The Mitochondrial Oxidative Phosphorylation Proteome of *Chlamydomonas reinhardtii* Deduced from the Genome Sequencing Project[1]

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Mitochondria originated from an endosymbiotic process that involved an α-proteobacterium (Martin and Muller, 1998; Gray et al., 1999). The evolution of the organelle has been associated with the massive migration of the endosymbiont genes to the nucleus of the host and with the acquisition of new proteins that were originally present in the host (Karlberg et al., 2000; Andersson et al., 2003; Richly et al., 2003). Mitochondrial proteomes are thus a mosaic of mitochondria- and nucleus-encoded proteins, the latter having a combined eubacterial/eukaryotic origin. The increasing number of complete genome sequences has naturally led to an attempt to establish a relationship between these genomes and their corresponding transcriptomes and proteomes. Of particular interest for bioenergeticists are the efforts performed toward establishing the proteomes of chloroplasts and mitochondria.

Mitochondria are the site of oxidative phosphorylation (OXPHOS). This process comprises an electron-transfer chain that is driven by substrate oxidation and is coupled to the synthesis of ATP through an electrochemical transmembrane gradient. Therefore, the OXPHOS proteome (or more simply the OXPHOSome) will be the anatomical description of the protein components that participate in this process (complexes I–V and additional oxidoreductases).

THE STANDARDS OF REFERENCE: THE BOVINE, YEAST, AND PLANT OXPHOSOMES

Historically, the bovine OXPHOS complexes were the first ones to be isolated and characterized (Hatefi et al., 1979), and three-dimensional structures of the complexes III (Xia et al., 1997; Zhang et al., 1998), IV (Tsukihara et al., 1996), and V (Abrahams et al., 1994) are now available. The genes coding for the subunits of the complexes have also been identified, and the N-terminal sequences of the corresponding mature proteins have been determined (Yanamura et al., 1988; Collinson et al., 1994; Iwata et al., 1998; Hirst et al., 2003). Currently, the organization of the different bovine OXPHOS complexes into supercomplexes is being actively explored (Schägger and Pfeiffer, 2000). The bovine system constitutes, therefore, an obliged reference for comparative studies. In other animal organisms, including human (http://www.sanger.ac.uk/HGP/) and the nematode *Caenorhabditis elegans* (Tsang and Lemire, 2003), the availability of genome sequences has allowed an immediate identification of most of the respiratory-chain components by comparison to bovine sequences.

A considerable amount of information is also available in the yeast *Saccharomyces cerevisiae*. Both its mitochondrial and nuclear genomes have been completely sequenced (The Saccharomyces Genome Database at http://www.yeastgenome.org/; Goffeau et al., 1996), and an inventory of the yeast gene products that are present in mitochondria has been drawn up (Schon, 2001). Furthermore, the OXPHOS complexes II (Lemire and Oyedotun, 2002), III (Ljungdahl et al., 1987), IV (Poyton et al., 1995), and V (Velours and Arselin, 2000) have been isolated and characterized. In addition, the crystallographic structures of complex III (Lange and Hunte, 2002) and of a complex V subcomplex (Stock et al., 1999) have been obtained, and supramolecular structures associating complexes III and IV have been described (Schägger and Pfeiffer, 2000). *S. cerevisiae* cannot, however, be considered as the reference simply because its mitochondria are deprived of complex I. Nevertheless, mitochondria of

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other fungi such as *Neurospora crassa* (Friedrich et al., 1998) and *Yarrowia lipolytica* (Kerscher et al., 2001) that possess a complex I have been successfully used to study the structure and function of this enzyme.

The higher plant Arabidopsis (*Arabidopsis thaliana*) can also be considered as a model system. Its three genomes have been sequenced (The Arabidopsis Information Resource at http://arabidopsis.org/info/agi.jsp), and the different respiratory complexes have been isolated by Blue-Native PAGE (BN-PAGE; Jansch et al., 1996). The presence of OXPHOS supercomplexes in plant mitochondria has also been detected (Eubel et al., 2003). In addition, 416 proteins of the mitochondrial proteome have been identified by liquid chromatography-tandem mass spectrometry (Heazlewood et al., 2004), and the constructed database was made available to the public at http://www.mitoz.bcs.uwa.edu.au.

THE CHLAMYDOMONAS OXPHOSOME DATA

The green alga *Chlamydomonas reinhardtii* allows the study of the OXPHOSome of a photosynthetic, unicellular organism. The sequence of its 15.8-kb linear mitochondrial genome is known (Michaelis et al., 1990), and a draft of the complete sequence of its nuclear genome is now available (http://www.jgi.doe.gov; Shrager et al., 2003). Moreover, the recent development of procedures to isolate Chlamydomonas mitochondria almost free of thylakoid contaminants (Eriksson et al., 1995; Nurani and Franzen, 1996; Cardol et al., 2002), to separate the major mitochondrial complexes in BN-PAGE (van Lis et al., 2003; Cardol et al., 2004), and to analyze by bioinformatics the algal-nucleic acid sequences (http://www.chlamy.org/) allowed us to initiate the characterization of the algal OXPHOSome (van Lis et al., 2003; Cardol et al., 2004).

In this review, using a genomic approach and taking into account previous published data, we present a compilation of the proteins that could be components of the Chlamydomonas OXPHOSome or could participate in its biogenesis. We found that, among polypeptidic sequences identified, the large majority have counterparts in mammals, fungi, and higher plants, whereas the remaining proteins are unique to *C. reinhardtii* or only common to two or three lineages.

COMPLEX I

Complex I (rotenone-sensitive NADH:ubiquinone oxidoreductase; EC 1.6.5.3) is the largest and most complicated enzyme of the mitochondrial respiratory chain. In the bovine complex I, 45 different subunits have been characterized and build up into a membrane-bound assembly with a molecular mass of approximately 980 kD (Hirst et al., 2003). The detailed dissection of complex I from the higher plants Arabidopsis and rice (*Oryza sativa*; Heazlewood et al., 2003a), and the fungi *N. crassa* (Videira and Duarte, 2002) and *Y. lipolytica* (Abdrakhmanova et al., 2004), have confirmed the complexity of the enzyme. A simpler membrane-associated type-I NADH dehydrogenase has been characterized in bacteria. It comprises 14 subunits, all being conserved among eukaryotes. These subunits bind the FMN and the eight iron-sulfur clusters of the enzyme. Seven of these subunits are the homologs of the hydrophobic ND1, 2, 3, 4, 4L, 5, and 6 subunits encoded in the mitochondrial genome of eukaryotes (Dupuis et al., 1998; Friedrich et al., 1998). The additional subunits present in mitochondrial complex I, the so-called supernumerary subunits, are considered to participate in the assembly, the stability, or the regulation of the enzyme (Friedrich et al., 1998; Heazlewood et al., 2003a).

