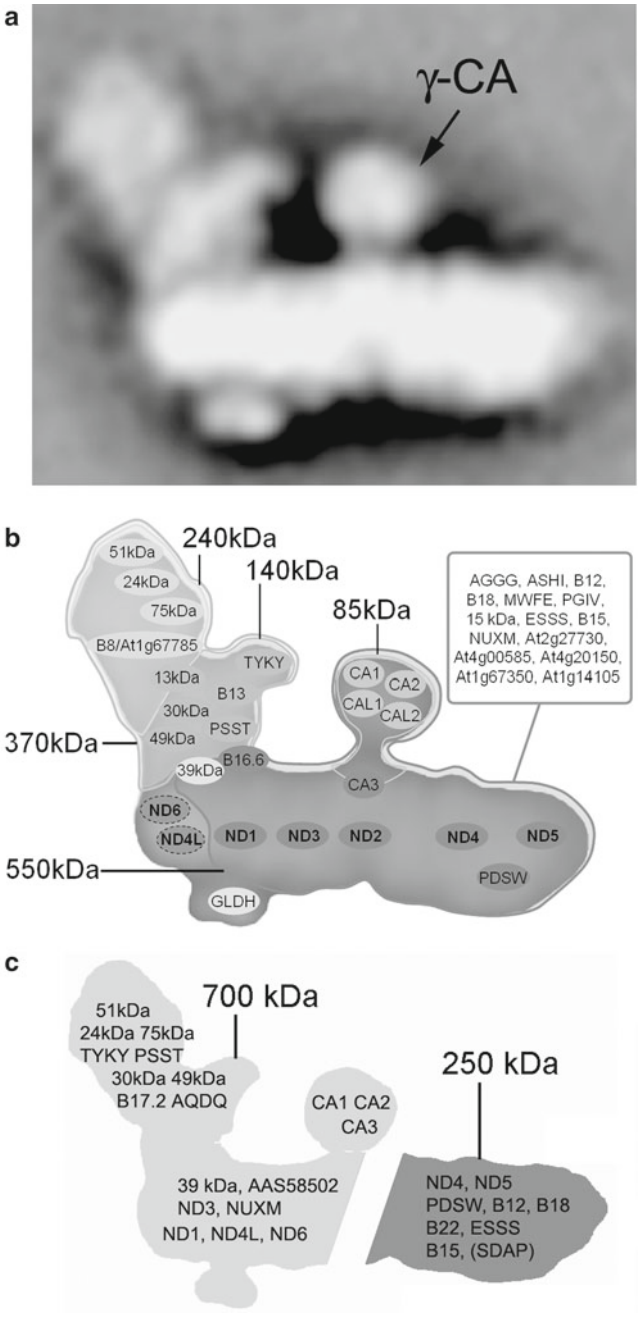

Fig. 11.1 Overall structure and subunit localization of plant mitochondrial complex I. (**a**) Projection map from *A. thaliana* complex I obtained by EM single-particle analysis (From Sunderhaus et al. 2006, Courtesy H.P. Braun and E.J. Boekema) The carbonic anhydrase domain is annotated (γ-CA). (**b**) Schematic model representation of complex I from *A. thaliana* (Modified from Klodmann et al. 2010, Courtesy H.P. Braun). (**c**) Schematic model representation of complex I from *C. reinhardtii* (Adapted from Cardol et al. 2008. With permission from Elsevier). Outline shape side-views are drawn from (Sunderhaus et al. 2006)

wild-type (Cardol et al. 2004) and in a subcomplex lacking the distal part of the membrane arm (Cardol et al. 2008). This subunit has putative homologs in land plants and shares also some similarities with ferredoxin and ferredoxin-like proteins from various sources. It was hypothesized that recruitment of a ferredoxin-like protein within plant complex I could drive electrons to the carbonic anhydrase domain (Cardol et al. 2008). But the lack of evidence of the association of such a protein with *Arabidopsis* complex I (Meyer et al. 2008; Klodmann et al. 2010) does not support this view.

