

Study of the mitochondrial-respiratory chain complexes from photosynthetic algae

ULg, Liège



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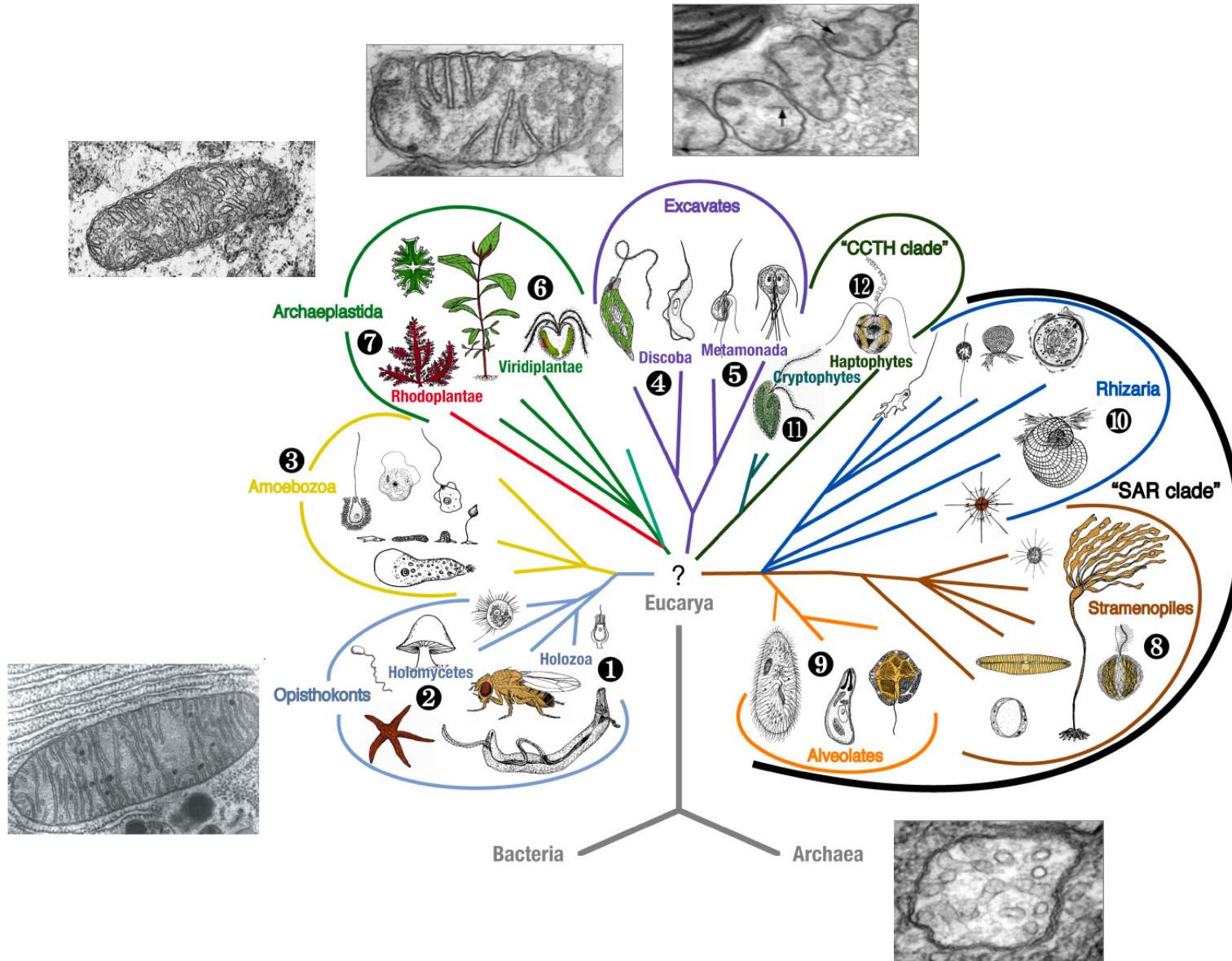


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Mexico 12/02/2013

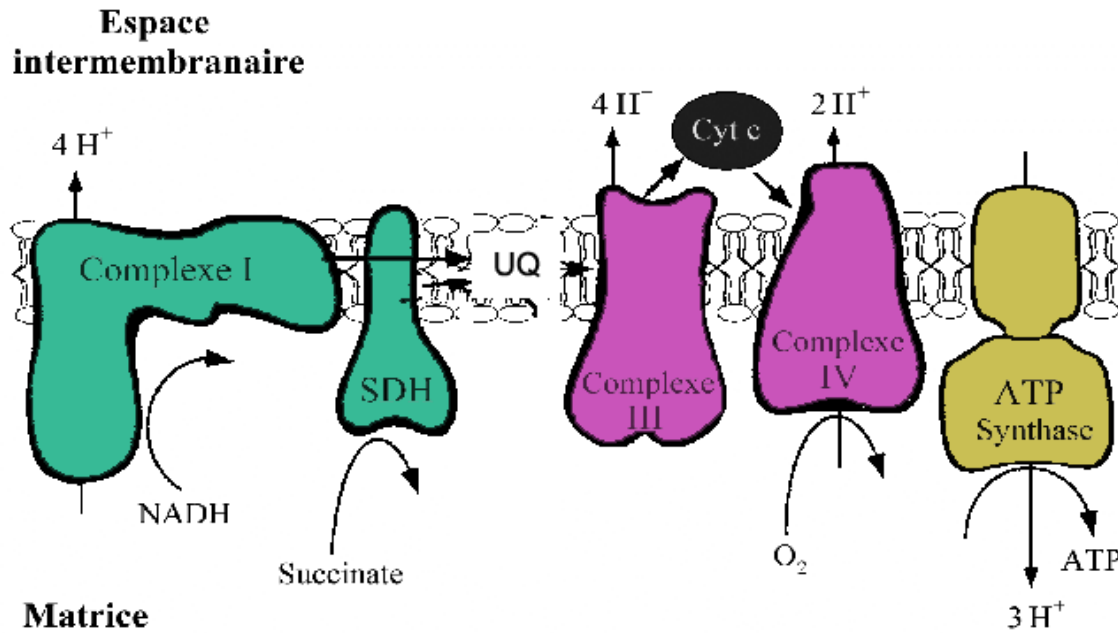
Mitochondria : a common feature in eukaryotes



Mitochondria : function

Anabolic and catabolic pathways

Aerobic respiration



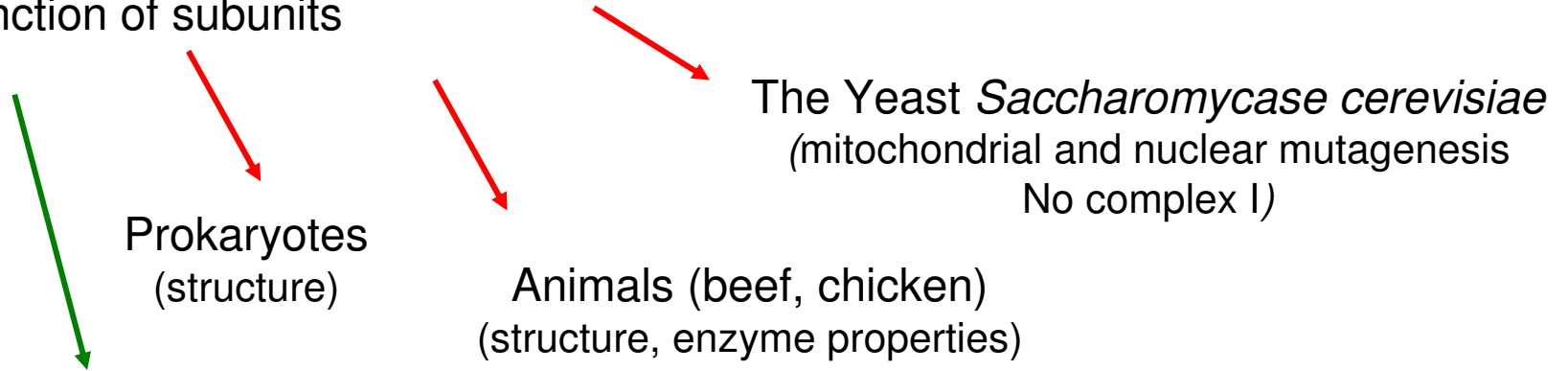
Peter D. Mitchell
The Nobel Prize
in Chemistry 1978

	R.a.	P.w.	M.p.	A.t.	C.r.	B.t.	N.c.	S.c.
Complex I								
nd1	+	+	+	+	+	+	+	-
nd2	+	+	+	+	+	+	+	-
nd3	+	+	+	+	-	+	+	-
nd4	+	+	+	+	+	+	+	-
nd4L	+	+	+	+	-	+	+	-
nd5	+	+	+	+	+	+	+	-
nd6	+	+	+	+	+	+	+	-
nd7	+	+	+	+	-	-	-	-
nd8	+	-	-	-	-	-	-	-
nd9	+	+	+	+	-	-	-	-
nd10	+	-	-	-	-	-	-	-
nd11	+	-	-	-	-	-	-	-
Complex II								
sdh2	+	-	-	-	-	-	-	-
sdh3	+	-	+	-	-	-	-	-
sdh4	+	-	+	+	-	-	-	-
Complex III								
cob	+	+	+	+	+	+	+	+
Complex IV								
cox1	+	+	+	+	+	+	+	+
cox2	+	+	+	+	-	+	+	+
cox3	+	+	+	+	-	+	+	+
Complex V								
atp1	+	+	+	+	-	-	-	-
atp3	+	-	-	-	-	-	-	-
atp6	+	+	+	+	-	+	+	+
atp8	+	-	-	-	-	+	+	+
atp9	+	+	+	+	-	-	+	+

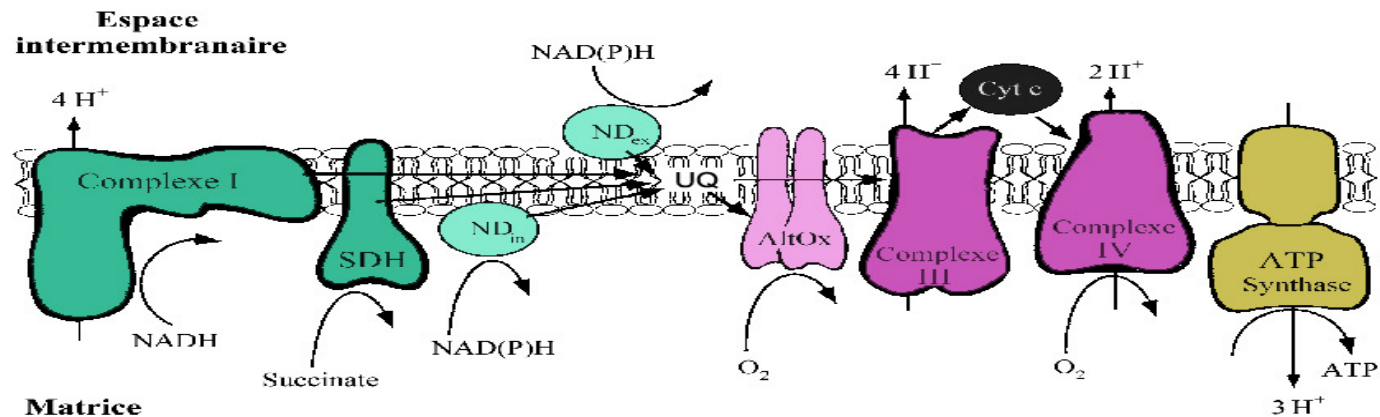
Tableau 1. Constituants de la chaîne respiratoire mitochondriale encodés par le génome mitochondrial chez divers organismes (adapté de Vedel *et al.*, 1999). R.a., *Reclinomonas americana*; P.w., *Prototheca wickerhamii*; M.p., *Marchantia polymorpha*; A.t., *Arabidopsis thaliana*; C.r., *Chlamydomonas reinhardtii*; B.t., *Bos taurus*; N.c., *Neurospora crassa*; S.c., *Saccharomyces cerevisiae*. + ou - indique la présence ou l'absence du gènes mitochondrial.