Recently, the composition of the *C. reinhardtii* complex I has been analyzed (Cardol et al., 2004). Combining proteomic and genomic approaches, 42 proteins of molecular masses ranging from 7 to 77 kD were identified for a molecular mass totaling 950 to 1,000 kD (Table I). Comparison of complex I subunit compositions from *C. reinhardtii*, mammals, fungi, and higher plants revealed that all eukaryotic enzymes contained 31 common components, including the 14 highly conserved subunits homologous to the prokaryotic type-I NADH dehydrogenase enzyme (Cardol et al., 2004).

Studies involving larger numbers of organisms now allow us to determine whether the remaining subunits are real lineage-specific components or could be poorly conserved orthologs. For example, the NUVM and NUWM gene products considered to be specific to the complex I of *Y. lipolytica* (Abdrakhmanova et al., 2004) actually possess counterparts in mammals, *N. crassa*, Arabidopsis, and *C. reinhardtii* (Table I). As a matter of fact, we have found that the NUWM gene product belongs to the complex I ESSS-subunit family that is conserved in all eukaryotes examined to date (Cardol et al., 2004). In other respects, NUVM shares similarities, both at sequence and hydropathy profile (Kyte and Doolittle, 1982) levels, with the mammal B15/NDUFB4 subunit (data not shown). As indicated in Table I, homologous sequences of NUVM/B15/NDUFB4 are found in Arabidopsis (At2g31490) and in Chlamydomonas (*v2.0*/C_120199 and GenBank/AAS48193), and the corresponding polypeptides have been independently identified in complex I preparations from both organisms (Heazlewood et al., 2003a; Cardol et al., 2004). The B15 subunit homologs could thus represent a new family of complex I components conserved among all eukaryotes. Moreover, considering that the Arabidopsis/At2g42210 gene product belongs to the weakly conserved B14.7-subunit family, including the *N. crassa* 21.3b subunit (GenBank/P25710) and the Chlamydomonas *v2.0*/C_180167 protein (GenBank/AAS58499), we now propose that the mitochondrial complexes I from eukaryotes actually share 33 common components (Table I).

Among the 43 identified or putative subunits of the *C. reinhardtii* complex I (including NUOS4b, which is

Table I. *Genomic analysis of mitochondrial OXPHOS components from C. reinhardtii*

Identification of homologous sequences in eukaryotic organisms. Protein sequences from mammals, fungi, and higher plants were obtained from ENTREZ at the National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov/) and from the Arabidopsis Information Resource (http://arabidopsis.org/info/agi.jsp) or were identified using the PSI-BLAST tool available at the NCBI server. Names of proteins are based on published papers and reviews (see text). *C. reinhardtii* homologous sequences were identified using BLAST facilities with fungal, mammal, and higher plant protein sequences against a draft version of the *C. reinhardtii* genome (v2.0 released on February 4, 2004; distributed by DOE Joint Genome Institute, http://genome.jgi-psf.org/chlre2/chlre2.home.html), against the Chlamydomonas expressed sequence tag contig databases (20021010 and ACEG, http://www.chlamy.org/), or against the sequences of individual expressed sequence tag clones (http://www.kazusa.or.jp/en/plant/chlamy/EST/). Chlamydomonas gene model accession number (v2.0), protein name, and GenBank accession numbers are given when applicable. The identity percentages between Chlamydomonas amino acid sequences and their homologs in human (% Hs) and Arabidopsis (% At) were computed from a full-length (global) alignment built with ClustalW (v1.8) tool (Thompson et al., 1994) at the European Bioinformatics Institute (http://www.ebi.ac.uk/clustalw/). N.I. (not identified) indicates that no sequence of significant similarity was found. MM, Predicted molecular mass with the compute pI/MW tool (Bjellqvist et al., 1993) from the ExPaSy molecular Biology Server (http://au.expasy.org/). When a mitochondrial targeting sequence was identified (number of amino acid residues are shown inside parentheses), its mass was deduced from the total molecular mass of the preproteins. nd, Not determined. Biochemical evidence of the presence of protein was obtained in the references indicated by footnotes a to d.