In *Arabidopsis*, L -galactono-1,4-lactone dehydrogenase (GLDH) is also a structural component of complex I (Heazlewood et al. 2003; Klodmann et al. 2010). GLDH catalyses the final oxidation of galactono-γ−lactone to ascorbic acid (Ostergaard et al. 1997; Siendones et al. 1999). Its activity decreases with leaf age and is modulated by the availability of oxidized cytochrome *c* (Bartoli et al. 2000). Ascorbate, the major soluble antioxidant and redox buffer in mammals and plants, plays important roles in plant development, photoprotection, and cell expansion (*e.g.* Smirnoff 2000; Arrigoni and De Tullio 2002). Complex I from *Arabidopsis* occurs in two forms that are distinguished on the basis of their different mobility in BN-PAGE. Only the high-mobility form contains GLDH and bears an ascorbate synthesis activity that is sensitive to rotenone (Heazlewood et al. 2003). These observations pointed out towards the existence of a subpopulation of complex I whose function could regulate ascorbate synthesis by monitoring the rate of NADH-driven electron flow through complex I (Millar et al. 2003; Pineau et al. 2008). A homozygous T-DNA *Arabidopsis* mutant, deficient for GLDH, developed only when supplemented with ascorbate and was impaired in complex I content (Pineau et al. 2008). There is a GLDH protein homolog encoded in *Chlamydomonas* (Genbank accession number EDP08950), and its possible association with complex I remains to be explored.

A small fraction of the mitochondrial acyl carrier proteins (ACP) involved in the synthesis of type II fatty acids, localizing primarily in the mitochondrial matrix (Cronan et al. 2005), is associated with mammal and fungal complex I (Zensen et al. 1992; Triepels et al. 1999; Carroll et al. 2003, 2005; Schilling et al. 2005; Hinttala et al. 2005). As a consequence, depletion of mitochondrial ACP in these organisms leads to complex I impairment, in addition to a defect in fatty acid biosynthesis (Schneider et al. 1995; Feng et al. 2009; Dobrynin et al. 2010). By similarity to complex I composition in mammals and fungi, one ACP subunit has been proposed to be a part of complex I in plants and algae (Heazlewood et al. 2003; Cardol et al. 2004). In *Arabidopsis*, it has been further shown that five mitochondrial ACP isoforms exist but ACP activity occurs predominantly as two soluble isoforms (mtACP1 and mtACP2) in the mitochondrial matrix (Meyer et al. 2007). However, recent proteomic analyses of *Arabidopsis* complex I failed to detect the presence of any of the five mitochondrial ACPs (Meyer et al. 2008; Klodmann et al. 2010). Thus ACP does not seem to be a *bona fide* complex I subunit in plants and its role in complex I biogenesis awaits further experimental testing.

11.1.2 Genetic Studies

In vascular plants, the consequences of complex I defects have been mainly studied in three experimental models, *A. thaliana*, *Nicotiana sylvestris* and *Z. mays*. Mutants in nuclear genes encoding complex I subunits in *Arabidopsis* include the plant specific γ-CA2 subunit (Perales et al. 2005) discussed previously and the eukaryotic specific 18-kDa subunit (NDUFS4). Unexpectedly, this latter mutant has been isolated from a mutagenized population screened for altered responses to different stress conditions (Ishitani et al. 1997). The mutant exhibited a reduced capacity for cold acclimation and increased superoxide production, although the impact on complex I activity and assembly was not investigated (Lee et al. 2002). A T-DNA linked mutation in the same nuclear gene was then further studied (Meyer et al. 2009). This insertional mutant showed no assembly or activity of complex I and delayed germination and growth. Comparative mitochondrial proteome analysis showed that modification was restricted to the abundance of complex I subunits without significant changes in other mitochondrial proteins. Metabolite changes predominated at night and ATP level was also lower in the dark period. Overall, the decreased efficiency of ATP production by OXPHOS lead to broad rearrangements in cellular metabolism and development and to altered tolerance to abiotic stresses.