Aerobic respiration : Model systems

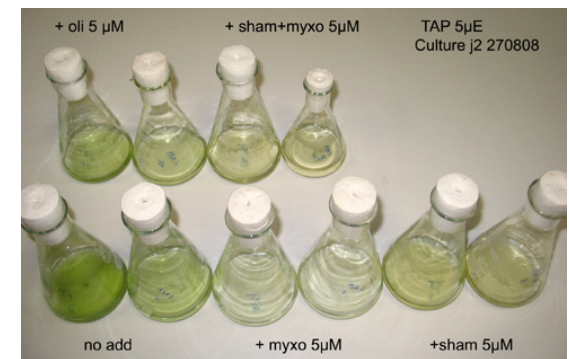
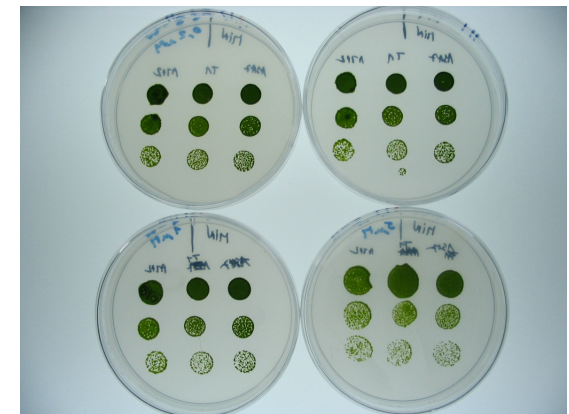
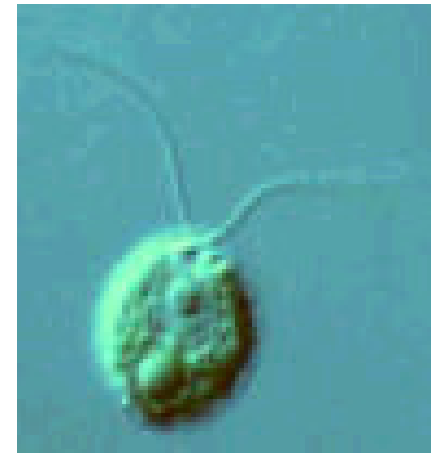
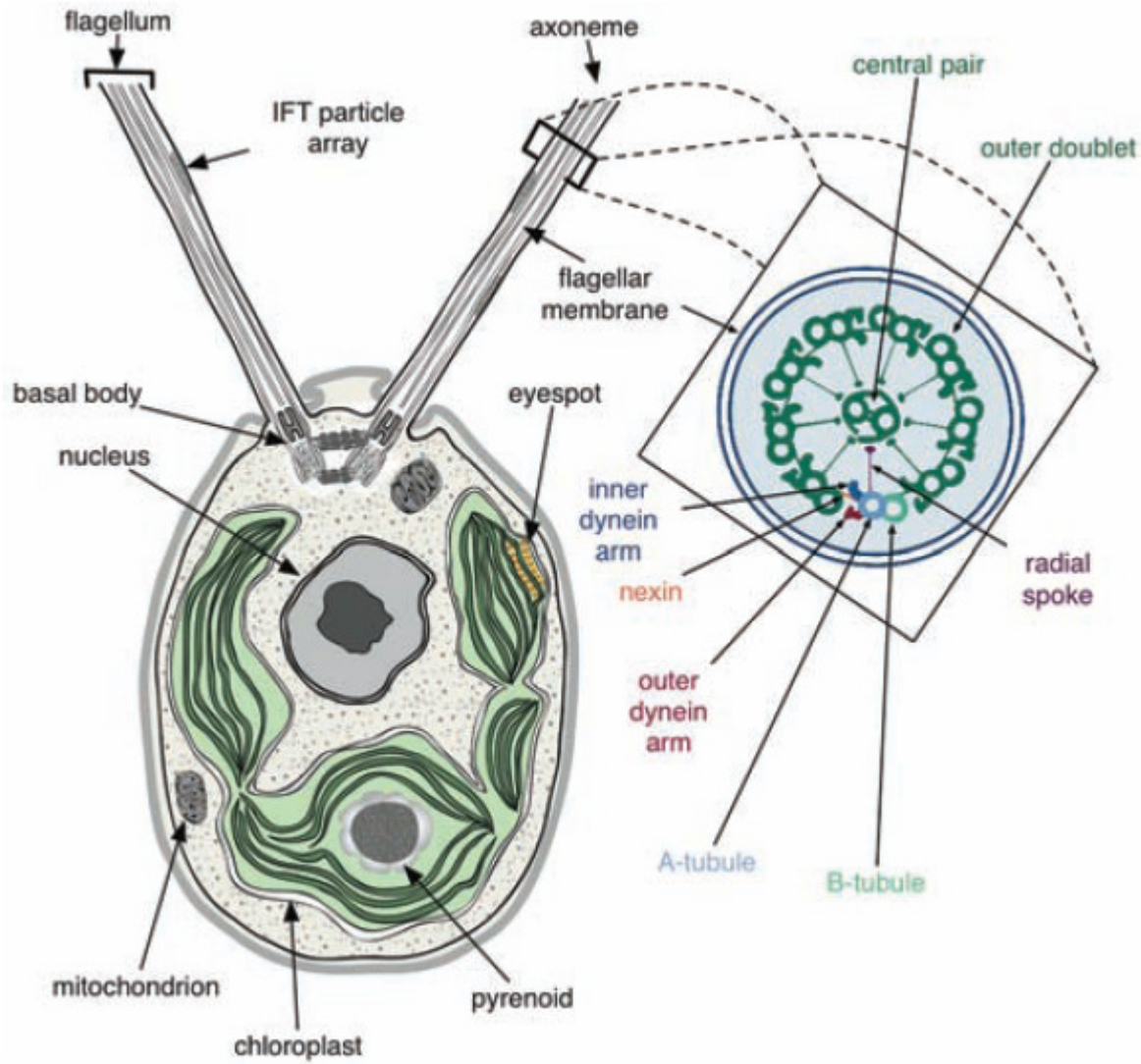
Structures of Complex Enzymes
Function of subunits



Green alga *Chlamydomonas reinhardtii*:
Heterotroph (ability to use acetate in the dark)
Phototroph
Alternative pathways of respiration



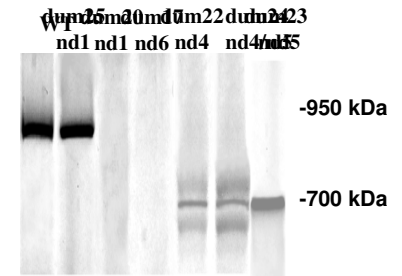
Chlamydomonas reinhardtii



Summary

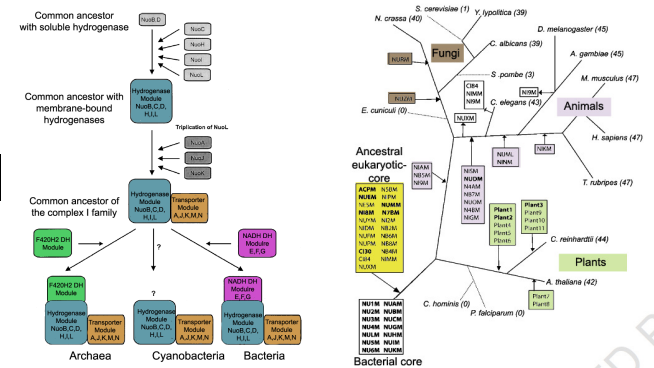
Part I :

Chlamydomonas as a model organism to study the function of Complex I subunits



Part II :

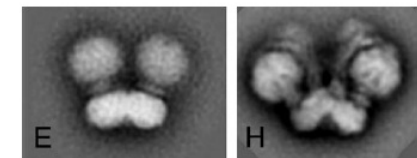
The modular evolution of Complex I



Part III :

Complex V (ATP synthase) evolution in eukaryotes

N.V. Dudkina et al. | FEBS Letters xxx (2006) xxx-xxx



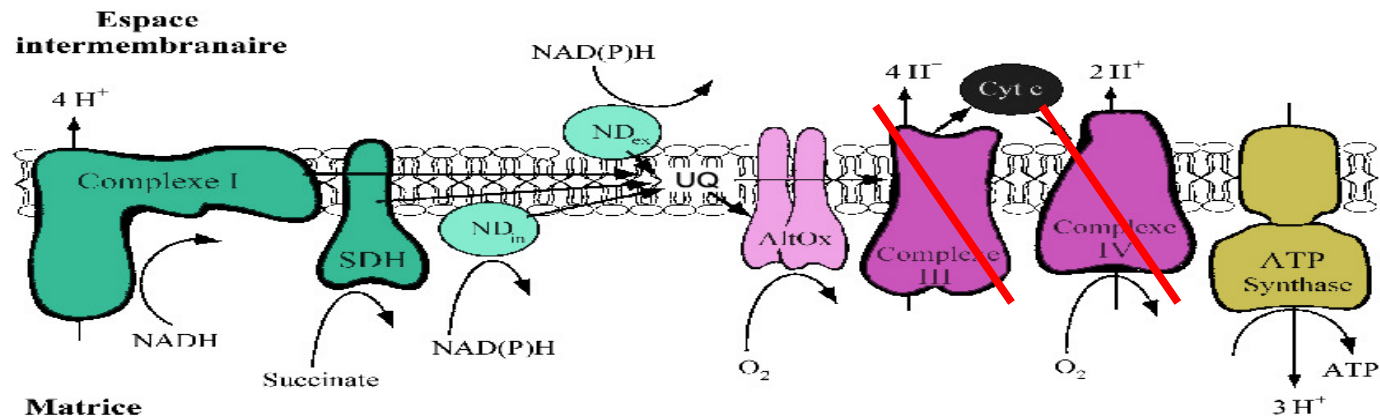
Chlamydomonas mitochondrial mutants : dk- phenotype

NUCLEAR MUTATIONS AFFECTING MITOCHONDRIAL
STRUCTURE AND FUNCTION IN *CHLAMYDOMONAS*

THE JOURNAL OF CELL BIOLOGY · VOLUME 73, 1977 · pages 56-77

ANDREW WISEMAN, N. W. GILLHAM, and J. E. BOYNTON

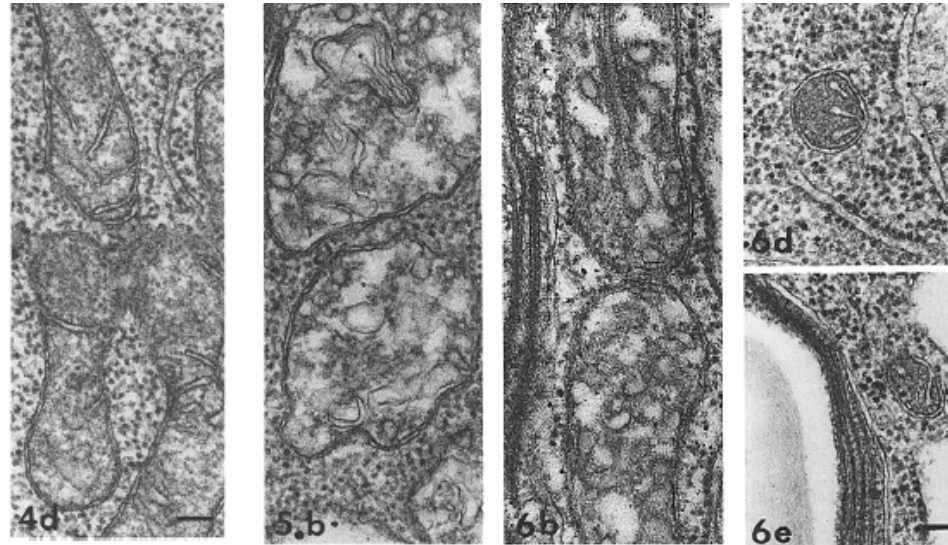
Chlamydomonas mitochondrial mutants : dk- phenotype



Genotype	Fluoroacetate sensitivity	Acetate incorporation	Isocitrate lyase activity	Cyanide-sensitive respiration	Cytochrome oxidase activity	tenone sensitive NADH-cytochrome c reductase	Whole cell ultrastructure (except mitochondria)	Mitochondria	
								Ultrastructure	DAB staining
<i>dk+</i>	yes	+	+	+	+	+	normal	normal	+
<i>dk-32</i>	yes	+	+	±	-	-	normal	grossly altered	-
<i>dk-34</i>	yes	+	+	±	-	-	normal	grossly altered	-
<i>dk-52</i>	no	±	+	+	±	+	reduced electron density of membranes	reduced electron density of membranes	-
<i>dk-76</i>	yes	+	+	±	±	±	normal	normal	-
<i>dk-80</i>	yes	±	+	-	-	+	swollen endoplasmic reticulum	swollen cristae	-
<i>dk-97</i>	yes	±	+	-	-	+	normal	normal	-
<i>dk-105</i>	yes	+	+	±	±	+	normal	normal	+
<i>dk-110</i>	yes	±	+	-	-	+	normal	precipitated matrix material	±
<i>dk-148</i>	yes	+	+	-	±	+	normal	precipitated matrix material	+

The biochemical and ultrastructural properties of nine mendelian *dk* mutants are summarized from Tables V to VII and Figs. 2 to 5, as well as unpublished observations. The symbol +, indicates >75% of wild type levels of activity, the symbol ±, ~25-75% of wild type levels of activity, and the symbol -, <25% of wild type levels of activity.

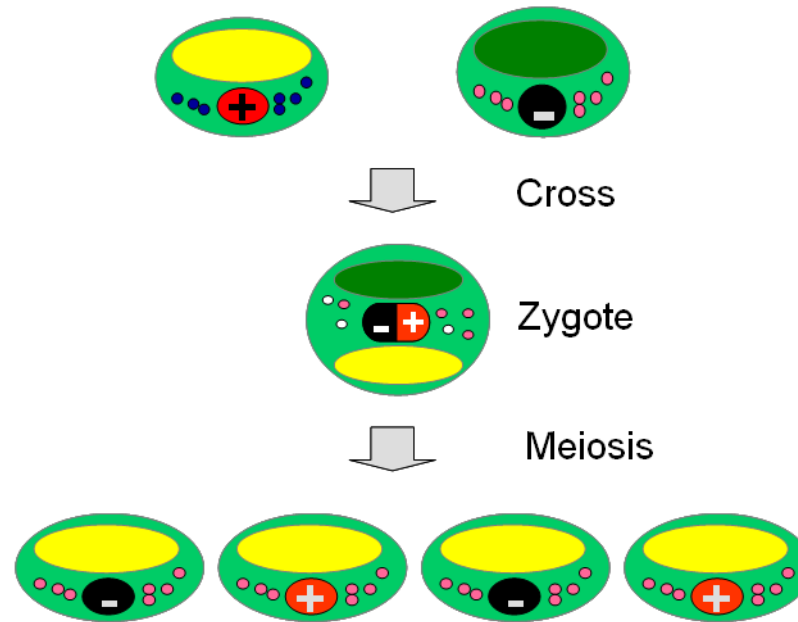
Chlamydomonas mitochondrial mutants : dk- phenotype



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								Ultrastructure	DAB staining
<i>dk+</i>	yes	+	+	+	+	+	normal	normal	+
<i>dk-32</i>	yes	+	+	±	-	-	normal	grossly altered	-
<i>dk-34</i>	yes	+	+	±	-	-	normal	grossly altered	-
<i>dk-52</i>	no	±	+	+	±	+	reduced electron density of membranes	reduced electron density of membranes	-
<i>dk-76</i>	yes	+	+	±	±	±	normal	normal	-
<i>dk-80</i>	yes	±	+	-	-	+	swollen endoplasmic reticulum	swollen cristae	-
<i>dk-97</i>	yes	±	+	-	-	+	normal	normal	-
<i>dk-105</i>	yes	+	+	±	±	+	normal	normal	+
<i>dk-110</i>	yes	±	+	-	-	+	normal	precipitated matrix material	±
<i>dk-148</i>	yes	+	+	-	±	+	normal	precipitated matrix material	+

The biochemical and ultrastructural properties of nine mendelian *dk* mutants are summarized from Tables V to VII and Figs. 2 to 5, as well as unpublished observations. The symbol +, indicates >75% of wild type levels of activity, the symbol ±, ~25-75% of wild type levels of activity, and the symbol -, <25% of wild type levels of activity.