H. sapiens/ *B. taurus*	*N. crassa*/ *Y. lipolytica*	Arabidopsis	*C. reinhardtii*	MM	% Hs	% At	References
Complex I							
NDUFS7/PSST	19.3/NUKM	At5g11770	C_740068, NUO10, AAQ63698	18.1	76	83	[a]
NDUFS8/TYKY	21.3c/NUIM	At1g16700, At1g79010	C_1010023, NUO8, AAQ63697	26.5	76	78	[a]
NDUFV2/24	24/NUHM	At4g02580	C_320064, NUO5, AAQ63695	26.4 (40)	54	53	[a,b]
NDUFS3/30	31/NUGM	AtMg00070	C_450014, NUO9, AAQ55457	32.1	55	60	[a]
NDUFS2/49	49/NUCM	AtMg00510	C_1430023, NUO7, AAQ63700	52.6	69	68	[a]
NDUFV1/51	51/NUBM	At5g08530	C_260120, NUO6, AAQ63696	49.6 (31)	68	75	[a,b]
NDUFS1/75	78/NUAM	At5g37510	C_910030, NUOS1, AAQ73136	77.3	51	50	[a]
ND1	ND1/NU1M	AtMg00516	ND1[f], AAB93446	31.6	45	50	
ND2	ND2/NU2M	AtMg00285	ND2[f], AAB93444	42.4	14	26	
ND3	ND3/NU3M	AtMg00990	C_1300011, NUO3, AAQ55461	30.2	36	43	[a]
ND4	ND4/NU4M	AtMg00580	ND4[f], AAB93441	48.7	34	38	
ND4L	ND4L/NULM	AtMg00650	C_690071, NUO11, AAO61142	24.2	28	31	
ND5	ND5/NU5M	AtMg00513	ND5[f], AAB93442	59	27	41	
ND6	ND6/NU6M	AtMg00270	ND6[f], AAB93445	17.7	15	28	
NDUFA1/MWFE	9.8/NIMM	At3g08610	[g]80:91611–91831, AAS48198	7.6	16	21	
NDUFAB1/SDAP	9.6/ACPM	At1g65290	C_650040, ACP1, AAQ73138	13.7	49	55	
NDUFA2/B8	10.5/NI8M	At5g47890	C_1140037, NUOB8, AAQ63699	11	51	49	[a]
NDUFB3/B12	10.6/NB2M	AK059007[h]	[g]36:249461–249659, AAS48194	6.5	33	37	
NDUFA5/B13	29.9/NUFM	At5g52840	C_930004, NUOB13, AAQ73139	18	24	39	[a]
NDUFS6/13A	18.4/NUMM	At3g03070	C_860009, NUOS6, AAQ64639	16.3	28	53	[a]
NDUFA6/B14	14.8/NB4M	At3g12260	C_950015, NUOB14, AAQ84469	16.1	40	31	[a]
NDUFA11/B14.7	21.3b/N.I.	At2g42210	C_180167, AAS58499	28.7	15	23	[a]
Q8WZ96/ESSS	11.7/NUWM	At3g57785	C_1650005, NUO17, AAS48192	19.6	10	31	[a]
NDUFS5/PFFD	11.5/NIPM	At3g62790, At2g47690	[g]49:431003–432988, AAQ98888	9.5	28	36	
NDUFB4/B15	7/NUVM	At2g31490	C_120199, NUOP2, AAS48193	16.2	16	33	[a]
NDUFA12/B16.6	13.5/NB6M	At1g04630, At2g33220	C_970017, NUOB16, AAQ64637	16.4	41	45	[a]
DAP13/B17.2	13.4/N7BM	At3g03100	C_140183, NUO13, AAQ64638	18	42	34	[a]
NDUFB7/B18	89.7/NB8M	At2g02050	C_430007, NUOB18, AAQ73135	10.3	34	44	[a]
NDUFS4/AQDQ	21/NUYM	At5g67590	C_540057, NUOS4, AAQ64640[i]	20.6	46	44	[a]
			C_560059, NUOS4b[i]	22	32	31	
NDUFA8/PGIV	20.8/NUJM	At5g18800	C_720061, NUOA8, AAQ55460	12.5	26	31	
NDUFB9/B22	18 3/N.I.	At4g34700	C_470021[j], NUOB22, AAQ73134	13.9	32	35	
NDUFB/PDSW	12.3/NIDM	At1g49140, At3g18410	C_950013, NUOB10, AAQ55459	17.9	14	20	[a]
NDUFA9/39	40/NUEM	At2g20360	C_60046, NUOA9, AAQ55458	43.7	46	41	[a]
N.I.	NUO20.9/NUXM	At4g16450	C_270165, NUO21, AAQ64641	13.5	N.I.	29	[a]
N.I.	N.I.	At4g20150	C_930030, NUOP1, AAS58501	9.4	N.I.	25	[a]
N.I.	N.I.	At3g07480	C_610041, NUOP3, AAS58502	22.3	N.I.	25	[a]
N.I.	N.I.	Carbonic anydrase-like protein family:	C_160093, FBP1, AAS48195	24.3	N.I.	~45	[a]
			C_190012, FBP2, AAS48196	28.4 (26)	N.I.	~50	[a,b]
		At5g63510, At3g48680, At1g47260, At5g66510	C_710033[j], C_7420003[l], CAH9, AAS48197	32.7	N.I.	~50	[a]
N.I.	N.I.	At1g67350	N.I.				
N.I.	N.I.	At2g27730[m]	N.I.				
N.I.	N.I.	N.I.	C_1300007, NU0P4, AAS58498	12.1	N.I.	N.I.	[a]

(*Table continues on following page.*)

Table I. (*Continued from previous page.*)

H. sapiens/ *B. taurus*	*N. crassa*/ *Y. lipolytica*	Arabidopsis	*C. reinhardtii*	MM	% Hs	% At	References
N.I.	N.I.	N.I.	C_1300001, NUOP5, AAS58503	15.3	N.I.	N.I.	[a]
N.I.	N.I.	N.I.	C_710036, NUOP6, AAS58500[n]	19.6 (26)	N.I.	N.I.	[a,c]
NDUFB8/ASHI	20.1/NIAM	N.I.	N.I.				
NDUFA3/B9[k]	N.I.	N.I.	N.I.				
NDUFC1/KFYI	N.I.	N.I.	N.I.				
NDUFB1/MNLL	N.I.	N.I.	N.I.				
NDUFB2/AGGG	N.I.	N.I.	N.I.				
NDUFA4/MLRQ	N.I.	N.I.	N.I.				
NDUFV3/10	N.I.	N.I.	N.I.				
NDUFA7/B14.5A	N.I.	N.I.	N.I.				
NDUFC2/B14.5B	N.I.	N.I.	N.I.				
NDUFB5/SGDH	N.I.	N.I.	N.I.				
NDUFB6/B17	N.I.	N.I.	N.I.				
NDUFA10/42	N.I.	N.I.	N.I.				
N.I.	9.5/NI9M[k]	N.I.	N.I.				
N.I.	17.8/N.I.	N.I.	N.I.				
N.I.	21.3a/NUZM	N.I.	N.I.				
Assembly Factors							
NDUFAF1/CIA30	CIA30	At1g72420	C_50184, NUOAF1	25.3	24	23	
N.I.	CIA84	N.I.	N.I.				
H. sapiens/B. taurus	*S. cerevisiae*	Arabidopsis	*C. reinhardtii*	MM	% Hs	% At	References
Complex II							
SDHA	SDH1	At2g18450, At5g66760	C_240095, SDH1A[o]	nd	nd	nd	
			C_240093, SDH1B[o]	nd	nd	nd	
SDHB	SDH2	At5g40650, At3g27380	C_200010, SDH2	32.3	65	59	
SDHC (QPS1)	SDH3	At5g09600	C_350099, SDH3	19.5	24	13	[d]
SDHD	SDH4	At2g46505	C_350036[p], SDH4, BK005613	12.8	23	13	
N.I.	N.I.	At1g47420	N.I.				
N.I.	N.I.	At1g08480	N.I.				
Assembly Factors							
N.I.	TCM62	N.I.	N.I.				
Complex III							
UQCRC1/Core1	COR1	At3g02090	C_1290016, QCR1	52.7 (21)	47	57	[b,d]
UQCRC2/Core2	QCR2	At3g16480, At1g51980	C_490077, QCR2	49.6	24	29	[d]
			C_10187, MPPA	54.3	32	38	
CYB/III	COB	AtMg00220	COB[f], AAB93440	42.3	49	59	
CYC1/IV	CYT1	At3g27240, At5g40810	C_2110015, CYC1, AAG44483	16.5 (71)	43	37	[d,e]
UQCRCFS1/RISP[g], IX[t]	RIP1	At5g13440, At5g13430	C_180142, RIP1, CAC86460	22.8 (54)	39	50	[d,e]
UQCRB/VI–14 kDa	QCR7	At4g32470, At5g25450	C_200033, QCR7	14.0	36	53	
UQCRQ/VII	QCR8	At3g10860, At5g05370	[g]11:563654–564261, QCR8, BK005618	8.7	25	23	
UQCRH/VIII	QCR6	At2g01090, At1g15120	[g]6:450163–451396, QCR6, BK005616	8	34	58	
UQCR10/X	QCR9	At3g52730	[g]150:92955–93974, QCR9, BK005617	7	29	38	
6.4 kDa/XI	QCR10	At2g40765	[g]52:408822–410003, QCR10, BK005619	6.5	21	26	
Assembly Factors							
N.I.	CBP1, 2, 4, 6	N.I.	N.I.				
CBP3	CBP3	At5g51220	C_180140, CBP3	31.1	25	26	
N.I.	CBS1, 2	N.I.	N.I.				
BCS1	BCS1	At5g17760	C_1490020, BCS1	68.3	21	20	
ABC1	ABC1	At4g01660	C_130084, ABC1	70.1	39	45	