Mitochondrial mutants lacking some of the *nd* genes as a result of genome rearrangements have been described in *Z. mays* and *N. sylvestris* (tobacco). In maize, mitochondrial rearrangements yield abnormal growth called the NCS (non chromosomal stripe) phenotype, defined as striped sectors of pale-green tissues on the leaves. The NCS2 mutant, affected for both *nd4* and *nd7* genes, is male-sterile and heteroplasmic for the mutation (Marienfeld and Newton 1994). Assembly of complex I is compromised (Karpova and Newton 1999, see Sect. 11.1.3) and expression of one the three genes encoding the mitochondrial alternative oxidase (Aox2) is specifically increased (Karpova et al. 2002). In addition, chloroplast function and structure are also altered (Roussell et al. 1991). In tobacco, mitochondrial rearrangements also lead to abnormal growth and male sterility, causing the CMS (cytoplasmic male sterility) phenotype. The CMSII mutant has been extensively studied. It has been first described as having lost the *nd7* gene (Pla et al. 1995; Gutierres et al. 1997) and was next shown also to be affected in the expression of *nd1* (Gutierres et al. 1999). However, complementation with a nuclear version of *nd7* alone, which expresses a protein targeted to mitochondria restored the wild-type phenotype (Pineau et al. 2005), showing that the defect in *nd7* was the cause of the complex I defect. Similar to the maize NCS mutants, the capacity of the alternative oxidase and the amount of Aox are increased (Gutierres et al. 1997; Sabar et al. 2000), which is linked to the specific overexpression of one of the three genes encoding the mitochondrial alternative (Aox1.2) (Vidal et al. 2007; Liu et al. 2008). In parallel, the activity and the amount of alternative NAD(P)H dehydrogenases also increase contrary to the situation found in NCS maize mutants (Gutierres et al. 1997; Sabar et al. 2000). Photosynthetic efficiency is reduced and carbon assimilation under different stress conditions is modified (Sabar et al. 2000; Dutilleul et al. 2003a; Cardol et al. 2010;

Galle et al. 2010). Metabolomic studies showed an accumulation of nitrogen-rich amino acids (Dutilleul et al. 2005). Finally, the expression of many stress-related genes was changed, resulting in modified tolerance to stresses (Dutilleul et al. 2003b; Galle et al. 2010). Mitochondrial rearrangements leading to altered complex I have also been described in cucumber but the molecular nature of these rearrangements is not known (Juszczuk and Rychter 2009). Finally, several mutants have been identified in genes encoding splicing factors involved in the expression of *nd4* in *N. sylvestris* (Brangeon et al. 2000) and *A. thaliana* (Nakagawa and Sakurai 2006), and *nd1* and *nd9* in *A. thaliana* (de Longevialle et al. 2007; Jonietz et al. 2010).

In conclusion, all the plant complex I mutants present a retarded growth phenotype, sometimes male sterility, usually enhanced alternative oxidase activity and modified photosynthetic performances. Although proteomic and metabolomic studies revealed certain modifications, a general trend in the changes could not be discerned. This situation contrasts with *Chlamydomonas* complex I mutants where neither the apparent capacity of the alternative oxidase nor the photosynthetic efficiencies seem severely affected (Cardol et al. 2003).

Chlamydomonas respiratory mutants can be obtained and are viable if maintained under phototrophic conditions (where they rely on photosynthesis in the chloroplast). 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 only one active proton-pumping site is left, complex I mutants, which retain two active proton-pumping sites are still able to grow in these conditions, although at a significant lower rate (Remacle et al. 2001a). 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, usually frameshift mutations, in the mitochondrial *nd* genes (*nd1*, *nd4*, *nd5* and *nd6*). They are homoplasmic and directly amenable to biochemical studies which have led to propose a model for complex I assembly and subunit localization within the *Chlamydomonas* enzyme (see below) (Cardol et al. 2002, 2008; Remacle et al. 2001a, b). In addition, biolistic transformation of the mitochondrial genome of *Chlamydomonas* has also been employed to reconstruct mutations in the mitochondrial *nd* genes (Remacle et al. 2006).

Concerning nuclear genes, RNA interference technology allowed the isolation of mutants deficient for the ND3 and ND4L subunits (Cardol et al. 2006). These two subunits are usually encoded in the mitochondrial genome except in *Chlamydomonas* where their corresponding genes have migrated to the nucleus (Cardol et al. 2006). Recently an insertional mutagenesis was conducted to isolate loss-of-function nuclear mutations in complex I genes. 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). To date, this screen allowed the recovery of two tagged mutants: one mutant is deficient for the

PDSW subunit (*NUOB10* gene), a non-core subunit of the distal part of the hydrophobic arm of complex I (Barbieri et al. 2011), the other is deficient for the 24 kDa subunit (*NUO5* gene), a core subunit, binding a (2Fe-2S) cluster in the soluble arm of complex I (unpublished data).