Chlamydomonas mitochondrial genome : Transmission mode and Sequence



Boynton et al., 1987
Matagne et al., 1993

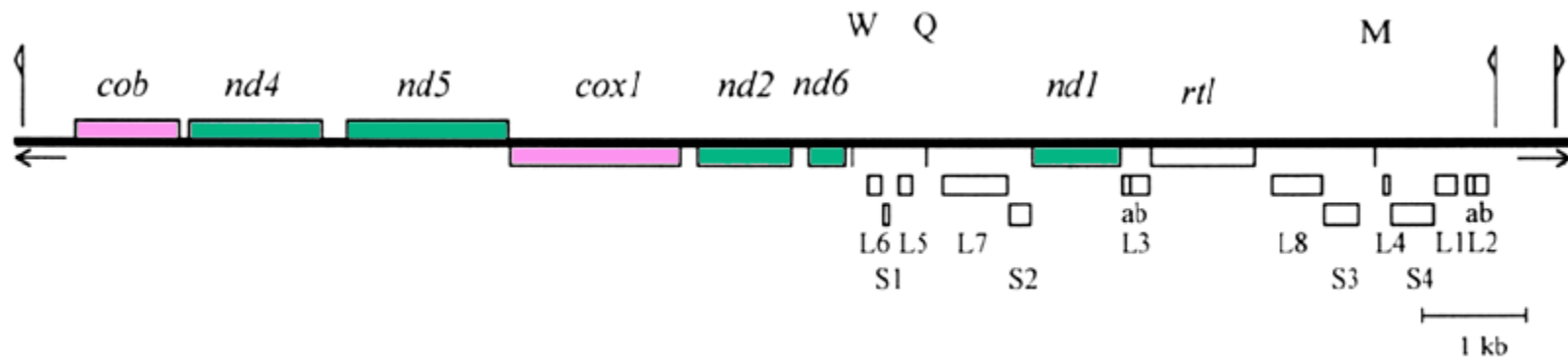


Figure 14. Carte génétique du génome mitochondrial de *Chlamydomonas reinhardtii* (Vahrenholz et al., 1993).

Human mitochondrial genome : 7 genes coding for Complex I subunits

Nature, 1985 Apr 18-24;314(6012):592-7.

Six unidentified reading frames of human mitochondrial DNA encode components of the respiratory-chain NADH dehydrogenase.

Chomyn A, Mariottini P, Cleeter MW, Ragan CI, Matsuno-Yagi A, Hatefi Y, Doolittle RF, Attardi G.

Science 31 October 1986:

Vol. 234, no. 4776, pp. 614 - 618

DOI: 10.1126/science.3764430

URF6, last unidentified reading frame of human mtDNA, codes for an NADH dehydrogenase subunit

A Chomyn, MW Cleeter, CI Ragan, M Riley, RF Doolittle, and G Attardi

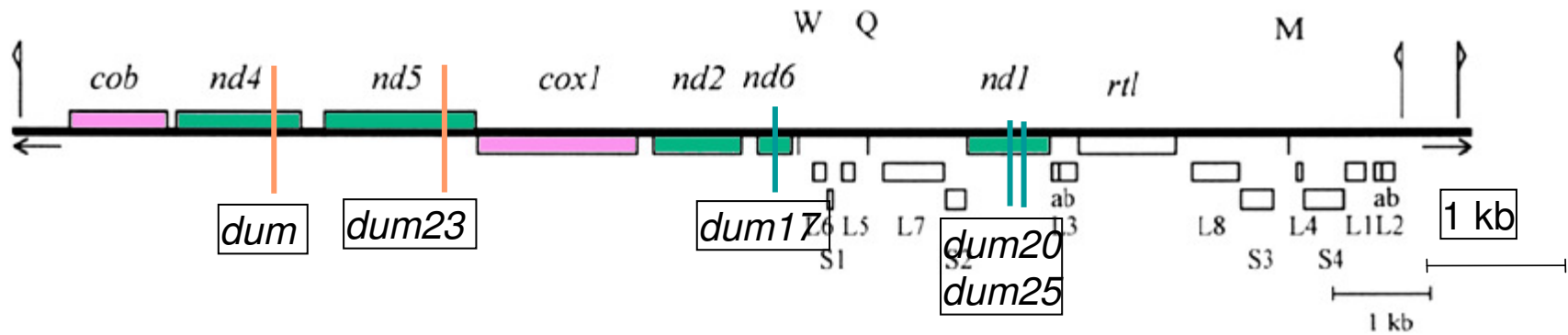
Chlamy Animals Yeast

Complex I	C.r.	B.t.	N.c.	S.c.
<i>nd1</i>	+	+	+	-
<i>nd2</i>	+	+	+	-
<i>nd3</i>	-	+	+	-
<i>nd4</i>	+	+	+	-
<i>nd4L</i>	-	+	+	-
<i>nd5</i>	+	+	+	-
<i>nd6</i>	+	+	+	-

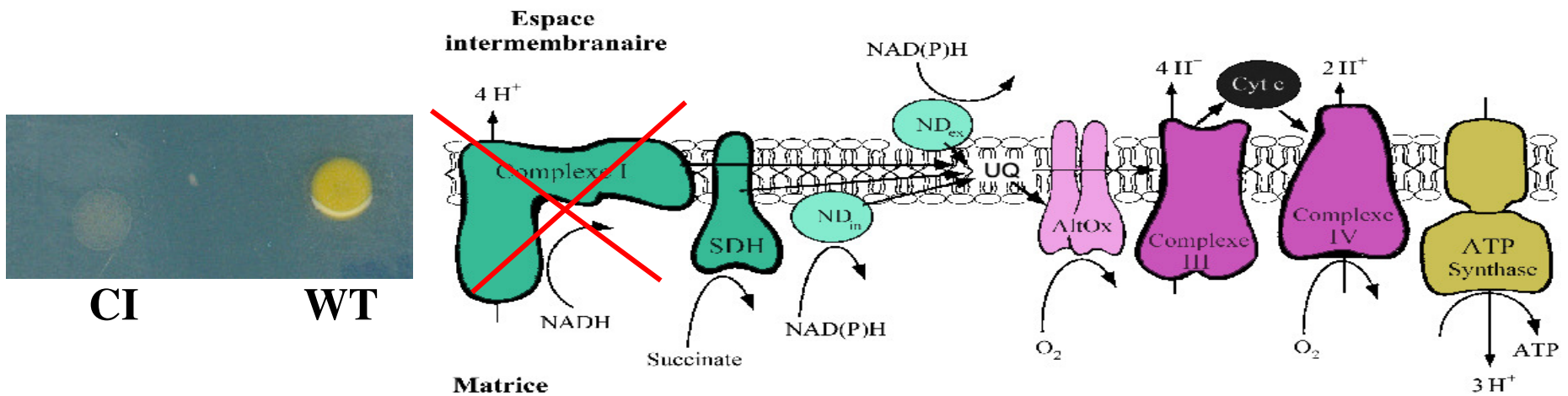
Isolation of mitochondrial complex I mutants

Random mutagenesis

Site-targeted mutagenesis



Remacle et al, 2001, 2006; Cardol et al, 2002, 2008



Study of nucleus-encoded subunits



The Chlamydomonas Genome Reveals the Evolution of Key Animal and Plant Functions
 Sabeeha S. Merchant, *et al.*
Science 318, 245 (2007);
 DOI: 10.1126/science.1143609

EST and raw data already available in 2002

→ ND3, ND4L, ND7 and ND9 are encoded in the nucleus

Complex I	Plants			Animals			Yeast	
	R.a.	P.w.	M.p.	A.t.	C.r.	B.t.	N.c.	S.c.
<i>nd1</i>	+	+	+	+	+	+	+	-
<i>nd2</i>	+	+	+	+	+	+	+	-
<i>nd3</i>	+	+	+	+	-	+	+	-
<i>nd4</i>	+	+	+	+	+	+	+	-
<i>nd4L</i>	+	+	+	+	-	+	+	-
<i>nd5</i>	+	+	+	+	+	+	+	-
<i>nd6</i>	+	+	+	+	+	+	+	-
<i>nd7</i>	+	+	+	+	-	-	-	-
<i>nd8</i>	+	-	-	-	-	-	-	-
<i>nd9</i>	+	+	+	+	-	-	-	-

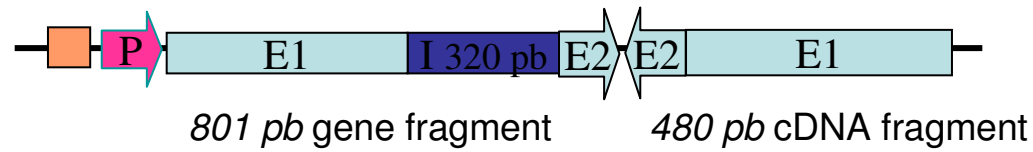
Strategy : RNA interference

Principle

Expression of double-stranded RNA
 Specific degradation of endogenous RNA

Study of nucleus-encoded subunits : RNA interference

Construction of an inactivation vector for *Nd9*



Co-transformation : Arginine auxotrophy (*ARG7*)