(*Table continues on following page.*)

Table I. (*Continued from previous page.*)

H. sapiens/B. taurus	*S. cerevisiae*	Arabidopsis	*C. reinhardtii*	MM	% Hs	% At	References
Cytochrome *c*							
CYC/CytC	CYC1	At1g22840, At4g10040	C_160106[j], CYC, S29514	12	64	71	
Assembly Factors							
CCHL	CYT2 (CC1HL)	N.I.	C_3680005, HCS1	31.3	39	N.I.	
	CYC3		C_650035, HCS2[p],	nd	nd	N.I.	
			C_12500002, HCS3	31.6	15	N.I.	
N.I.	N.I.	AtMg00830, AtMg00900, AtMg00960, AtMg00110, AtMg00180	N.I.				
Complex IV							
COI	COI	AtMg01360	COX1[f], AAB93443	55.2	50	61	
COII	COII	AtMg00160	C_110207, COX2a, AAK30367	16.5 (143)	34	45	b
			C_180065, COX2, AAK32117	17.2	61	58	b,d
COIII	COIII	AtMg00730	C_40035, COX3, AAG17279	30 (110)	32	34	b
COX4	Va, Vb (COX5)	N.I.	N.I.				
COX5A	VI (COX6)	N.I.	N.I.				
COX5B	IV (COX4)	At3g15640, At1g80230	C_1270018, COX5b	13.1 (58)	30	22	b,d
N.I.	N.I.	At3g62400, At2g47380 COX5C	C_110052, COX5c	6.8	N.I.	26	
COX6A	VIa (COX13)	At4g37830	C_2910004, COX13	12.3 (17)	24	31	b
COX6B	VIb (COX12)	At1g22450, At4g28060	C_90043, COX12	16.7 (11)	34	29	b
COX6C	VIIa (COX9)	N.I.	N.I.				
COX7A	VII (COX7)	N.I.	N.I.				
COX7B	N.I.	N.I.	N.I.				
COX7C	VIII (COX8)	N.I.	N.I.				
COX8	(VIIa)	N.I.	N.I.				
N.I.	N.I.	N.I.	C_810006, COX90, AAM88388	11.7	N.I.	N.I.	
Assembly Factors							
Surfeit1	SHY1	At3g17910	C_970016, SUR1	41.2	24	24	
SCO1, SCO2	SCO1, SCO2	At3g08950	C_1080032, SCO1	22	32	40	
COX10	COX10	At2g44520	C_160214, COX10	48.3	33	35	
COX11	COX11	At1g02410	C_1190009[j], COX11, BK005614	28.5	36	47	
N.I.	COX14, COX20	N.I.	N.I.				
COX15	COX15	At5g56090	C_50022, COX15	47.3	37	42	
AAH01702	COX16	At4g14145	C_340132, COX16	13	14	21	
COX17	COX17	At1g53030	C_2180002[j], COX17, AAF82382	8.3	55	56	
OXA1	COX18, OXA1 (PET1402)	At5g62050	C_650075[p], COX18, BI529086	nd	nd	nd	
COX19	COX19	At1g66590	C_320109[j], COX19, BK005615	10.3	33	39	
NP_077276	COX23	At1g02160	C_1100043, COX23	16.5	13	19	
N.I.[q]	PET100	At4g14615, At1g52821	N.I.				
CSRP2BP	PET117	N.I.	N.I.				
N.I.	PET54, 111, 122, 494	N.I.	N.I.				
AAH47722	PET191	BAC43353	[g]119:54609–54976, PET191, BK005620	7.5	27	47	
LRPPRC	PET309	PPRC protein family (At1g73710, At1g79490, At3g22470, etc.)	C_190150, PPRC1, C_180166, PPRC2, C_150087, PPRC3, C_490098, PPRC4, C_1230044, PPR	84.1–132.9	nd	nd	
N.I.	MSS2, MSS51	N.I.	N.I.				
Complex V							
Fo Subcomplex							
ATP6/A	ATPA (ATP6)	AtMg00410, AtMg01170	C_350120, ATP6, AAL79815	24.6 (107)	28	23	c
ATP5F1/B	ATPB (ATP4)	AtMg00640	N.I.				
ATP5G3/C	ATPC (ATP9)	AtMg01080, At2g07671	C_1080025, ATP9A[r]	15.2	34	57	b
			C_1080045, ATP9B[r]	15.7	40	60	b
ATP5H/D	ATPD (ATP7)	At3g52300	N.I.				
ATP5I/E	ATPE (ATP21)	At5g15320	N.I.				
ATP5J2/F	ATPF (ATP17)	At4g30010	N.I.				
ATP5L/G	ATPG (ATP20)	At2g19680	N.I.				