11.1.3 Internal Architecture and Biogenesis

The localization of subunits within complex I subcomplexes (or domains) has been investigated in *Chlamydomonas* and *Arabidopsis*. In *Chlamydomonas*, the analysis of mutants deficient in the synthesis of mitochondrial-encoded hydrophobic components ND1, ND4, ND5, and ND6 (Cardol et al. 2002, 2008; Remacle et al. 2001a, b, 2006), or mutants that have lost the expression of nuclear genes encoding ND3, ND4L (Cardol et al. 2006) or PDSW (Barbieri et al. 2011) led to the identification of a 200- and 700-kD subcomplexes. The 200-kD soluble subcomplex carries the rotenone insensitive NADH dehydrogenase activity and could correspond to a fragment of the matrix-exposed arm. In PDSW, ND4 and ND5-deficient cells, the 700-kD membrane-associated subcomplex also displays NADH dehydrogenase activity (Barbieri et al. 2011; Cardol et al. 2002, 2008; Remacle et al. 2006). This subcomplex is less tightly bound to the membrane than the wild-type enzyme, and is mainly composed of subunits belonging to the matrix-exposed arm (Cardol et al. 2008) (see Fig. 11.1c). In maize, the NCS2 mutant affected in ND4 also displays a partially assembled complex I loosely attached to the mitochondrial inner membrane (Karpova and Newton 1999). In *Arabidopsis*, ten subcomplexes were obtained by destabilizing the enzyme by means of various treatments (Klodmann et al. 2010). Their individual subunit composition was resolved and the major building blocks were deduced from this analysis (Fig. 11.1b). Based on the subcomplexes identified in various mutants or following destabilizing treatments, models of complex I architecture and assembly have also been proposed in *Neurospora* (Videira and Duarte 2002) and human (Hirst et al. 2003; Antonicka et al. 2003; Ugalde et al. 2004; Lazarou et al. 2009; Dieteren et al. 2008; Vogel et al. 2007a; McKenzie and Ryan 2010). Localization of conserved subunits within these subcomplexes is generally conserved among the different models for complex I assembly. Assembly models for plant complex I also include additional small modules, most remarkably a matrix-exposed domain including γ-type carbonic anhydrases (see paragraph 1.1 for further details). The current hypothesis is that complex I assembly occurs by a stepwise mechanism during which preformed modules, or assembly intermediates, are combined. The hydrogenase and the membrane modules are joined together and both are expanding through the recruitment of non-core subunits.

The biogenesis of complex I requires numerous chaperones and assembly factors, most of which are conserved in plants and algae (Table 11.2). The first two assembly factors, CIA30 and CIA84, were identified in *N. crassa*. CIA30 and CIA84 are chaperones that have been shown to be directly involved in the assembly process

Table 11.2 Assembly factors in human, higher plants and *C. reinhardtii*

Complex I assembly factor				
H. sapiens	*Higher plants*	*C. reinhardtii*	Function	Reference
NDUFAF1 (CIA30)	CIA30 (AT1G17350)	NUOFAF1	Chaperone (early assembly)	Kuffner et al. (1998)
NUBPL (Ind1)	INDL	Ind1	Assembly of Fe–S cofactors	Bych et al. (2008)
Foxred1	NP_180034 Sarcosine oxidase family protein	XP_001692123 FAD-dependent oxidoreductase	Redox reactions	Calvo et al. (2010)
C8ORF38	Os06g0104100	XP_001693265	Abundance and activity of complex I	Pagliarini et al. (2008)
C20ORF7	OsI_37783	XP_001693605	Assembly early stage of complex I	Sugiana et al. (2008)
NDUFAF2 (B17.2L)	–	–	Chaperone (late assembly)	Vogel et al. (2007b)
NDUFAF3	OsJ_32539	XP_001702394	Cooperation between NDUFAF3 and NDUFAF4 from early to late stages complex I assembly	Saada et al. (2009)
NDUFAF4 (C6ORF66)	–	XP_001701912		