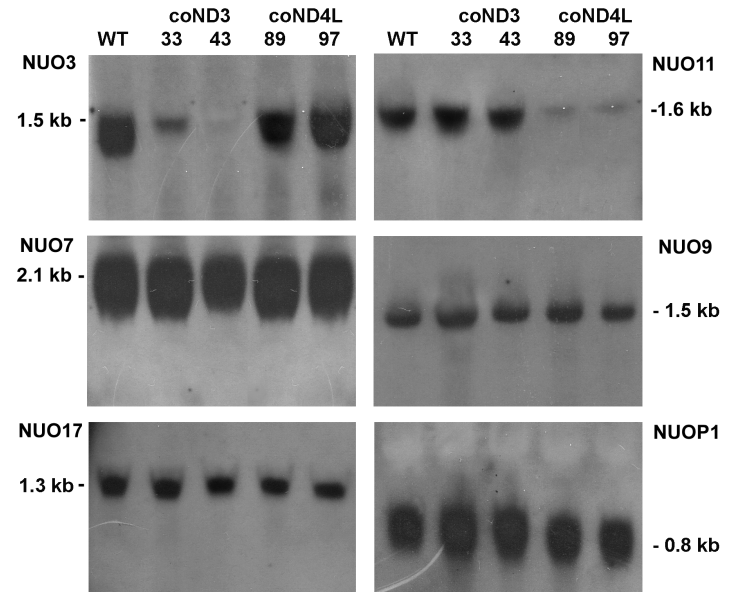
Selection of Arg⁺

Analysis of 100 Arg⁺ transformants by PCR

- Rate of Co-transformation is ~50 %

Complex I activity and assembly

- 3 clones



Complex I assembly

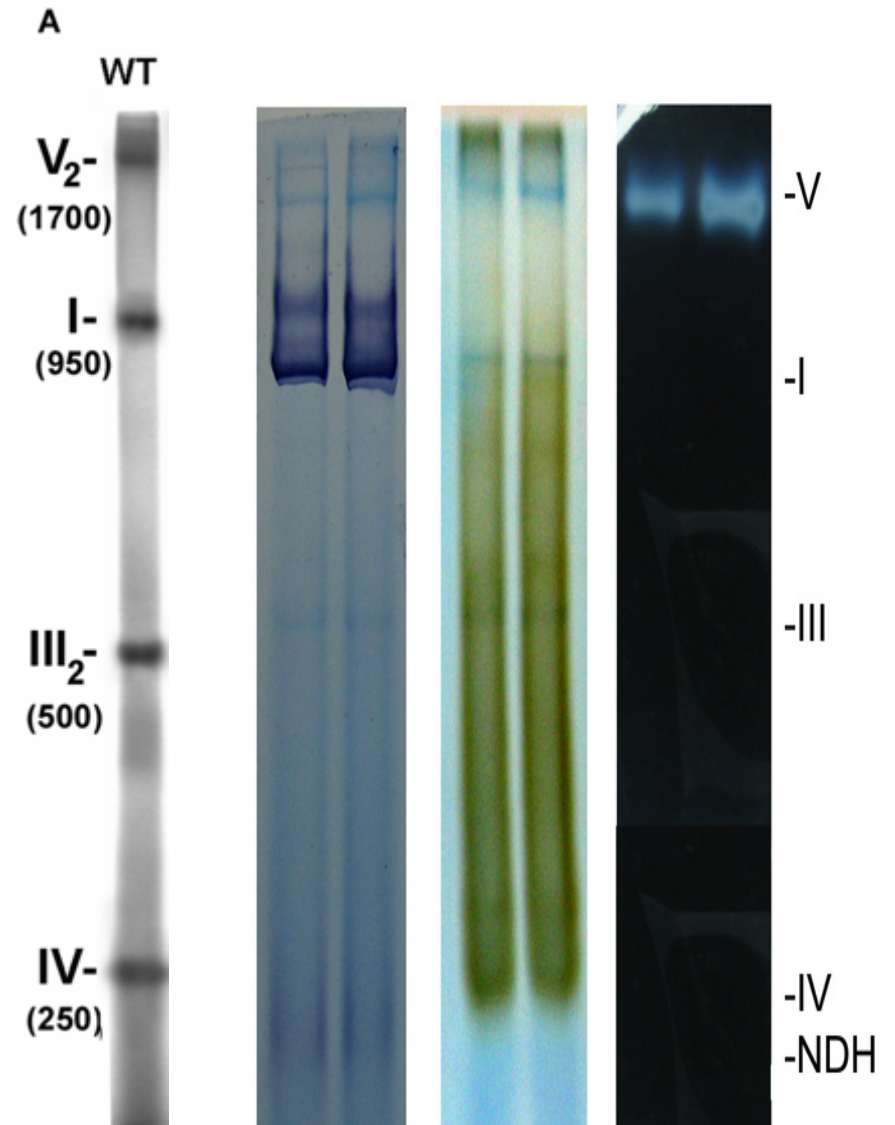
Generation of wall-less strain

Purification of mitochondria

Solubilization by mild-detergent

Blue-Native PAGE

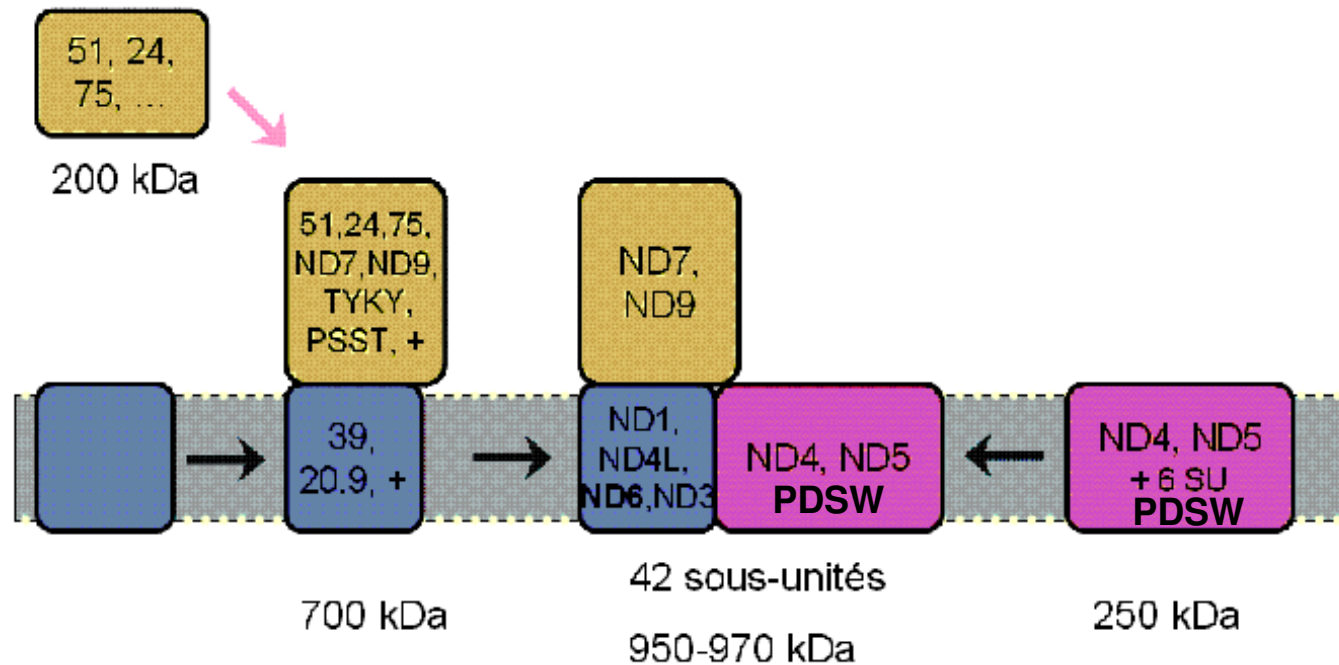
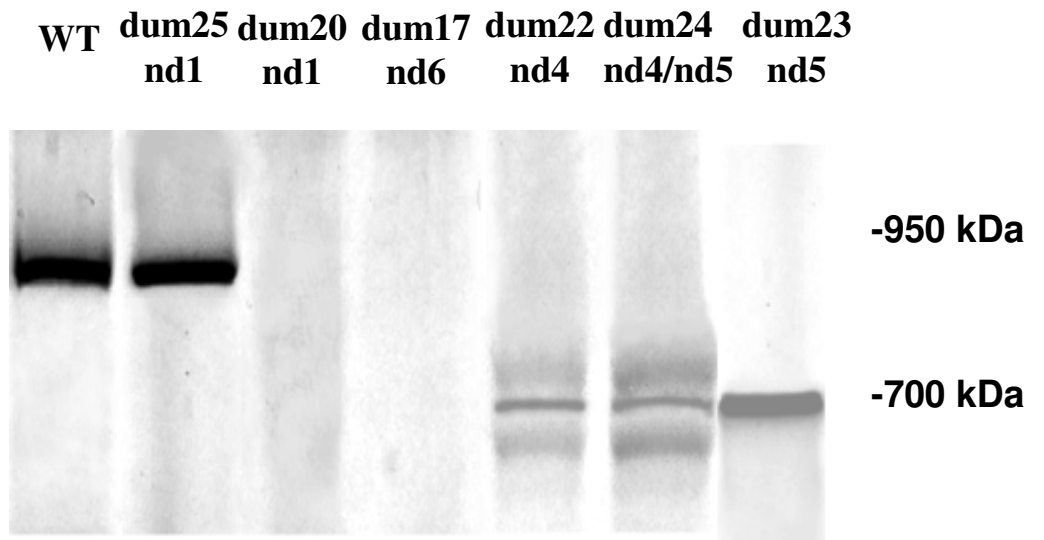
Complex enzyme detection



Assembly of complex I in the mutants

Mutants mitochondriaux		Activité du complexe I
<i>dum5</i>	-1T 3' UTR <i>nd5</i>	12%
<i>dum17</i>	-1T <i>nd6</i> (codons 143-144)	0
<i>dum23*</i>	-1T <i>nd5</i> (codons 145-146)	0
<i>dum20</i>	-1T <i>nd1</i> (codon 243)	0
<i>dum25</i>	-6 NT <i>nd1</i> (codons 199-200)	0
<i>dum22</i>	délétion <i>cob</i> + 3' <i>nd4</i>	0
<i>dum24</i>	délétion <i>cob</i> + <i>nd4</i> + 3' <i>nd5</i>	0
<i>dum28</i>	-69 NT <i>nd4</i>	0
Mutants nucléaires		
<i>Co-Nd3</i>	Inactivation of <i>Nd3</i> expression	0
<i>Co-Nd4L</i>	Inactivation of <i>Nd4L</i> expression	0
<i>Co-Nd7</i>	Inactivation de <i>Nd7</i> expression	0
<i>Co-Nd9</i>	Inactivation of <i>Nd9</i> expression	0
<i>nuo9</i>	Insertion of HygR. Loss of <i>Nd9</i>	0
<i>nuob10</i>	Insertion of HygR (<i>Nuob10/PSSW</i>)	0
<i>nuop4</i>	Insertion of HygR in <i>NUOP4</i> gene	50%

Complex I modular assembly deduced from mutant analysis



Modular Evolution of Complex I - part I : to prokaryote

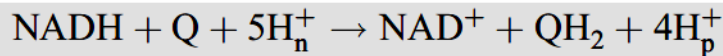
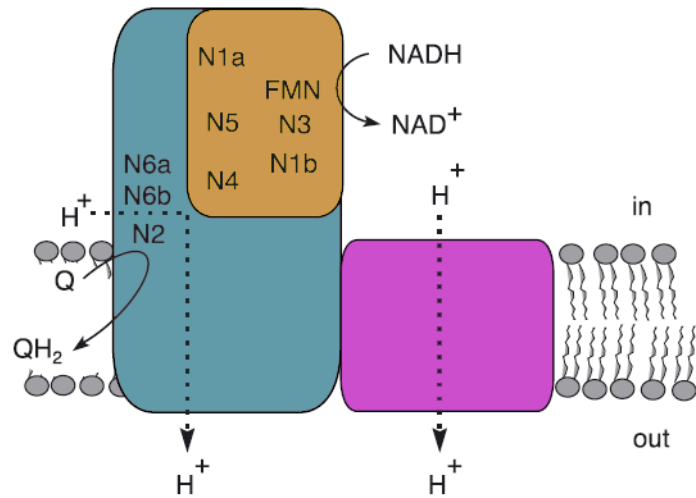
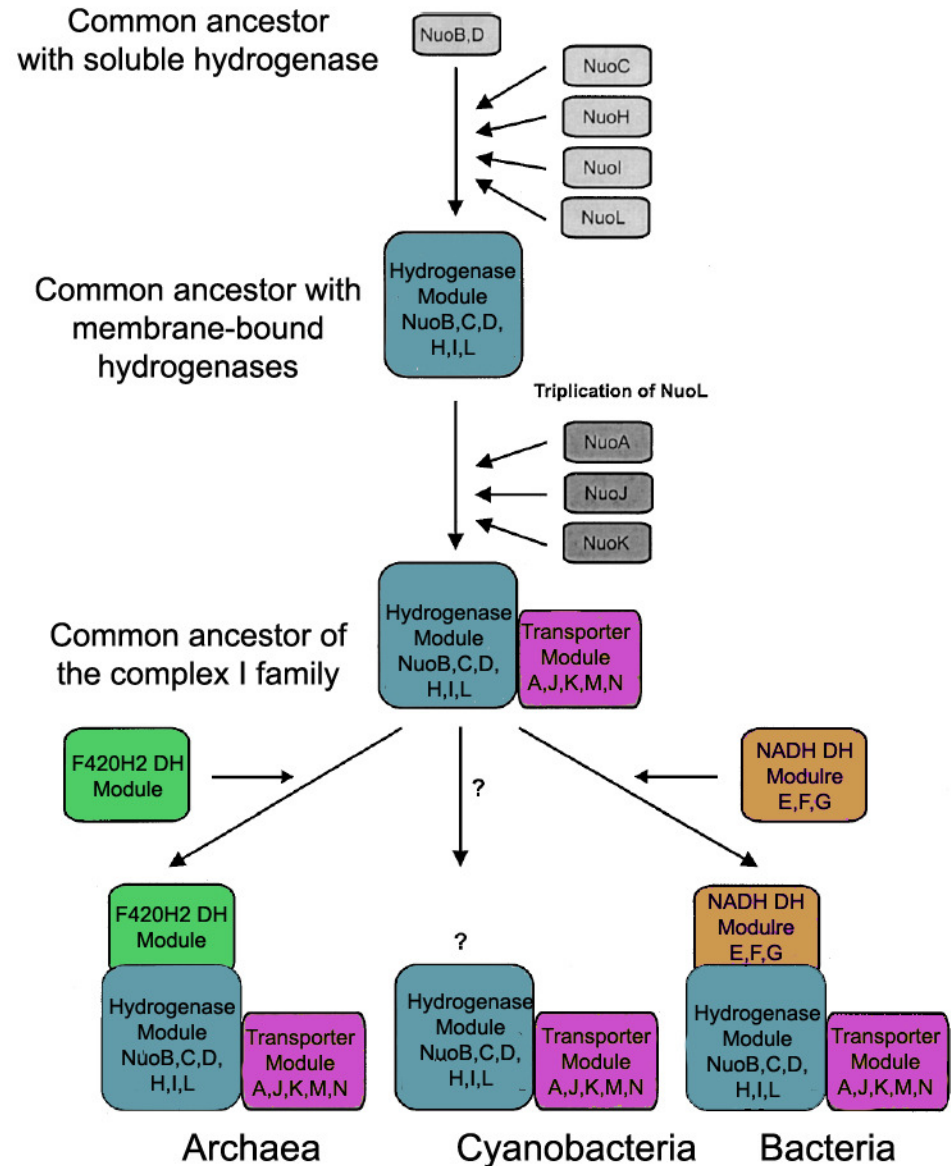


Fig. 2. Cartoon of complex I, showing the three modules. The NADH dehydrogenase module is drawn in yellow, the hydrogenase module in blue and the transporter module in red. The Fe/S clusters are denoted as N1a, N1b, N2, N3, N4, N5, N6a, and N6b. The two proposed coupling sites are indicated, see text for details.

Fig. 1. Hypothetical scheme of the modular evolution of complex I. An ancestral hydrogenase made up by the progenitors of NuoB and D (upper block) evolved by addition of a ferredoxin (NuoI), a transport protein (the progenitor of NuoL, M, and N), a quinone-binding protein (NuoH), and a protein of yet unknown function (NuoC). This led to the formation of the common ancestor of complex I and the membrane-bound hydrogenases. This enzyme was equipped with further proteins by triplication of the transporter protein NuoL and addition of NuoA, J, and K leading to the common ancestor of complex I of bacteria, cyanobacteria, and archaea. The bacterial and the archaeal complex I emerged by acquisition of the NADH dehydrogenase module (NuoE, F, and G) and the F₄₂₀H₂ dehydrogenase module, respectively. The nature of the module adapted to build the cyanobacterial complex is not yet known. It cannot be excluded



Modular Evolution of Complex I - Role of core subunits

Table 1

Nomenclature, localization and properties of the 14 'minimal' complex I subunits

Designation of subunit (Bacteria/Eucarya)	Localization	Predicted function
NuoA/ND3	membrane arm	?
NuoB/PSST	peripheral arm	[4Fe-4S]; N2 ubiquinone-binding?
NuoC/30 kDa (IP) ^a	peripheral arm	?
NuoD/49 kDa (IP) ^a	peripheral arm	Ubiquinone binding?
NuoE/24 kDa (FP)	peripheral arm	[2Fe-2S]; N1a
NuoF/51 kDa (FP)	peripheral arm	NADH- and FMN-binding [4Fe-4S]; N3
NuoG/75 kDa (IP)	peripheral arm	3 × [4Fe-4S]; N1c ^b , N4, N5 [2Fe-2S], N1b
NuoH/ND1	membrane arm	Ubiquinone binding?
NuoI/TYKY	peripheral arm	2 × [4Fe-4S]; N6a, N6b
NuoJ/ND6	membrane arm	?
NuoK/ND4L	membrane arm	Proton translocation?
NuoL/ND5	membrane arm	Ubiquinone binding? Proton translocation?
NuoM/ND4	membrane arm	Ubiquinone binding? Proton translocation?
NuoN/ND2	membrane arm	Proton translocation?

^a NuoC and D are fused in some bacteria.

^b This cluster is only present in some bacteria.