(*Table continues on following page.*)

Table I. (*Continued from previous page.*)

H. sapiens/B. taurus	*S. cerevisiae*	Arabidopsis	*C. reinhardtii*	MM	% Hs	% At	References
ATP5J/F6	ATPH (ATP14)	N.I.	N.I.				
ATP8/A6L	ATP8	AtMg00480	N.I.				
ATP5O/OSCP	ATP5	At5g13450	C_1140001, ATP5	28.2 (31)	30	24	[c,d]
ATPI/IF1	INH1, STF1	At5g04750	N.I.				
N.I.	STF2	N.I.	N.I.				
N.I.	ATPJ/I (ATP18)	N.I.	N.I.				
ATP5S/ATPW	N.I.	N.I.	N.I.				
F_1 Subcomplex							
ATP5A1/α	α (ATP1)	AtMg01190, At2g07698	C_20064, ATP1, T08113	56.8 (45)	71	70	[c,d]
			C_260094, ATP1B	74.8	21	21	
ATP5B/β	β (ATP2)	At5g08670, At5g08680, At5g08690	C_3890001, ATP2, P38482; C_170034[s]	59 (26)	79	81	[c,d]
ATP5C1/γ	γ (ATP3)	At2g33040	C_1380005[p], ATP3	nd (44)	nd	nd	[b,c,d]
ATP5D/δ	δ (ATP16)	At5g47030	C_470090, ATP16	21.2	32	33	[b,c,d]
ATP5E/ε	ε (ATP15)	At1g51650	N.I.				
N.I.	ATPK (ATP19)	N.I.	N.I.				
N.I.	N.I.	At2g21870 (ATP7, F_Ad)	N.I.				
N.I.	N.I.	N.I.	C_420010, ASA1 (MASAP), CAD29654	63.2	N.I.	N.I.	[b,d]
N.I.	N.I.	N.I.	C_710028, ASA2	46 (29)	N.I.	N.I.	[c]
N.I.	N.I.	N.I.	C_750022, ASA3	36.3 (32)	N.I.	N.I.	[c]
N.I.	N.I.	N.I.	C_230150, ASA4	31.2 (28)	N.I.	N.I.	[c]
N.I.	N.I.	N.I.	C_710036, NUOP6, AAS58500[n]	19.5 (26)	N.I.	N.I.	[c]
N.I.	N.I.	N.I.	C_10209, ASA5	14.3 (0)	N.I.	N.I.	[c]
N.I.	N.I.	N.I.	C_50224, ASA6	13.3 (27)	N.I.	N.I.	[c]
Assembly Factors							
N.I.	ATP10	AAF18252	N.I.				
ATPAF1	ATP11	At2g34050	C_150089, ATP11	23.5	25	29	
ATPAF2	ATP12	At5g40660	C_10143, ATP12	30.6	27	30	
N.I.	ATP13 (AEP2)	N.I.	N.I.				
N.I.	ATP22 (TCM10)	N.I.	N.I.				
N.I.	AEP1, AEP3	N.I.	N.I.				
N.I.	FMC1	N.I.	N.I.				
Alternative Oxidase Family							
N.I.	AOX - Q9Y711[u]	At3g22370 (AOX1a), At3g22360 (AOX1b), At3g27620 (AOX1c), At1g32350 (AOX1d), At5g64210 (AOX2)	C_330029, AOX1, AAC05743 C_340013, AOX2, AAG02081	38.4 37.6	N.I. N.I.	~40 ~40	
Type-II NAD(P)H Dehydrogenase Family							
N.I.	NDAe1	At1g07180 (NDA1)	C_310108, NDA1	58.7	N.I.	nd	
	NDAe2	At2g29990 (NDA2)	C_1890016, NDA2[p]	nd	N.I.	nd	
	NDAi1	At4g28220 (NDB1),	C_1170009, NDA3	77.3	N.I.	nd	
		At4g05020 (NDB2),	C_5950001, NDA4[p]	nd	N.I.	nd	
		At4g21490 (NDB3),	C_820024, NDA5[p]	nd	N.I.	nd	
		At2g20800 (NDB4),	1450028, NDA6[j]	nd	N.I.	nd	
		At5g08740 (NDC1)	C_1450029, NDA7[j]	nd	N.I.	nd	

[a]Cardol et al. (2004). [b] van Lis et al. (2003). [c] Funes et al. (2002). [d] P. Cardol and C. Remacle (unpublished data). [e] Atteia (1994) and Atteia et al. (2003). [f] Mitochondria-encoded subunit. [g] When no gene model is available, scaffold number and positions are given (e.g. 6:450163–451396). [h]Rice sequence accession number. [i]Sequences are 45% identical. [j]The gene model is probably wrong. [k]The comparison of the amino acid hydropathy profiles does not support the protein alignment (Cardol et al., 2004). [l]Overlaps with C_710033 (the gene model probably results from a misassembly). [m]Erroneously assigned in GenBank as Complex V-ATPI subunit. [n]Identified independently both as a complex I component (Cardol et al., 2004) and as a complex V component (Funes et al., 2002). [o]Subunit SDH1 is apparently split into 2 parts, but, unfortunately, 1 of the sequences (C_240095) is incomplete, and since both gene fragments are separated by only 42 kb in the same scaffold (scaffold_24), the apparent gene splitting most probably represents a sequence assembly artefact and not a real heterodimeric SDH1 subunit. [p]Incomplete sequence. [q]Homologous sequences were, however, identified in nematodes (*Caenorhabditis elegans* NP_497099), insects (*Drosophila melanogaster* NP_611235), and fish (*Tetraodon nigroviridis* CAF92199). [r]ATP9A and ATP9B gene models are adjacent on the genome and divergently transcribed (scaffold_108:57000–61000). [s]Matches 3′ end of ATP2 gene for mitochondrial ATP synthase β chain. [t]The mitochondrial targeting presequence of the subunit the Rieske iron-sulphur protein is processed after insertion into the cytochrome bc_1 complex in mammals and retained as a subunit IX in the complex (Brandt et al., 1993). [u]*Pichia stipitis* sequence accession number.

closely related to NUOS4), one (NUO21) is present in higher plants and fungi but has no counterpart in the bovine enzyme (Table I). Five subunits seem to be common to higher plants (Heazlewood et al., 2003a) and Chlamydomonas only (Cardol et al., 2004): NUOP1 (GenBank/AAS58501), NUOP3 (GenBank/AAS58502), and the three so-called ferripyochelin-binding proteins FBP1, FBP2, and CAH9 (GenBank/AAS48195, AAS48196, and AAS48197) that are related to the γ carbonic anhydrase (Parisi et al., 2004). Furthermore, three subunits, NUOP4 (GenBank/AAS58498), NUOP5 (GenBank/AAS58503), and NUOP6 (GenBank/AAS58500), could be specific of the green alga lineage (Cardol et al., 2004).

The presence of chaperones involved in the assembly of complex I has also been investigated. To date, only two chaperones, the CIA30 and CIA84 proteins, have been described in *N. crassa* (Kuffner et al., 1998; Table I). The homologous gene of the CIA30 chaperone is present in human (Janssen et al., 2002), higher plants (Arabidopsis/At1g72420), and Chlamydomonas (*Chlamy v2.0*/C_50184), whereas we did not find any homolog of CIA84 in other organisms (Table I).