of complex I, through their association with a large membrane domain in a mutant unable to assemble the holoenzyme (Kuffner et al. 1998). CIA30 is well conserved among eukaryotes and homologs were found not only in vascular plants and algae but also in humans (Table 11.2). In fact, in humans, CIA30 plays a crucial role in the early assembly of complex I and mutations in the CIA30 encoding gene are responsible for a complex I-linked mitochondrial disease (Dunning et al. 2007). To date, CIA84 seems specific to *N. crassa* and was not found in vascular plants or in algae. In 2008, Ind1 was identified as participating in the assembly of Fe–S cofactors and subunits of complex I in the yeast *Y. lipolytica* (Bych et al. 2008). As expected for such a role, Ind1 is well conserved among eukaryotes. In humans, where it is also known as NUBPL, it is critically required for the assembly of complex I with a possible role in the delivery of one or more Fe/S clusters to complex I subunits (Sheftel et al. 2009). In the past few years, the discovery of six assembly factors (C20orf7, C8ORF38, FOXRED1, NDUFAF2, NDUFAF3, and NDUFAF4) provided a significant insight into the assembly process of human complex I. Bioinformatics analysis reveals that homologs of these assembly factors are detected in algae and vascular plants, except for NDUAF2 and NDUAF4 (Table 11.2). C20orf7 is peripherally associated with the matrix side of the mitochondrial inner membrane and plays a crucial role in the early stage of complex I assembly but in a different manner than CIA30 (Sugiana et al. 2008). Knocking-down *C8orf38* in mice resulted in a reduction of both abundance and activity of complex I (Pagliarini et al. 2008). FOXRED1 is an uncharacterized protein that derives its name from a FAD-dependent oxidoreductase protein domain. A lack of FOXRED1 leads to severe complex I deficiency in humans, but its biochemical activity in the assembly process remains unclear. Four human homologs of FOXRED1 (DMGDH, SARDH, PIPOX and PDPR) perform redox reactions in amino acid catabolism, suggesting a potential link between amino acid metabolism and complex I (Calvo et al. 2010). NDUFAF2, also known as B17.2 L is a paralog of a complex I subunit (B17.2), which is not incorporated into the holoenzyme, and plays a role in a late step of the assembly/stability of complex I (Vogel et al. 2007b). Recent studies showed that NDUFAF3 and NDUFAF4 cooperate from early to late stages of complex I assembly in association with at least the highly conserved subunits NDUFS2, NDUFS3, and NDUFS8, and non-core subunit NDUFA5 (Saada et al. 2009).

Analyses of supercomplexes in plants have been investigated after solubilization with digitonin and migration in BN-PAGE (*e.g.* Eubel et al. 2004; Krause et al. 2004) and by single particle electron microscopy (*e.g.* Peters et al. 2008; Bultema et al. 2009). Different supercomplexes could be detected : $I+III_{(2)}$ (the most abundant), $III_{(2)}+IV_{(1)}$, $V_{(2)}$, $I_{(2)}+III_{(2)}$ and respirasome $I+III_{(2)}+IV_{(1)}$. In *Chlamydomonas*, solubilization of mitochondria with dodecyl-maltoside allows to detect $I+III_{(2)}$ (Cardol et al. 2008) but detailed architecture was not investigated further. As far as complex I is concerned, it is worth mentioning that complex I mutants in *Chlamydomonas* usually exhibit a higher activity of succinate:cytochrome *c* oxidoreductase (complexes II+III) (Barbieri et al. 2011; Cardol et al. 2002, 2006, 2008; Remacle et al. 2001a), which could be viewed as a compensation effect for the loss of complex I. This suggests that in some circumstances preferential association

between complexes II and III exists although these two are usually not detected in supercomplexes.

11.2 Complex I in the Chloroplast

The thylakoid membranes of photosynthetic eukaryotes harbor a complex I-like enzyme, also referred to as NDH-1, that is related to cyanobacterial complex I (Battchikova and Aro 2007). Evidence for such an enzyme in the green lineage was suspected from the discovery of *ndh* genes encoding proteins with similarity to known complex I subunits in the first sequenced plastid genomes (Ohyama et al. 1986; Shinozaki et al. 1986). Based on the occurrence of the *ndh* genes in chloroplasts, NDH-1 is proposed to be present in all land plants (including ferns and mosses) (Sugiura et al. 2003; Gao et al. 2009) with the exception of some gymnosperm species (Wakasugi et al. 1994). Plastid *ndh* genes are also present in primitive green algae such as *Nephroselmis* or *Mesostigma* (Lemieux et al. 2000; Turmel et al. 1999) but absent from other microalgae such as *Chlamydomonas*, *Chlorella* or *Ostreococcus* (Wakasugi et al. 1997; Maul et al. 2002; Robbens et al. 2007). The observation that the plastid *ndh* genes are absent from the nuclear genomes of microalgae was taken as evidence that the plastid complex I was lost in such organisms (Derelle et al. 2006; Merchant et al. 2007; Palenik et al. 2007). In *Chlamydomonas* (and probably other algae or gymnosperms missing the plastid *ndh* subunits), it has been shown that type II NAD(P)H dehydrogenase enzymes operate instead of plastid complex I (Desplats et al. 2009; Jans et al. 2008; Peltier et al. 2010).