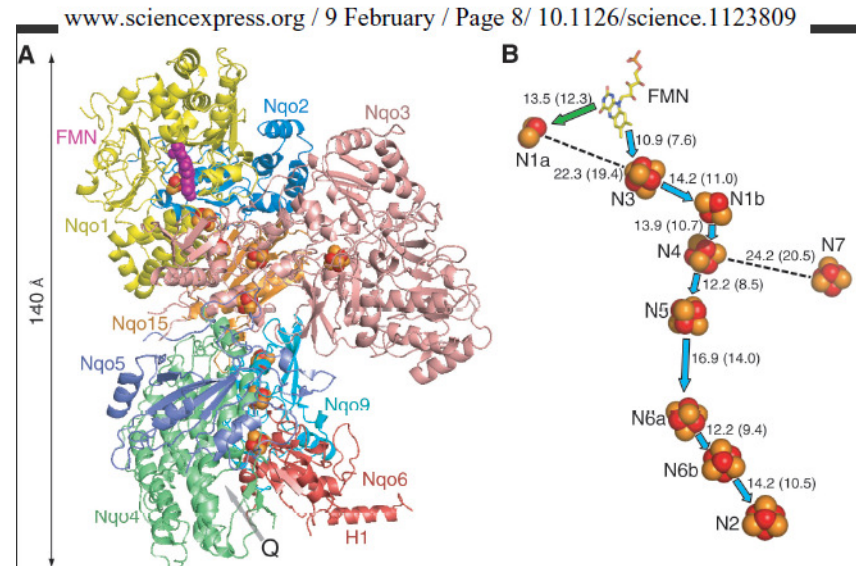
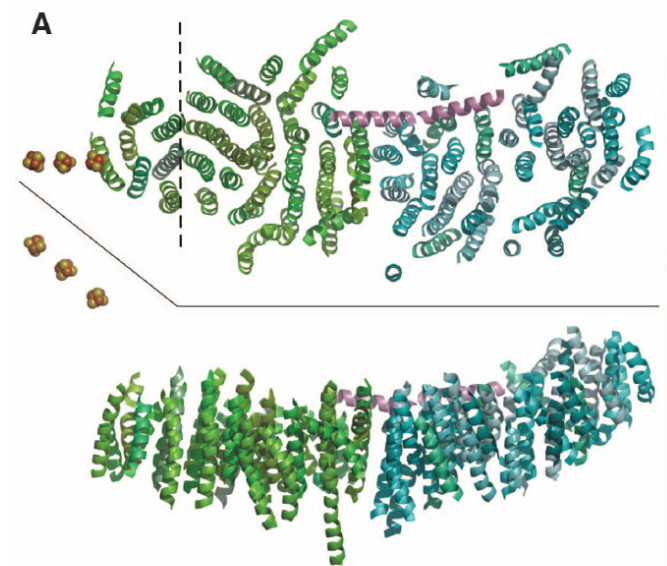
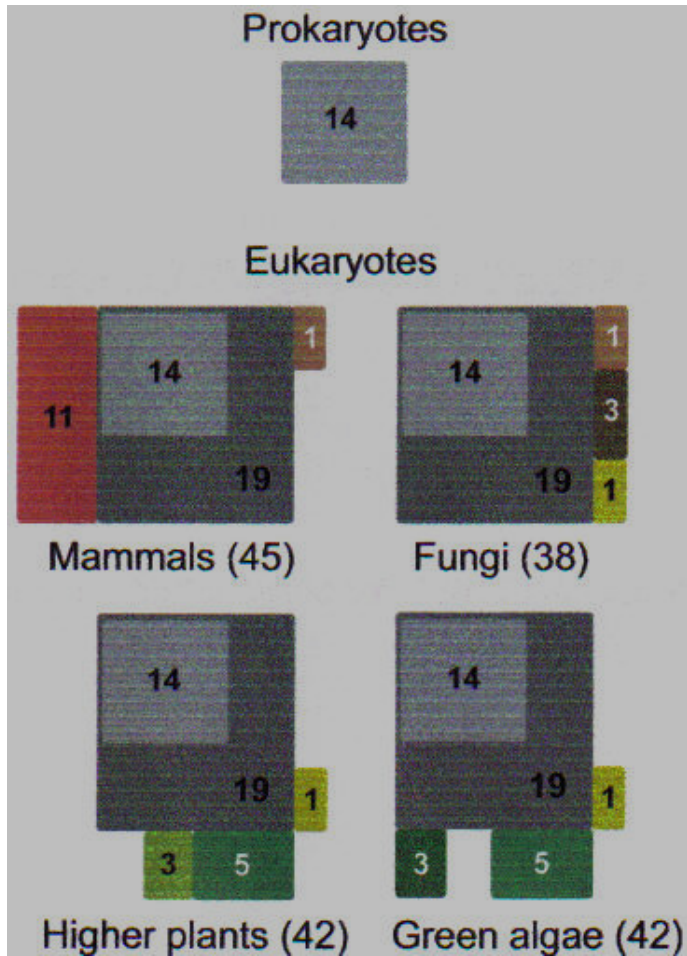


Fig. 2. Transmembrane segments and interface of the membrane arm and the peripheral arm. (A) Top view from matrix side and side view of the α -helical model of the transmembrane segments (upper and lower panel, respectively) fitted with helices arranged in a proximal (green) and a distal (cyan) domain. The border of the docking area with the peripheral arm is indicated by a dashed line (top). The three iron-sulfur clusters of the Q module are shown in space-filled representation. An extended helical element (magenta) may be critical for energy transmission within the membrane arm.

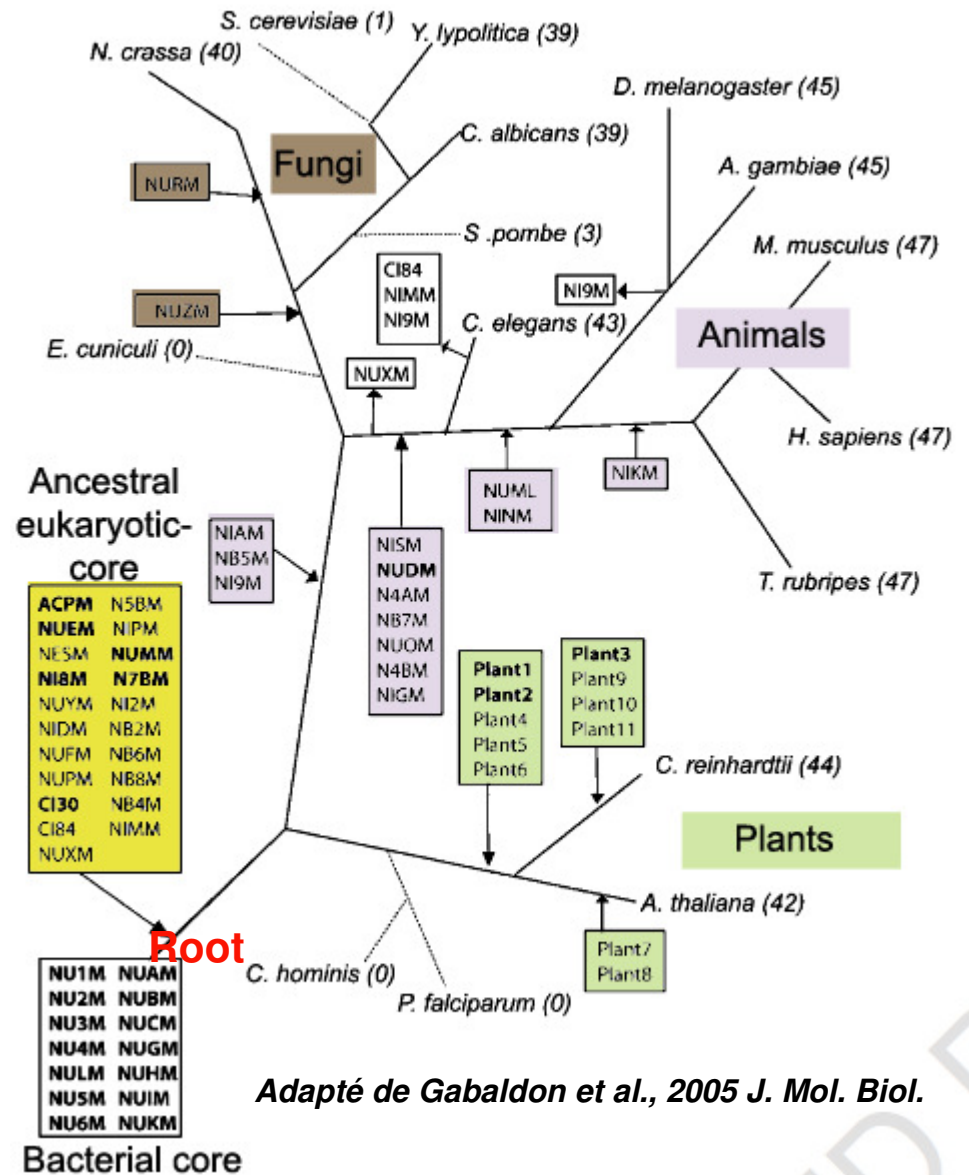


Hunte et al, 2010, Hinchcliffe and Sazanov., 2006, Science; Efremov and Sazanov, 2011, Nature

Modular Evolution of Complex I - part II : to eukaryotes



Adapté de Cardol et al, 2004, BBA bioenergetics

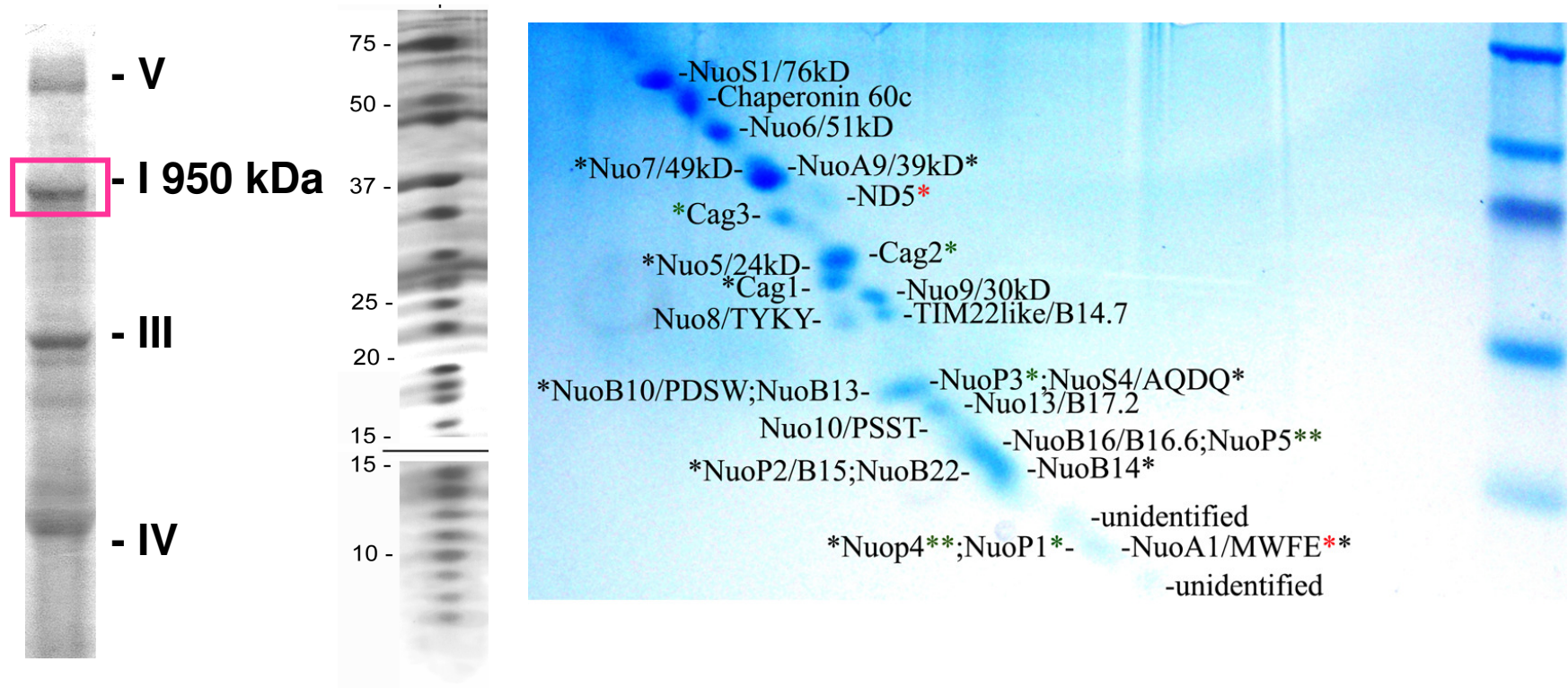


Adapté de Gabaldon et al., 2005 J. Mol. Biol.

Modular Evolution of Complex I - part II : to eukaryotes

Genomic and proteomic characterization of complex I

- Purification of mitochondria from wall-less cells
- Separation of solubilized mitochondrial complexes by BN-PAGE
- Separation of complex I components by SDS-PAGE
- Identification of proteins by mass spectrometry



Identification of orthologs to lineage-specific complex I subunits

1/ % of amino acid identities/similarities

→ BLASTp

→ Psi (Position specific iterative) BLAST (*Altschul et al., 1997*)

-profile on initial multiple alignment

-position specific scoring matrix

-iterative BLAST search

-the results of each "iteration" is used to refine

2/ protein size (+/- 50%)

3/Hydrophathy profiles (Kite and Doolittle scale, Protoscale)

4/ Putative transmembrane helices ?

5/ Structural motifs ?