COMPLEX II

Complex II, or succinate:ubiquinone oxidoreductase (EC 1.3.99.1), is the respiratory-chain complex enzyme with the lowest molecular mass. This complex is considered as a bifunctional enzyme that participates both in the mitochondrial electron-transport chain and in the Krebs cycle. Classically, it contains only four polypeptides, all encoded in the nucleus: the flavoprotein SDH1 subunit, the iron-sulfur SDH2 subunit, and two hydrophobic membrane anchors, the SDH3 and SDH4 subunits. The corresponding genes are found in the Chlamydomonas genome database (Table I).

As judged by BN-PAGE, complex II from Arabidopsis contains four additional subunits of unknown function. Two of these subunits have been identified (Arabidopsis/At1g47420 and At1g08480; Eubel et al., 2003). Whereas no homolog of At1g08480 gene product has been found in the Chlamydomonas genome, the Arabidopsis/At1g47420 gene product shares 50% and 31% identities with the Arabidopsis/At1g47260 and the *Chlamy v2.0*/C_160093 gene products, respectively. Surprisingly, these two proteins are complex I components (Heazlewood et al., 2003a; Cardol et al., 2004) and are structurally related to carbonic anhydrases (Parisi et al., 2004).

Only one complex II chaperone, the Tcm62 protein related to the Hsp60 chaperone, has been identified in mitochondria of *S. cerevisiae* (Dibrov et al., 1998). This chaperone could be involved in mitochondrial gene expression at elevated temperatures (Klanner et al., 2000). We did not find any counterpart of Tcm62 in the databases of animals, algae, or plants.

COMPLEX III OR BC_1 COMPLEX

The mitochondrial complex III (ubiquinol-cytochrome *c* oxidoreductase; EC 1.10.2.2) from mammals, yeast, and higher plants is an oligomeric membrane protein complex made of 10 highly conserved subunits (Braun and Schmitz, 1995; Iwata et al., 1998; Eubel et al., 2003).

The *C. reinhardtii* bc_1 complex has been isolated and found to be composed of 9 subunits, with molecular masses ranging from 10 to 50 kD (Atteia, 1994; van Lis et al., 2003). Among these polypeptides, subunit core I (53 kD) has been identified by N-terminal sequencing and corresponds to the *Chlamy v2.0*/C_1290016 sequence (Table I). Before the genomic approach, the sequences of the mitochondrial cytochrome *b* gene (GenBank/NC_001638; Gray and Boer, 1988), the nuclear genes encoding the Rieske iron-sulfur protein (RIP1;GenBank/AJ320239), and the cytochrome c_1 (GenBank/AJ417788) have also been characterized (Atteia et al., 2003). The search of the genome sequences of *C. reinhardtii* allowed us to find the 10 classical subunits of complex III: core I and core II proteins (QCR1 and QCR2), cytochrome *b*, cytochrome c_1, RIP1, and the five additional subunits: QCR7 (equivalent to the bovine 14-kD subunit VI or QP-C), QCR8 (equivalent to bovine 9.5-kD subunit VII or ubiquinol-binding protein), QCR6 (equivalent to the bovine 7.8-kD subunit VIII or hinge protein), QCR9 (equivalent to the bovine 7.2-kD subunit X), and QCR10 (equivalent to bovine 6.4-kD subunit XI; Table I). The *C. reinhardtii* putative QCR8 and QCR10 protein sequences present a very low identity with the corresponding sequences of the other eukaryotes, but using a window of seven residues (Kyte and Doolittle, 1982), we have found good similarities between the hydropathy profiles (data not shown), which supports the idea that all these proteins are orthologs. Thus, on the basis of this putative subunit composition, the complex III of *C. reinhardtii* would be very similar to the complex III of the other eukaryotes.

The QCR1 and QCR2 homologs from higher plants possess a proteolytic activity (mitochondrial processing peptidase, or MPP) that cleaves the transit peptide of preproteins when imported into mitochondria (Glaser and Dessi, 1999). In Chlamydomonas, QCR1 exhibits consensus sequences typical of β-MPP proteins, while QCR2 does not share consensus sequences for α-MPP activity (van Lis et al., 2003). Nevertheless, more than two polypeptide bands are resolved in the core region of Chlamydomonas complex III in two-dimensional SDS-PAGE after BN-PAGE (R. van Lis, A. Atteia, and D. González-Halphen, unpublished data). Therefore, a putative isoform of QCR2 (*Chlamy v2.0*/C_10187) could be responsible for catalyzing the α-MPP activity.

Homologs of proteins ABC1, CBP3, and BCS1 that act in yeast as chaperones essential for proper conformation and functioning of the complex (Bousquet et al., 1991; Nobrega et al., 1992; Cruciat et al., 1999;

Shi et al., 2001) are present in the Chlamydomonas sequence database (Table I). Besides these chaperones, several proteins of *S. cerevisiae* were found to be required for proper expression, maturation, assembly, and stability of cytochrome *b* (CBP1, CBP2, CBP6, CBS1, and CBS2; Dieckmann et al., 1982; McGraw and Tzagoloff, 1983; Dieckmann and Tzagoloff, 1985; Rodel, 1986) or would be involved in the assembly of complex III (CBP4; Crivellone, 1994). None of these proteins was found to possess counterparts in animals or plants (Table I).

CYTOCHROME *C*

The mitochondrial cytochrome *c* from *C. reinhardtii* is encoded by a single nuclear gene (*Swissprot*/S29514), and its structure is very similar to that of higher plant cytochromes *c* (Amati et al., 1988).

Three distinct pathways of cytochrome *c* biogenesis have been described (Kranz et al., 1998). System I occurs in mitochondria from higher plants and protozoa. System II takes place in many gram-positive bacteria, in cyanobacteria, and in chloroplasts from higher plants and green algae. System III involves heme lyase enzymes in yeasts (CYC3 and CYT2) and animals (CCHL). Whereas no system I counterparts could be identified, three putative homologous enzymes of system III were found to be encoded in the Chlamydomonas genome (Table I).

COMPLEX IV

In yeast (Taanman and Capaldi, 1992) and mammals (Yanamura et al., 1988), complex IV (cytochrome *c* oxidase; EC 1.9.3.1) is composed of 12 or 13 well-characterized subunits. In Arabidopsis, the enzyme is made of 10 to 12 subunits, but only 7 have been identified (Eubel et al., 2003; Table I).