Based on electron microscopy of cyanobacterial complex I, plastid NDH-1 is proposed, similarly to bacterial and mitochondrial complex I, to display a L-shape with a hydrophobic core in the thylakoid membrane and a hydrophilic arm facing the stroma (Arteni et al. 2006). NDH-1 bearing plastids usually encode 11 NDH subunits (NdhA-NdhK), seven of which (NdhA-NdhG) are found in the membrane-embedded hydrophobic subcomplex. The counterparts of the bacterial NuoE, F and G subunits that are involved in NADH binding and oxidation (*i.e.* NADH dehydrogenase module) in bacterial/mitochondrial complex I are missing in both cyanobacterial and plastid NDH-1 (Friedrich et al. 1995). Because NADPH is the major stromal reductant, it is not clear how electrons enter plastid complex I considering that NDH-1 appears to use NADH as its preferred electron donor (Sazanov et al. 1998; Rumeau et al. 2005). However, NDH-1 might be operating as a ferredoxin-plastoquinone oxido-reductase based on the recent finding that stromal ferredoxin can act as an electron donor to NDH-1 (Yamamoto et al. 2011). The site of ferredoxin oxidation is currently unknown and it is conceivable that NDH-1 can accept electrons from several stromal donors.

It should be noted that the low abundance and instability of plastid NDH-1 has been a major challenge for the biochemical and enzymatic characterization of this protein complex (Sazanov et al. 1996, 1998). Nevertheless, a combination of genetic and biochemical approaches including the partial purification of NDH-1 led to the discovery of 18 additional nuclear-encoded structural subunits of the plastid complex I, some of which are shared with cyanobacteria while others appear