Identification of orthologs to lineage-specific complex I subunits

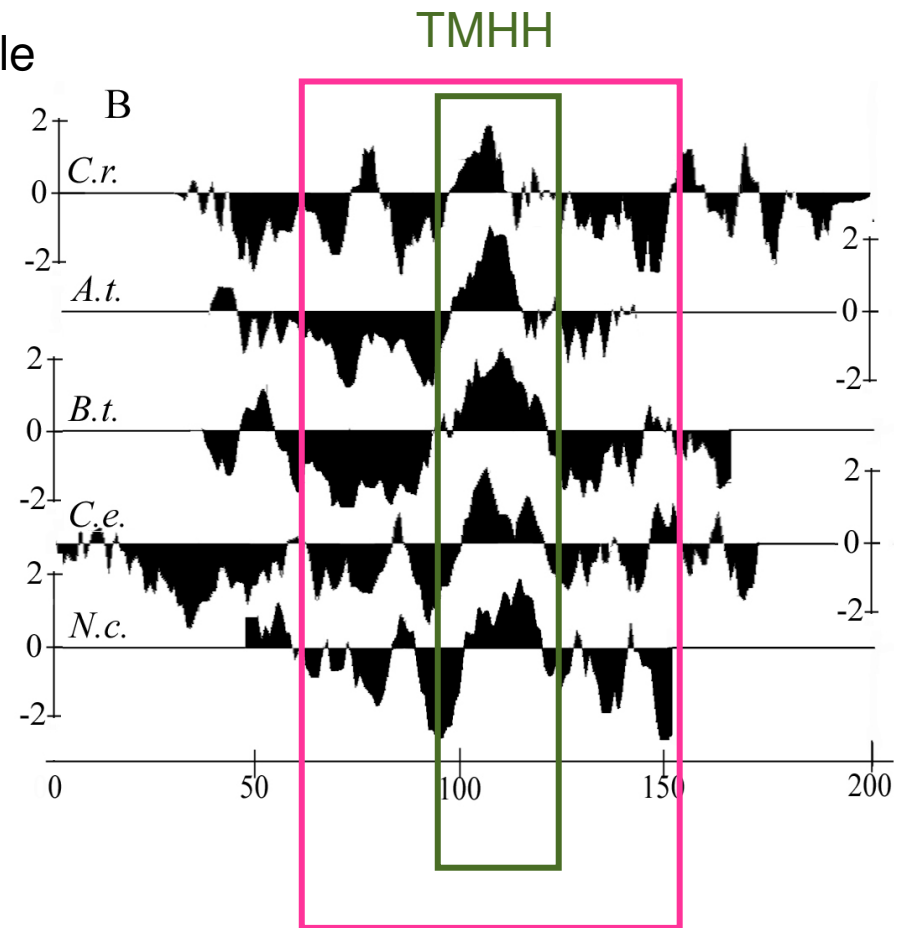
Protein NUO17 is ortholog to
 mammalian complex I ESSS subunit
 Arabidopsis complex I

subunit Fungi NUWM complex I

- Similarity between hydropathy profile
- One conserved putative TMHH

A

Cr	11	R	V	G	A	Q	Y	L	R	N	Q	R	V	R	G	G	G	E	Y	P	G	G	S	F	W	S	E	G	T	Q	T	G	K	N	G	F	L	F	G	E	V	I	N							
At	9	V	A	A	K	T	L	R	N	R	I	H	F	R	S	G	S	T	S	T	G	P	S	R	W	A	T	P	G	H	E	E	R	P	K	G	Y	F	M	N	R	T	E	P	P					
Bt	11	S	S	S	R	A	V	I	A	P	S	T	L	A	C	K	R	P	S	E	P	T	L	R	W	Q	E	D	P	E	P	E	D	E	N	L	Y	E	K	N	P	D	S	H	G	Y				
Ce	45	S	Y	A	Y	E	N	P	W	P	K	L	N	G	R	L	D	W	L	F	G	D	G	W	R	R	P	L	A	K	D	Q	G	A	K	M	R	R	E	W	I	W	F	S	Q					
Nc	3	A	P	T	I	L	R	A	G	A	L	A	S	R	R	A	F	S	T	S	R	A	V	R	S	G	A	P	H	Y	D	P	P	S	G	W	L	F	G	V	R	E	---							
Cr	55	G	Q	P	R	K	T	L	W	E	P	---	Y	W	Y	A	G	F	G	G	M	G	V	G	V	Y	L	I	Y	H	A	K	P	L	E	A	L	D	I	K	Y	W	A							
At	54	G	Q	S	R	K	W	E	D	W	E	L	---	P	C	Y	I	T	S	F	L	T	I	V	I	L	G	V	G	L	N	A	K	---	P	D	L	---	S	I	E	T	W	A						
Bt	56	D	K	D	P	A	V	D	I	W	N	M	---	V	V	F	F	F	G	F	S	I	V	L	V	L	G	S	T	F	V	A	Y	L	---	P	D	Y	---	R	M	Q	E	W	A					
Ce	91	I	A	H	D	E	H	K	D	W	A	R	F	---	H	Q	A	A	F	L	L	F	T	V	L	T	T	W	F	T	C	W	I	M	F	A	R	---	P	D	W	P	M	G	R	E	W	A		
Nc	45	G	E	E	Y	K	R	E	G	W	E	I	---	P	F	F	Y	G	F	C	G	S	F	A	V	A	T	I	A	Y	A	F	K	---	P	D	T	---	S	I	Q	T	W	A						
Cr	97	A	P	R	A	A	K	E	L	E	T	E	M	R	M	L	D	K	L	N	E	R	P	D	L	K	E	R	L	V	A	V	C	K	D	L	N	M	I	E	D	E	A	Y	D	L				
At	95	H	Q	K	A	L	E	R	L	E	M	E	K	L	A	S	A	G	D	S	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
Bt	98	R	R	E	A	E	R	---	L	V	K	Y	R	E	A	H	G	L	P	I	M	E	S	N	C	F	D	P	S	K	I	Q	L	P	E	D	---	---	---	---	---	---	---	---	---	---	---			
Ce	136	L	R	E	A	H	L	E	I	A	R	R	E	K	A	G	L	P	L	I	S	P	D	L	I	P	R	D	R	V	A	A	T	L	P	S	D	E	E	L	R	D	F	D	V	L				
Nc	85	L	E	E	A	R	R	R	L	E	A	E	G	I	L	E	D	P	H	P	E	K	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---



Modular Evolution of Complex I - Supernumerary subunits

2003 : Conserved subunits 27 M/F/P (*Heazlewood et al., 2003*)

2005 : Conserved subunits 32-33 M/F/P, 34 M/F

(*Cardol et al. 2005; Gabaldon et al 2005*)

2010 : *Arabidopsis* : 48 subunits → 34 conserved subunits M/F/P

(*Klodmann et al., 2010*)

→ 14 plant-specific subunits

Pichia pastoris : 41 subunits → 36 conserved subunits M/F

(*Bridges et al., 2010*)

→ 5 ascomycete-specific subunits

→ 9 mammal-specific subunits

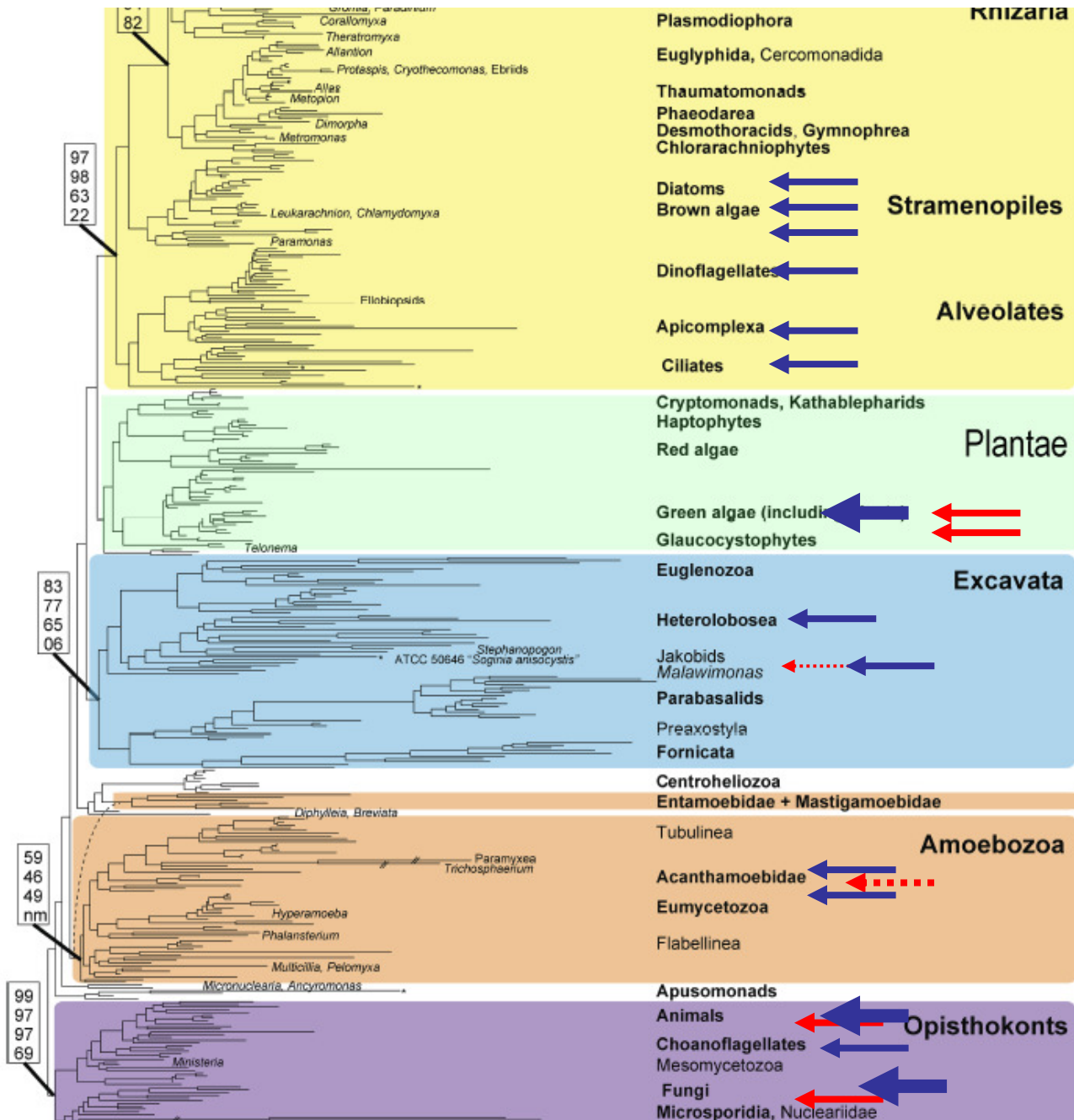
Acanthamoeba : 2 sous-unités « plant specific »

(*Gawryluk and Gray, 2010*)

→ Lineage specific subunits or Orthologs highly divergent ?

→ Most lineage-specific subunit are small hydrophobic proteins (~100 aa)

Modular Evolution of Complex I - part II : to eukaryotes



Downloaded from <http://journals.org>

→ Proteomic data
→ Genomic data

journals.org at University of Liege on August 12, 2010

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) ?

[Clear](#)

Query subrange ?

XP_001697702.1

From

To

Or, upload file

Parcourir... ?

Job Title

nuo17 Chlamydomonas reinhardtii

Enter a descriptive title for your BLAST search ?

 Align two or more sequences ?

Choose Search Set

Database

Non-redundant protein sequences (nr) ?

Organism

Optional

Enter organism name or id--completions will be suggested

 Exclude

+

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown. ?

Exclude

Optional

 Models (XM/XP) Uncultured/environmental sample sequences

Entrez Query

Optional

Enter an Entrez query to limit search ?

Program Selection

Algorithm

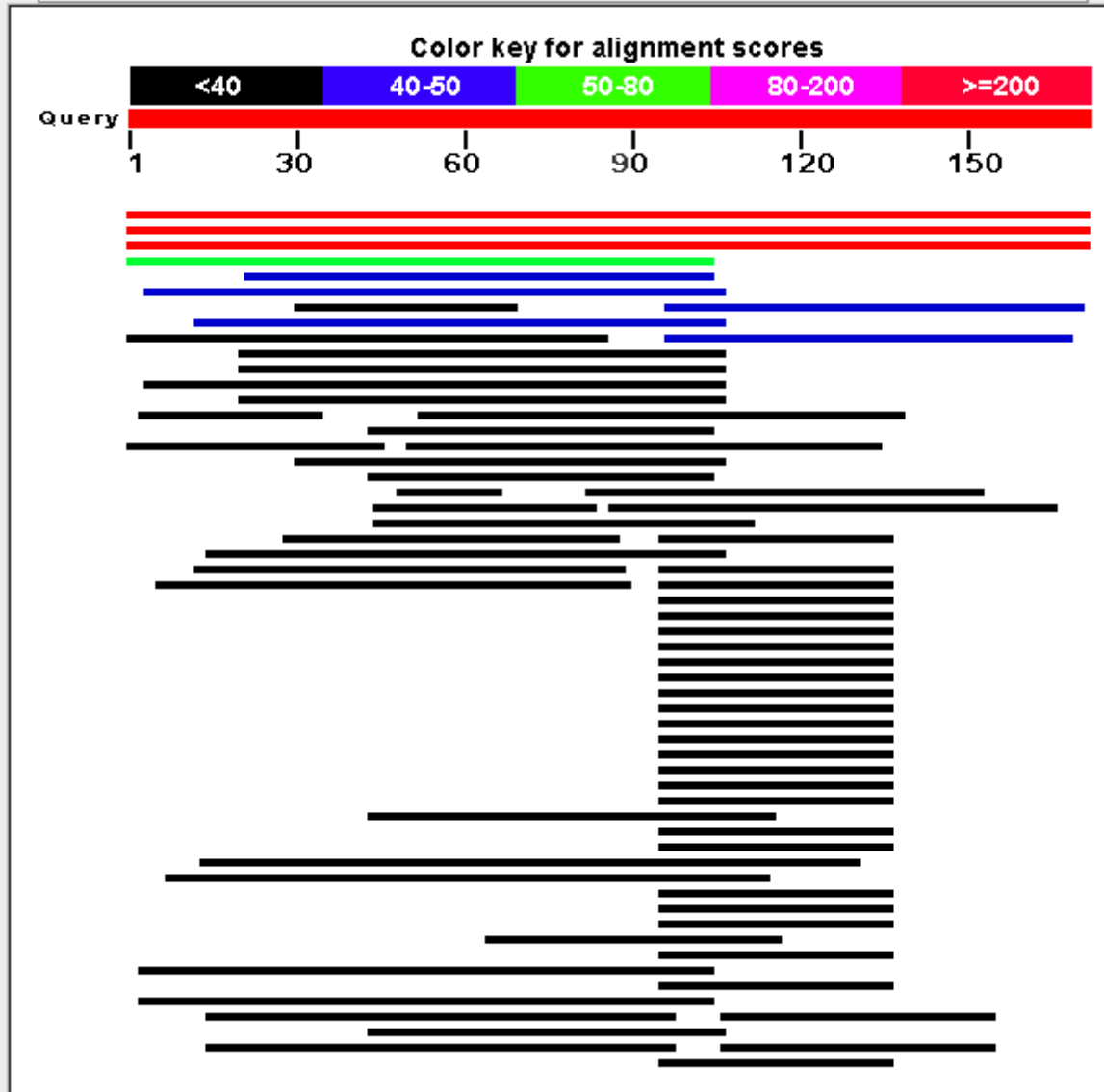
- blastp (protein-protein BLAST)
- PSI-BLAST (Position-Specific Iterated BLAST)
- PHI-BLAST (Pattern Hit Initiated BLAST)

Choose a BLAST algorithm ?