Most generally, COX1 (binding heme *a*, heme a_3, and Cu_B), COX2 (binding Cu_A), and COX3 subunits, which form the catalytic core of the enzyme, are encoded in the mitochondrial genome, whereas the other subunits, which are regulatory proteins, are encoded in the nucleus. Exceptions to this situation exist, however: in some legumes only subunits COX1 and COX3 are mitochondria encoded (Daley et al., 2002), whereas in *C. reinhardtii* (Michaelis et al., 1990) and in *Polytomella parva* (Fan and Lee, 2002), COX1 is the only subunit encoded in the mitochondrial genome.

The Chlamydomonas cytochrome *c* oxidase complex was resolved by BN-PAGE into 10 polypeptides of molecular masses ranging from 8 to 40 kD (van Lis et al., 2003). However, only eight coding sequences were found in the Chlamydomonas sequence database (Table I). Six encode homologs of proteins found in other eukaryotic complexes IV (orthologs of bovine subunits COX1, 2, 3, 5b, 6a, and 6b), whereas a seventh one (*Chlamy v2.0*/C_110052) encodes the homolog of the plant-specific COX5c subunit (Jansch et al., 1996; Hamanaka et al., 1999). We have also found the sequence corresponding to COX90 (GenBank/AAM88388), a Chlamydomonas-specific protein said to be essential for complex IV assembly and considered to belong to the enzyme complex (Lown et al., 2001).

The subunit composition of Chlamydomonas complex IV deduced from the genome sequencing project is thus quite similar to the one of complex IV from Arabidopsis (Table I). This raises the question whether sequences homologous to bovine COX4, 5a, 6c, 7a, 7b, 7c, and COX8 subunits (fungal COX5–9 subunits) are present in Chlamydomonas and Arabidopsis but are too divergent to be identified by sequence analysis, or whether the cytochrome *c* oxidase composition is actually different in photosynthetic organisms.

Chlamydomonas complex IV additional subunits show some unusual characteristics (R. van Lis, A. Atteia, and D. González-Halphen, unpublished data): (1) the first 60 residues of the mature subunit COX6b are highly hydrophobic and have counterparts in plant sequences, but not in animal or yeast sequences; (2) subunit COX5b lacks the 3 conserved cysteins that are known to bind a zinc atom in cytochrome *c* oxidase from other organisms (Rizzuto et al., 1991); and (3) subunit COX6a shows an N-terminal region with high similarity with COX5a from mammals, while the C-terminal region is more similar to the COX6a subunit.

In *S. cerevisiae*, more than 20 complex IV chaperones have been identified. The COX11, COX17, COX19, COX23, SCO1, and SCO2 proteins play a role in copper delivery to cytochrome *c* oxidase (Nobrega et al., 2002; Barros et al., 2004; Horng et al., 2004; Leary et al., 2004), whereas COX10 and COX15 are required for heme *a* biosynthesis (Glerum and Tzagoloff, 1994; Barros and Tzagoloff, 2002). Homologs of all these proteins are present in *C. reinhardtii*, mammals, and plants (Table I). A number of yeast proteins, such as SHY1, Pet54, Pet111, Pet122, Pet309, Pet494, Mss2, Mss51, COX14, and COX20, are supposed to act as translational activators of COX1, COX2, or COX3 mRNAs (Hell et al., 2000; Broadley et al., 2001; Barrientos et al., 2002, 2004; Naithani et al., 2003; Xu et al., 2004). Except for SUR1, the ortholog of SHY1, and for a protein presenting an Leu-rich pentatricopeptide repeat cassette motif found in Pet309, no homolog of these polypeptides has been identified in *C. reinhardtii* and Arabidopsis sequence databases. Concerning COX16, Pet100, and Pet191, three chaperones with no identified function in yeast (McEwen et al., 1993; Forsha et al., 2001; Carlson et al., 2003), two (COX16 and Pet191) have counterparts in human, Arabidopsis, and Chlamydomonas (Table I). Finally, a partial amino acid sequence that shares similarities with COX18 and OXA1 chaperones has also been found (Table I). These two proteins play a critical role in the biogenesis of yeast cytochrome *c* oxidase and are required for the insertion of various hydrophobic proteins in the inner mitochondrial membrane (He and Fox, 1997; Saracco and Fox, 2002; Funes et al., 2004).

COMPLEX V

The mitochondrial complex V (FoF_1-ATP synthase; EC 3.6.1.3) catalyzes the phosphorylation of ADP by inorganic phosphate using the proton motive force generated by the electron transport chain. The protein complex possesses two domains, the membrane-bound sector Fo involved in proton translocation and the extrinsic domain F_1 that catalyses ATP synthesis. In bacteria, while the Fo sector is composed of three subunits in a ratio ab_2c_{10-14}, the F_1-ATPase contains five subunits in a $\alpha_3\beta_3\gamma\delta\varepsilon$ stoichiometry (Weber and Senior, 2003). The two sectors are connected by two stalks: a central stalk ($\gamma\varepsilon$) that couples proton translocation with the catalytic region and a lateral stalk ($b_2\delta$), which is considered to be part of the stator of the enzyme. The eukaryotic complex V contains homologous components but possesses additional subunits: in mammals, ATPase is made of 16 subunits (Collinson et al., 1994), whereas the yeast enzyme comprises 20 components, 13 being essential for the structure of the complex (Velours et al., 1998; Velours and Arselin, 2000; Table I).

Surprisingly, whereas almost all the mammal ATPase proteins are conserved in Arabidopsis (Heazlewood et al., 2003b; Table I), our search in the Chlamydomonas database led to the identification of only seven homologs. They correspond to subunits ATP5, 6, and 9 of the Fo domain and to subunits α, β, γ, and δ of the F_1 domain (Table I). In other respects, the complex V of Chlamydomonas was found to be composed of 14 subunits of molecular masses ranging from 7 to 60 kD (van Lis et al., 2003). Eight of these subunits were identified (Funes et al., 2002; van Lis et al., 2003, P. Cardol, unpublished data): the seven typical subunits ATP5, ATP6, ATP9, α, β, γ, and δ, and the so-called P60 subunit (61.0 kD; *Chlamy v*2.0/C_420010) or mitochondrial ATP synthase-associated protein (ASA1), which is also present in *Polytomella* sp. (GenBank/AJ558193). ASA1 protein is believed to be responsible for the extraordinary stability of the chlorophycean complex V dimer in BN-PAGE (Atteia, 1994; van Lis et al., 2003). Besides these components, six N-terminal amino acid sequences were determined by Edman degradation (Funes et al., 2002). These partial-polypeptide sequences allowed us to identify the corresponding sequences in the Chlamydomonas sequence database: EESSVANLVKS matches to *Chlamyv2.0*/C_50224, MKLLPESLQQEAA to *Chlamyv2.0*/C_10209, LSTLVEKFTFGSAAD to *Chlamyv2.0*/C_710036, ATGAAPSKK to *Chlamyv2.0*/C_230150, GAPAGSDHDHP to *Chlamyv2.0*/C_750022, and ATATFVPGVSGDASG to *Chlamyv2.0*/C_710028 (Table I). Surprisingly, none of these mitochondrial ATP synthase-associated proteins has a counterpart in other eukaryotic genomes. Since some of the classical subunits missing in Chlamydomonas are associated to the structure of the stator (Velours and Arselin, 2000), one may speculate that these six constituents unique to Chlamydomonas could also play a role as a stator in a structure quite different from the one present in the conventional ATP synthase.