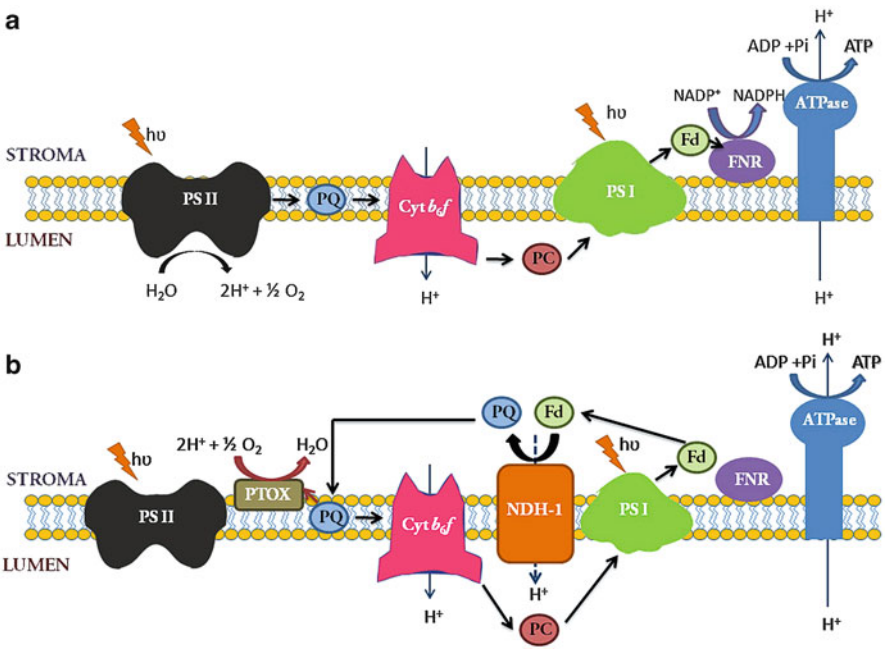

Fig. 11.2 Models for electron transfer routes in the thylakoid. (**a**) **Linear Electron Transfer**: The light dependent linear electron transfer at the thylakoid membrane starts from photo-excitation (hv) of two electrons obtained from water oxidation at Photosystem II (*PSII*). One electron is then transferred via plastoquinone (*PQ*) to cytochrome b_6f (Cyt b_6f). From Cyt b_6f, Plastocyanin(*PC*) transports the electron to Photosystem I (*PSI*) where it is again subjected to photo-excitation (hv). The electron is then received by Ferredoxin (*Fd*), which acts as the electron donor to Ferredoxin:NADP Reductase (*FNR*) for the production of NADPH. The proton gradient established by Cyt b_6f acts as the driving force for the synthesis of ATP by ATPase. (**b**) **Cyclic Electron Transfer and Chlororespiration**: In the light, cyclic electron transfer can also take place and leads to ATP synthesis with no net NADPH being produced. In this instance, the electrons flow from Fd to Plastid Complex I (NDH-1) instead of FNR. NDH-1 transfers the electrons from two Fd to one PQ. From PQ the electron directly flows into Cyt b_6f, bypassing the requirement for PSII. During chlororespiration (*arrows* represented in *brown*), the PQ pool is oxidized by a plastid terminal oxidase (*PTOX*). Chlororespiration can take place in the *dark*

to be plant specific (Rumeau et al. 2005; Munshi et al. 2006; Muraoka et al. 2006; Ishihara et al. 2007; Ishikawa et al. 2008; Majeran et al. 2008; Shimizu et al. 2008; Ishida et al. 2009; Sirpio et al. 2009a,b; Suorsa et al. 2009; Takabayashi et al. 2009; Yamamoto et al. 2011).

Genetic inactivation of several plastid-encoded subunits of NDH-1 led to the conclusion that plastid complex I is fully dispensable for plant growth under normal conditions (*e.g.* Burrows et al. 1998; Kofer et al. 1998; Shikanai et al. 1998; Horvath et al. 2000). Biochemical investigation of plastid complex I mutants showed that NDH-1 mediates chlororespiration in the dark and cyclic electron transfer around photosystem I in the light (Fig. 11.2a, b). The term "chlororespiration" was first

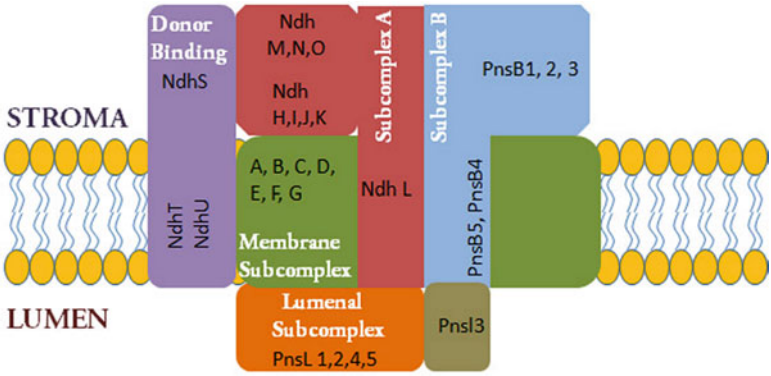

Fig. 11.3 The organization of structural subunits of Plastid Complex I. Plastid Complex I (NDH-1) is located in the thylakoid membrane of the plant chloroplast and consists of five subcomplexes: subcomplex A, subcomplex B, lumenal subcomplex, membrane subcomplex and the electron donor binding subcomplex. This diagram is based on Yamamoto et al. (2011) and Ifuku et al. (2011). We retained the terminology proposed by Ifuku et al. (2011)

coined to describe the activity of a putative electron transfer chain at the thylakoid membrane of chloroplasts to explain effects on the redox state of the plastoquinone pool (PQ) in the absence of photochemistry (Bennoun 1982). Similar to a respiratory chain, PQ was proposed to be reduced via the action of a NAD(P)H dehydrogenase and re-oxidized by oxygen via a terminal oxidase. The discovery of NDH-1 and PTOX (Plastid Terminal OXidase), a quinol oxidase related to mitochondrial alternative oxidase provided the first molecular evidence for the operation of such an electron transfer activity (Carol et al. 1999; Wu et al. 1999). It is likely that NDH-1 and PTOX constitute the components involved in chlororespiration but proof for a direct electron transfer from NDH-1 to PTOX is still missing. While loss of NDH-1 does not result in any visible phenotype, the absence of PTOX impacts carotenogenesis in leaves. Interestingly, a recent study in tomato suggests that NDH-1 controls carotenoid biosynthesis in fruit chromoplasts but not in chloroplasts (Nashilevitz et al. 2010). The NDH-1 dependent Cyclic Electron Flow around PSI (CEF) was originally defined as the transfer of electrons from stromal NADPH (that is reduced by the activity of PSI) back into the PQ pool (Rumeau et al. 2007; Peltier et al. 2010) (Fig. 11.2b). The physiological importance of NDH-1 is still unclear but several studies have highlighted the importance of the NDH-1 complex in stress conditions such as high light, drought or extreme temperatures (reviewed in Shikanai 2007; Suorsa et al. 2009; Johnson 2011). It is likely that the enzyme participates in regulating the ATP/NADPH ratio for optimal photosynthesis, a role already postulated for auxiliary electron transfer routes such as chlororespiration and CEF (Rumeau et al. 2007; Peltier et al. 2010).