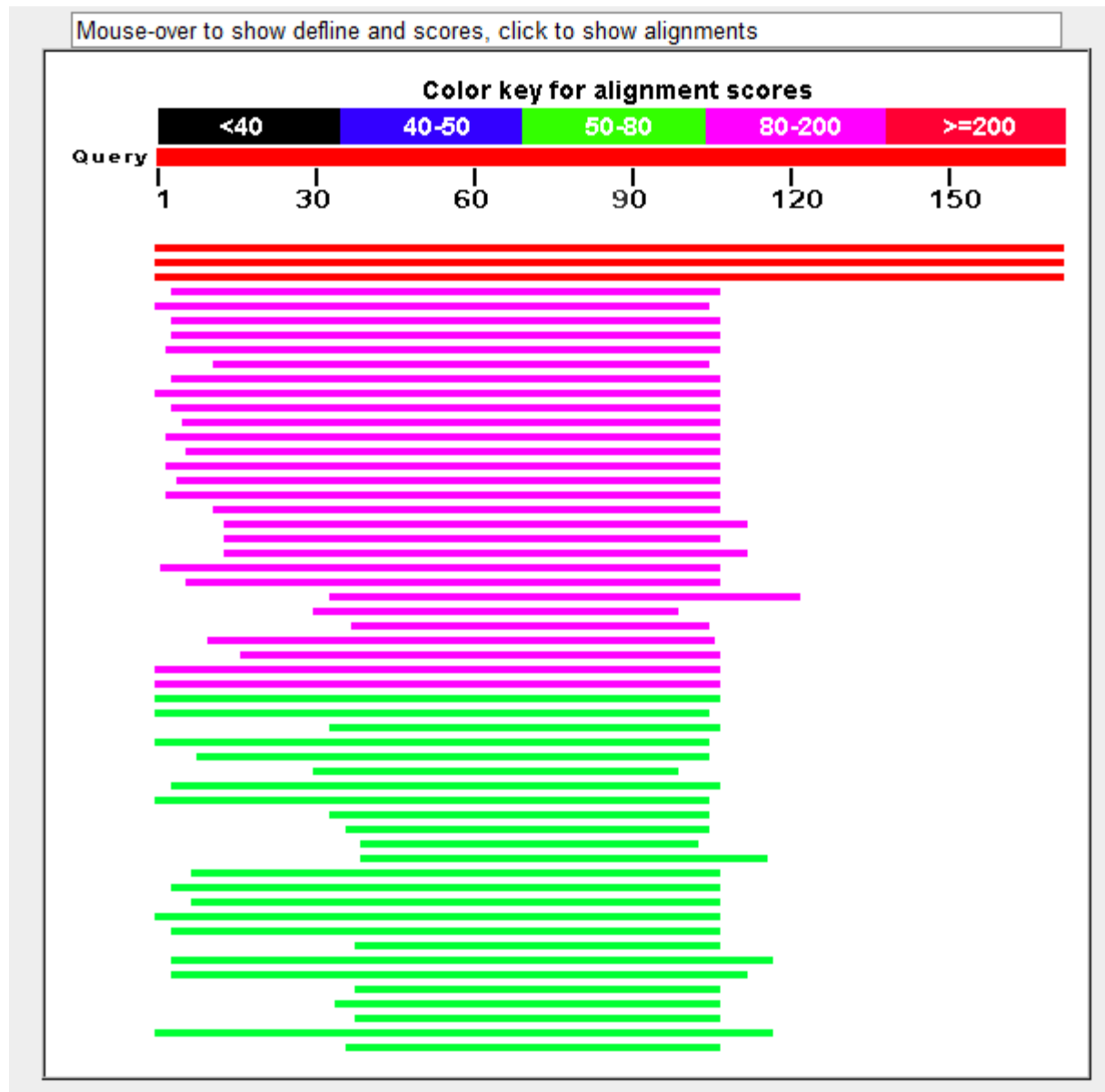
BLASTSearch **database Non-redundant protein sequences (nr)** using **PSI-BLAST (Position-Specific Iterated BLAST)**

Initial Blast

Mouse over to see the define, click to show alignments



Second iterative Blast



Identification of orthologs to lineage-specific complex I subunits

		NCBI	Mw	pl	Gravy	TMHH
36	AGGG / NDUFB2					
Mammals	<i>Homo sapiens NDUFB2</i>	CAG46918	12,1	5,5	-0,47	(55-75)
Fungi	<i>Neurospora crassa</i>	EAA26760	12,6	9,3	-0,76	45-65
Amoebozoa	<i>Dictyostelium discoideum</i>	XP_641411	6,4	9,7	-0,23	15-32
Viridiplantae	<i>Arabidopsis thaliana</i>	At1g76200	7,6	8,9	-0,54	19-40
Chromalveolates	<i>Phaeodactylum tricornutum</i>	XP_002178561	14,3	6,5	-0,91	(41-62)
Excavates	<i>Naegleria gruberi</i>	XP_002677670	17,6	5,2	-1,00	(60-80)

```

Excavate Ng 1 M V V K T E R M S L S S V I Q Q L N Q R R N F S O N N T N R G S S G H H E Q E H K Q H T K K T E F - E E L D D V L P E P
Fungi Nc 1 -----M A G D G E F E R V P R P D N Y P H G P E F Q P R T L H Q R L Q G K W W Y R V
Mammal Hs 1 -----M S A L T R I A S F A R V G G R L F R S G C A R T A G D G G V R H A G G G W H I E P R Y R Q F P Q L
Amoebozoa Dd 1 -----M A G G H H A P F -----H V
Stramenop Pt 1 -----M F S P R V S G V L R K S A Q A R S N V C ---R G P O V R R M A G S H Y L -----K I
Streptoph At 1 -----M G G G ---G H G G G I T Y K G V T V H -----T P
Chlorophy Ol 1 M S A A R G A L L R L G A R L R I A Q P M R P A S G A R G F A ---S G G G G V T H E G V T L H -----D A
consensus 1 . . . . .

```



```

Ng 60 P R E V -----I L A K I A G A S A M F W I L Y K M R O D ---G L I V F G - L E K E ---H W E H A M E D D E S D R
Nc 40 P P N H S K P I Y Q F L S I G L G A S M W F W N F L V I T N P A Y P G Q V S - A R I T E G R H Q S S F ----I R S
Hs 51 T R S Q V -----F Q S E F F S G I M W F W I L W R F W H D ---S E E V L G H F P Y P D P S Q W T D E E L G I P P D D
Dd 12 S K V H K -----L L S V G L G T T V W F W V F Y R A K O D ---W R T F V G - V N Y P W D - H I E -----
Pt 38 S K T H T -----M W G E A F G T V C W L W I F H R A R H D ---L P V V L G - F R H P W E - H A E D P F S P H T H H H
At 21 K T W H T -----V T G K G L C A M W F W I L Y R A K O D ---G P V V M G - W R H P W D G H G D -----H G H
Ol 49 S F A H K -----A L G T G E G A M W F W V F Y R F Y H D ---G D T L I Y - G H A P ---H F E -----H D D
consensus 61 * * * * *

```

Table 1 : Complex I subunit composition in eukaryotes

	Mammals	Ascomyceta	Amoebozoa	Streptophytes	Chlorophytes	Excavates	Stramenopiles
core subunits							
1 NDUFS7 / PSST	v	v	v	v	v	+	+
2 NDUFS8 / TYKY	v	v	v	v	v	+	+
3 NDUFV2 / 24 kDa	v	v	+	v	v	+	+
4 NDUFS3 / 30 kDa	v	v	v	v	v	+	+
5 NDUFS2 / 49 kDa	v	v	v	v	v	+	+
6 NDUFV1 / 51 kDa	v	v	v	v	v	+	+
7 NDUFS1 / 75 kDa	v	v	v	v	v	+	+
8 ND1	v	v	+	v	+	+	+
9 ND2	v	v	+	v	+	+	+
10 ND3	v	v	v	v	v	+	+
11 ND4	v	v	+	v	+	+	+
12 ND4L	v	v	+	+	+	+	+
13 ND5	v	v	v	v	+	+	+
14 ND6	v	v	+	v	+	+	+
conserved supernumerary subunits							
15 NDUFS6 / 13A	v	v	v	v	v	+	+
16 DAP13 / B17.2	v	v	+	v	v	+	+
17 NDUFS4 / AQDQ	v	v	+	v	v	+	+
18 NDUFA9 / 39 kDa	v	v	v	v	v	+	+
19 NDUFAB1 / ACPM	v	v	+	+	+	+	+
20 NDUFA2 / B8	v	v	v	v	v	+	+
21 NDUFA1 / MWFE	v	v	+	v	+	v	+
22 NDUFB3 / B12	v	v	v	v	v	+	+
23 NDUFA5 / B13	v	v	v	v	v	+	+
24 NDUFA6 / B14	v	v	+	v	v	+	+
25 NDUFA11 / B14.7	v	v	+	v	v	+	+
26 Q8WZ96 / ESSS	v	v	-	v	v	+	+
27 NDUFS5 / PFFD	v	v	+	v	+	+	+
28 NDUFB4 / B15	v	v	+	v	v	-	+
29 NDUFA12 / B16.6	v	v	+	v	v	+	+
30 NDUFB7 / B18	v	v	+	v	v	+	+
31 NDUFA8 / PGIV	v	v	v	v	+	+	+
32 NDUFB9 / B22	v	v	v	+	+	+	+
33 NDUFB10 / PDSW	v	v	-	v	v	+	+
34 NDUFB8 / ASHI	v	v	+	v	+	+	+

Identification of orthologs to lineage-specific complex I subunits

35	NDUFC2 / B14.5B	v	v	+	v	+	+	+
36	NDUFB2 / AGGG	v	+	+	v	v	+	+
37	NDUFA7 / B14.5A	v	v	-	+	+	+	+
38	NDUFA3 / B9	v	v	-	+	+	+	+
39	NDUFB1 / MNLL	v	v	+	v	+	+	+
40	NDUFC1 / KFYI	v	v	+	v	+	-	+
41	NDUFA4 / MLRQ	v	+	+	+	+	+	+
42	NDUFB5 / SGDH	v	v	-	v	-	-	-
(43)	NDUFA10 / 42 kDa	v	-	+	+	+	+	+
(44)	g-carbonic anhydrase	-	-	v	v	v	+	+
(45)	Rhodanese	+	v	+	+	+	+	+
(46)	Galactono-lactone DH	+	+	-	v	+	+	+

2005 : 33 conserved subunits M/F/P

2010 : conserved subunits : 34 M/F/P; 36 M/F

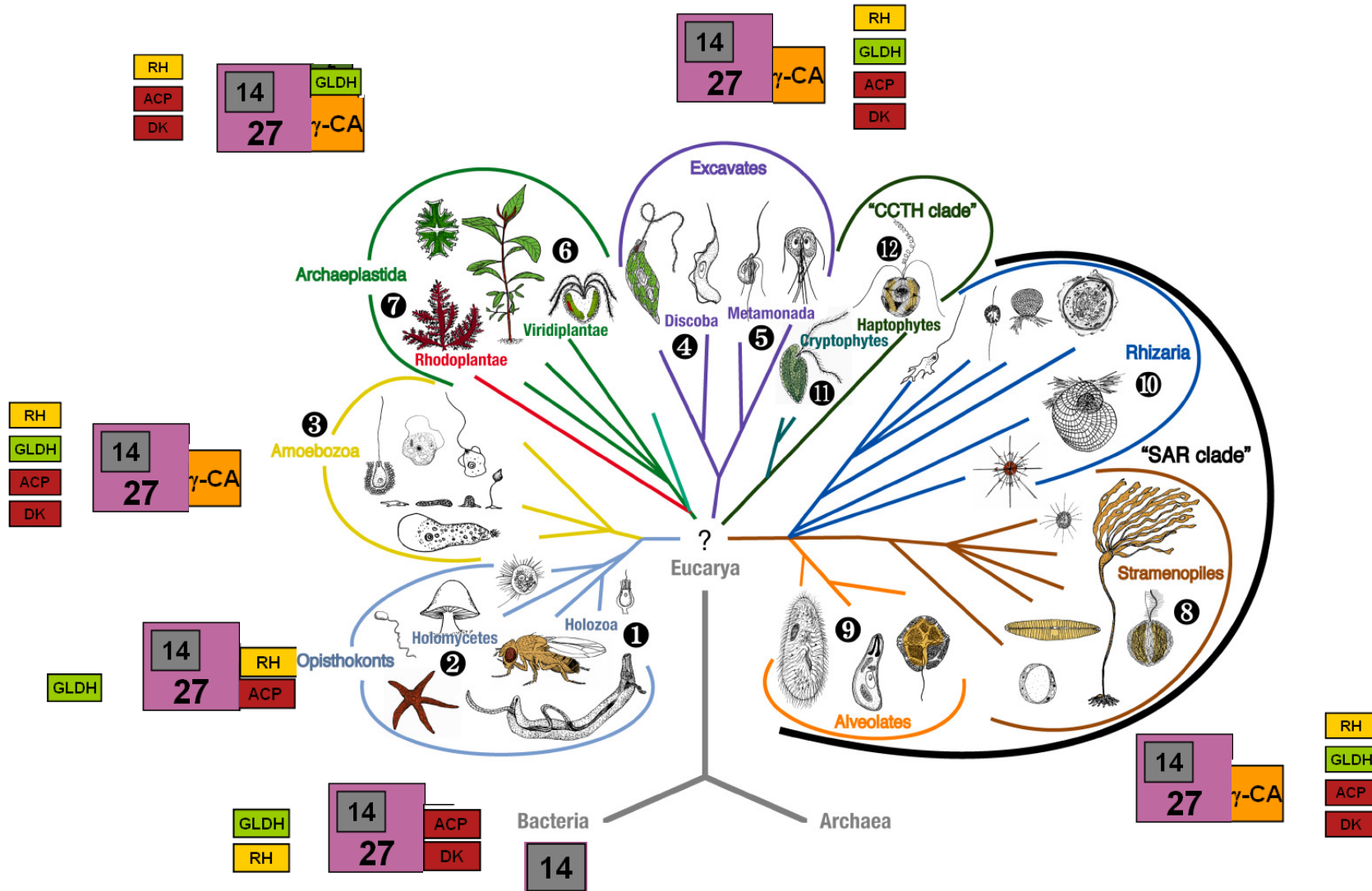
2011 : >43 conserved subunits in eukaryotes

Arabidopsis : 48 subunits → 43 conserved subunits in eukaryotes
 (Klodmann et al., 2010) → Gamma carbonic anhydrase domain
 → 2 plant-specific subunits ?