Finally, the Chlamydomonas complex V exhibits two additional peculiar features: (1) in contrast to other organisms, ATP6 is nucleus encoded (Funes et al., 2002); and (2) it could include two isoforms of ATP9 (subunit *c*), since two genes are predicted to encode a *c*-like subunit (Table I). These genes are located adjacent to each other in the genome (scaffold_108:57,000–61,000) and seem to be divergently transcribed (P. Cardol, unpublished data). The N-terminus sequence of the ATP9 subunit (SVLAASKMVGA; van Lis et al., 2003) does not allow us to predict whether only one polypeptide or both of them are present in the mature complex.

To our knowledge, eight assembly factors for complex V have been identified in yeast. Only two of them, ATP11 and ATP12, widely conserved chaperones for the F_1 domain (Ackerman and Tzagoloff, 1990b; Wang et al., 2001), are present in the Chlamydomonas genome (*Chlamy* v2.0/C_150089, C_10143; Table I). In contrast, FMC1, which is required for the assembly or stability of F_1 domain in heat stress conditions (Lefebvre-Legendre et al., 2001); ATP10 and ATP22, two proteins required for the assembly of the Fo sector (Ackerman and Tzagoloff, 1990a; Helfenbein et al., 2003; Tzagoloff et al., 2004); AEP1 and AEP2, both required for correct expression of ATP9 (Payne et al., 1991; Finnegan et al., 1995); and AEP3, which stabilizes mitochondrial bicistronic mRNA-encoding subunits 6 and 8 (Ellis et al., 2004), do not seem to have counterparts in photosynthetic organisms and animals. ATP10 homologous sequences have, however, been identified in higher plants (Arabidopsis/AAF18252, rice/AAT77344).

ADDITIONAL ENZYMES

In addition to the proton-pumping complex I, plant and fungal mitochondria contain several type-II NAD(P)H dehydrogenases. These additional enzymes, located at the surface of the mitochondrial inner membrane and facing either the intermembrane space or the matrix, allow electron transfer from NAD(P)H to ubiquinone. They are single, low-molecular-weight polypeptides that are insensitive to rotenone (Moller et al., 1993). In *S. cerevisiae*, three enzymes have been well characterized (Marres et al., 1991; Luttik et al., 1998), whereas in Arabidopsis, three gene families (*nda*, *ndb*, and *ndc*) with different evolutionary origins encode NADH dehydrogenases that are targeted to mitochondria (Michalecka et al., 2003).

Our searches in the Chlamydomonas sequence database revealed that seven amino acid sequences share similarities with known type-II NAD(P)H dehydrogenases (Table I). Since several genomic sequences present gaps (NDA2, 4, and 5) and since some of the gene models seem to be erroneously predicted (NDA6 and 7), it is difficult to clearly associate these sequences to any of the NADH dehydrogenase families.

Mitochondria from plants, several fungi, and several protists also possess an alternative oxidase (AOX) that drives the electrons from the ubiquinol pool directly to molecular oxygen. This nonphosphorylating enzyme is thought to regulate the mitochondrial respiratory electron flow and to protect plant cells from oxidative damage (Maxwell et al., 1999). In *C. reinhardtii*, evidence for the presence of an AOX has been provided (Matagne et al., 1989; Eriksson et al., 1995) and the expression of two genes, *AOX1* and *AOX2*, has been reported (Dinant et al., 2001). The *AOX1* gene is more actively transcribed than *AOX2* (Dinant et al., 2001) and its expression is strongly dependent on the nitrogen source, being downregulated by ammonium and stimulated by nitrate (Baurain et al., 2003). In contrast to Chlamydomonas, *Polytomella* sp. seems to lack an AOX, maybe in relation to the loss of photosynthesis in this colorless alga (Reyes-Prieto et al., 2002).

CONCLUDING REMARKS

Generally speaking, the OXPHOS components of eukaryotes can be classified into two categories: the core and the supernumerary subunits. The core subunits are the conserved components that usually bind redox components and prosthetic groups and seem to constitute the minimal functional unit of each complex. In general, they have counterparts in the bacterial OXPHOS complexes. The so-called supernumerary subunits may have structural or regulatory functions or a transient role during the biogenesis of each complex. These subunits could additionally be classified into conserved and lineage-specific to distinguish those subunits that are unique to certain species.

Out of 156 protein families that constitute the OXPHOSome or are involved in its biogenesis in eukaryotes, 106 were found to be encoded in Chlamydomonas. Of these algal sequences, 87 have counterparts in mammals, fungi, and higher plants: 65 are subunits of mitochondrial complexes I, II, III, IV, and V, and cytochrome *c*, while 22 correspond to biogenesis factors. The 19 remaining subunits (including additional enzymes) were found in two or three lineages only. Finally, 10 constituents of OXPHOS complexes seem to be unique to Chlamydomonas (Table I). In particular, seven of these algal-specific subunits pertain to the ATP synthase, which makes this enzyme the most divergent and intriguing OXPHOS complex of the algal-respiratory chain.

The recent release of the complete genome sequence of *C. reinhardtii* has thus allowed the construction of a comprehensive catalog of the OXPHOS components of the green alga, comprising 116 proteins. At this stage, however, the exhaustive reconstruction of the Chlamydomonas OXPHOSome is necessarily incomplete due to the following factors: (1) the presence of incomplete sequences, assembly, and sequencing errors in the ongoing *C. reinhardtii* genome sequencing project; (2) the possible presence of other lineage-specific mitochondrial components in the OXPHOSome of Chlamydomonas that escaped identification in database searches; and (3) the very partial biochemical characterization of the OXPHOS complexes in chlorophycean algae. Future biochemical studies will be necessary to get a better view of the OXPHOSome of photosynthetic organisms and of Chlamydomonas in particular.

Sequence data from this article have been deposited with the EMBL/GenBank data libraries under accession numbers given in Table I.

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