The assembly of NDH-1, like its mitochondrial counterpart is proposed to proceed via modular assembly of five subcomplexes (Fig. 11.3): a membrane subcomplex, a soluble stromal subcomplex A, a membrane attached stromal facing subcomplex B, an electron donor binding subcomplex and a lumenal subcomplex

(Peng et al. 2008, 2009; Ifuku et al. 2011). The electron donor binding subcomplex contains NdhS, NdhT and NdhU. NdhS is required for the high-affinity binding of Fd to NDH-1 in an in vitro Fd-dependent PQ reduction assay and was postulated to act as the Fd-docking site in NDH-1 (Yamamoto et al. 2011). The membrane subcomplex (NdhA-NdhG, PnsB4) and the soluble subcomplex A (NdhH-NdhK, NDHM-NDHO) contain the core subunits that are also conserved in bacterial complex I. The membrane subcomplex and the soluble subcomplex A are proposed to be connected by NDHL (Shimizu et al. 2008). The subcomplex B (PnsB1, 2, 3, 4, 5) is postulated to interact with the chlorophyll binding proteins of the light-harvesting complex, LHCA5/LHCA6, which are attached to PSI (Peng et al. 2009). The identification of a novel 300 kDa complex containing NdhS, the candidate Fd-binding subunit suggests the existence of additional structural subunits, which still remain unidentified (Yamamoto et al. 2011). It is expected that yet-to-be-discovered subunit(s) containing co-factors might function in the transfer of electrons from Fd to plastoquinone. The lumenal subcomplex (PnsL1, 2, 4, 5) contains subunits that are specific to plastid complex I and is required for the stability of subcomplex A. PnsL3 is a lumenal subunit that is not part of the lumenal subcomplex but is in close interaction with subcomplex B (Ifuku et al. 2011).

In the thylakoid membranes, NDH-1 occurs as a high molecular weight complex that was shown to correspond to an NDH-1/PSI supercomplex (Lennon et al. 2003; Peng et al. 2008, 2009). In addition to the NDH-1/PSI supercomplex, three distinct subcomplexes containing NDH-1 subunits have been detected after resolution of thylakoid membranes via BN-PAGE (Ishihara et al. 2007; Peng et al. 2008; Sirpio et al. 2009a). Because the abundance of such complexes varies upon plastid differentiation, it is not clear if they correspond to assembly intermediates or subcomplexes with specialized functions. Interestingly, cyanobacteria possess at least four NDH-1 complexes that are very distinct in terms of function. Such functional versatility is achieved through a modification in subunit composition (Battchikova and Aro 2007; Ogawa and Mi 2007).

The biogenesis of NDH-1 requires several nuclear encoded proteins, some of which are involved in the splicing and editing of plastid *ndh* mRNAs while others are required at a post-translational step of the enzyme biogenesis (Suorsa et al. 2009). So far four proteins CRR1, CRR6, CRR7 and PIFI have been implicated in the assembly and/or stabilization of plastid Complex I (Munshi et al. 2005, 2006; Shimizu and Shikanai 2007; Wang and Portis 2007; Peng et al. 2010). Because there is no motif in their sequences that indicate an enzymatic activity, these components were postulated to act as NDH1-specific chaperones (CRR1, 6, 7, NDF5) or a regulator of NDH-1 activity (PIFI). One notable exception is CRR1, a stromal protein whose pyridine nucleotide binding site suggests a possible redox activity for this assembly factor (Shimizu and Shikanai 2007).

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