Pichia pastoris : 41 subunits → no fungus-specific subunit
 (Bridges et al., 2010) → 2 metazoa-specific subunits

Modular Evolution of Complex I

- part II : to eukaryotes



Impact on Complex I studies

1) Extrapolation of *results obtained on N. crassa* mutants

Nuo21.3a/B14.5A (*Alves and Videira 1994*)

Nuo10.4/B14.5b (*Nehls et al. 1992; Marques et al. 2005*)

Nuo20.9/MNLL (*Schulte and Weiss 1995*)

→ *Use of non mammalian model systems to study subunit function*

2) newly-identified conserved components (e.g. SGD1, AGGG, KFYI) probably play yet to elucidate conserved functions

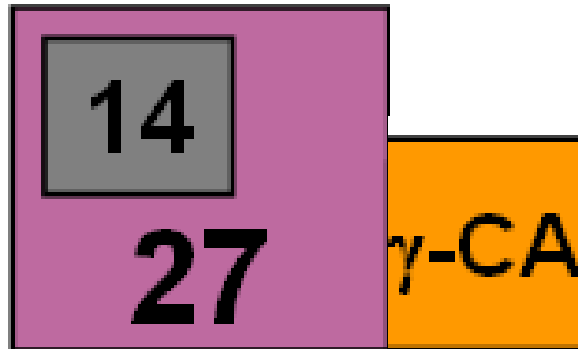
3) x-ray cartography performed on *Yarrowia* complex I (*Hunte et al. 2010*) highly relevant for understanding complex I from other sources.

4) machinery required for its assembly is well conserved among eukaryotes → *Use of non mammalian model systems to study assembly process*

5) complexification of mitochondrial complex I did not occur progressively during speciation of eukaryotic lineages

Modular Evolution of Complex I - supernumerary subunits

2. What is their function within Complex I



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Biochimica et Biophysica Acta 1604 (2003) 135–150



Review

The nuclear encoded subunits of complex I from bovine heart mitochondria

Judy Hirst*, Joe Carroll, Ian M. Fearnley, Richard J. Shannon, John E. Walker

Medical Research Council Dunn Human Nutrition Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 23Y, UK

Received 13 March 2003; received in revised form 14 May 2003; accepted 22 May 2003

The functions of the supernumerary subunits of complex I are unclear. In mitochondria, the cytochrome *bc*₁ complex and cytochrome *c* oxidase also possess a number of supernumerary subunits. The bovine enzymes have 11 and 13 subunits [30,67], whereas their equivalents in *P. denitrificans* have only 3 and 4 subunits, respectively [66,67]. This suggests that supernumerary subunits may have general roles, including protecting the complexes against oxidative stress or improving their structural stability. However, they may also play specific roles in regulating activity, or in the assembly of the complexes.

Modular Evolution of Complex I -

Carbonic anhydrase - Plant specific domain

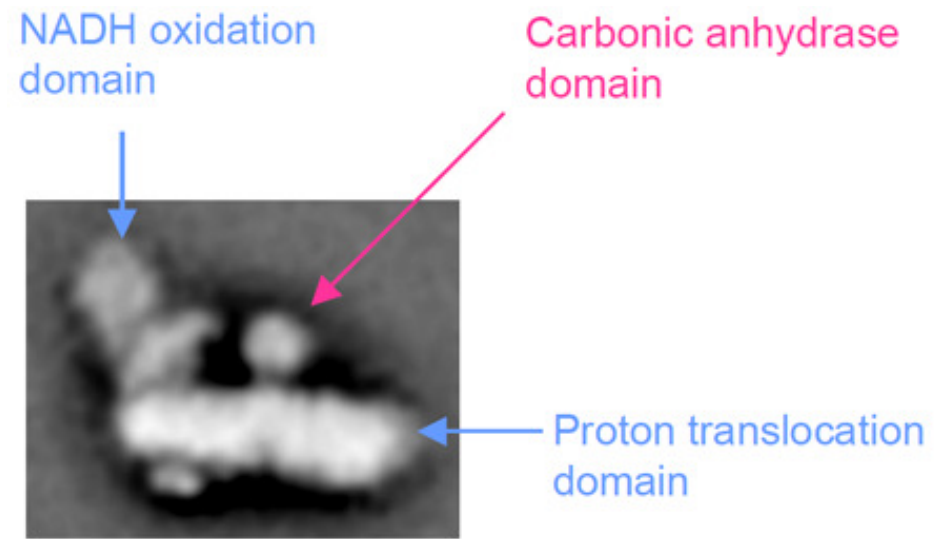
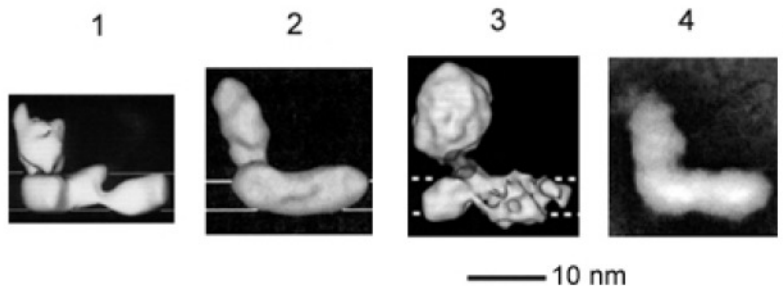
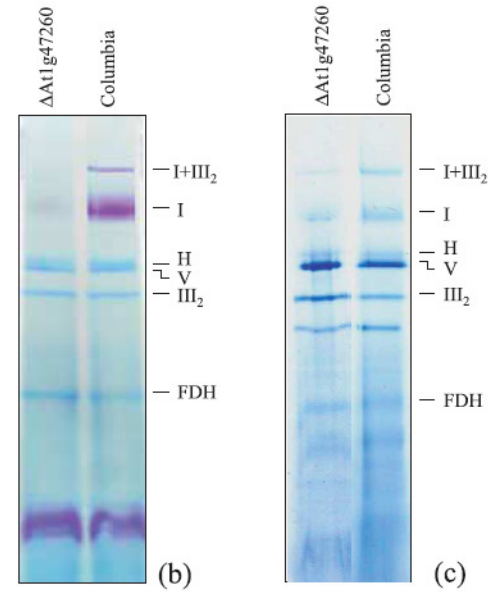
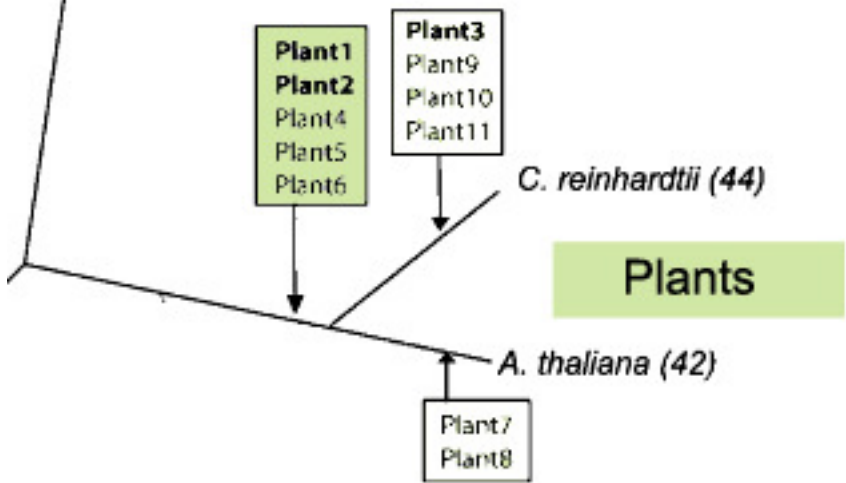


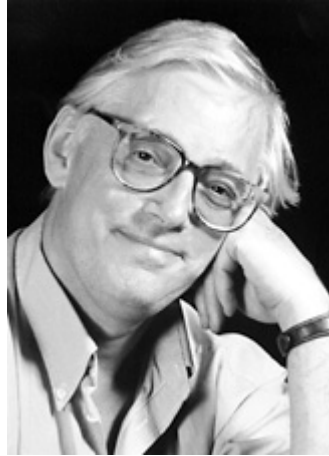
Figure 2. Structure établie en microscopie électronique des complexes I mitochondriaux de *N. crassa* (1,2) (Guenebaut *et al.*, 1997; Hofhaus *et al.*, 1991), de *B. taurus* (3) (Grigorieff, 1998) et de *Y. lipolytica* (4) (Djafarzadeh *et al.*, 2000). La membrane est schématisée par les traits.

Perales *et al.*, 2005
Dudkina *et al.*, 2005

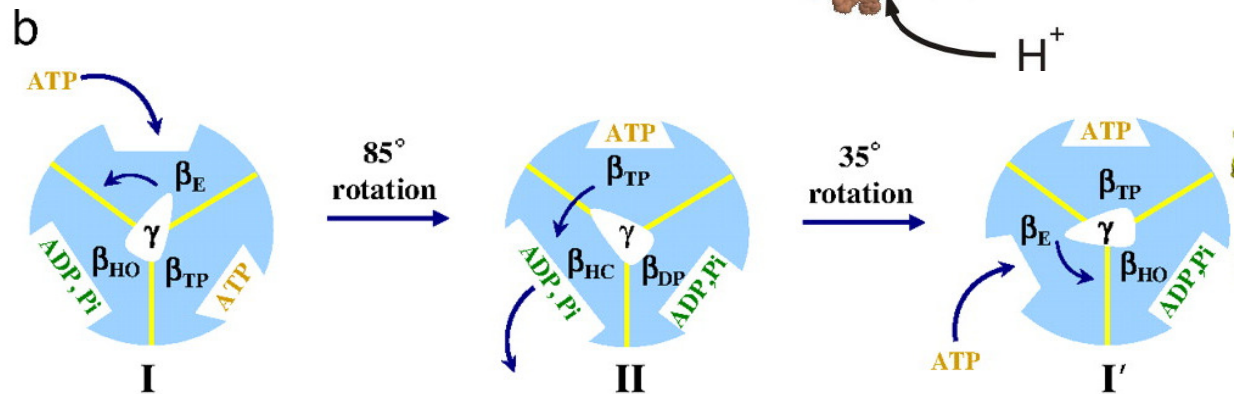
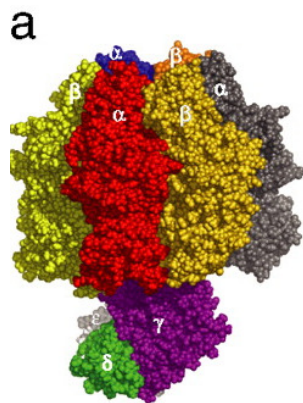
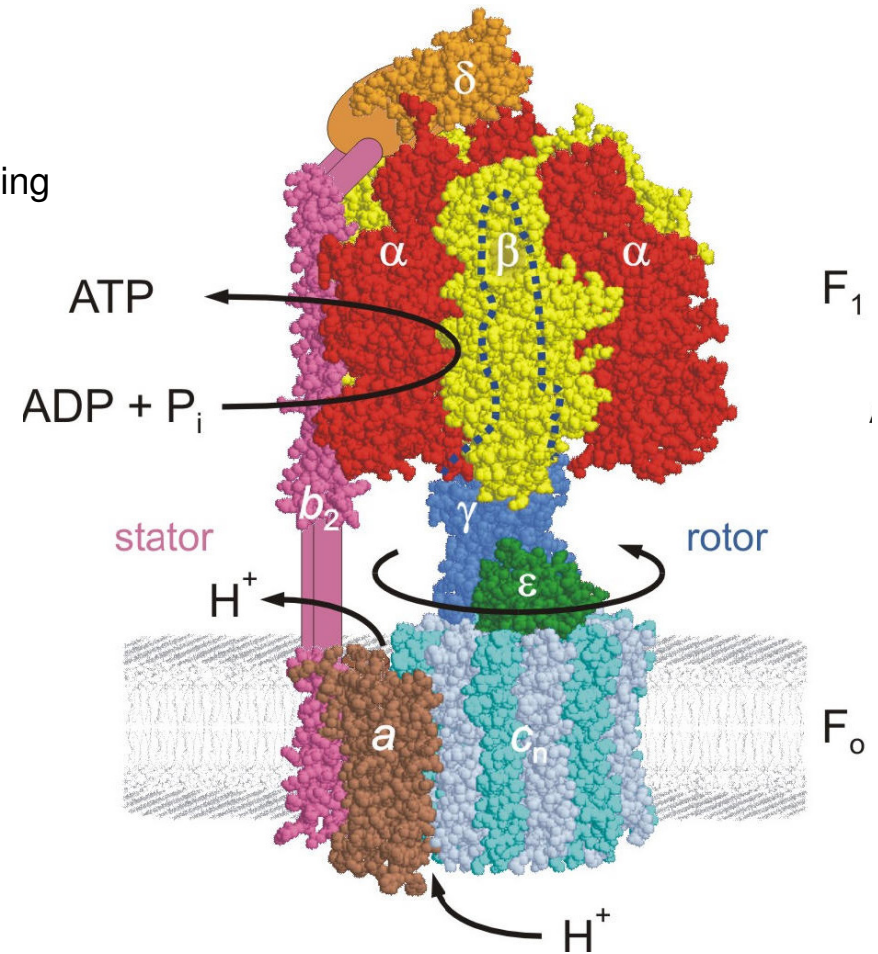
Structure and function of Complex V

The Nobel Prize in Chemistry 1997

"for their elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP)"

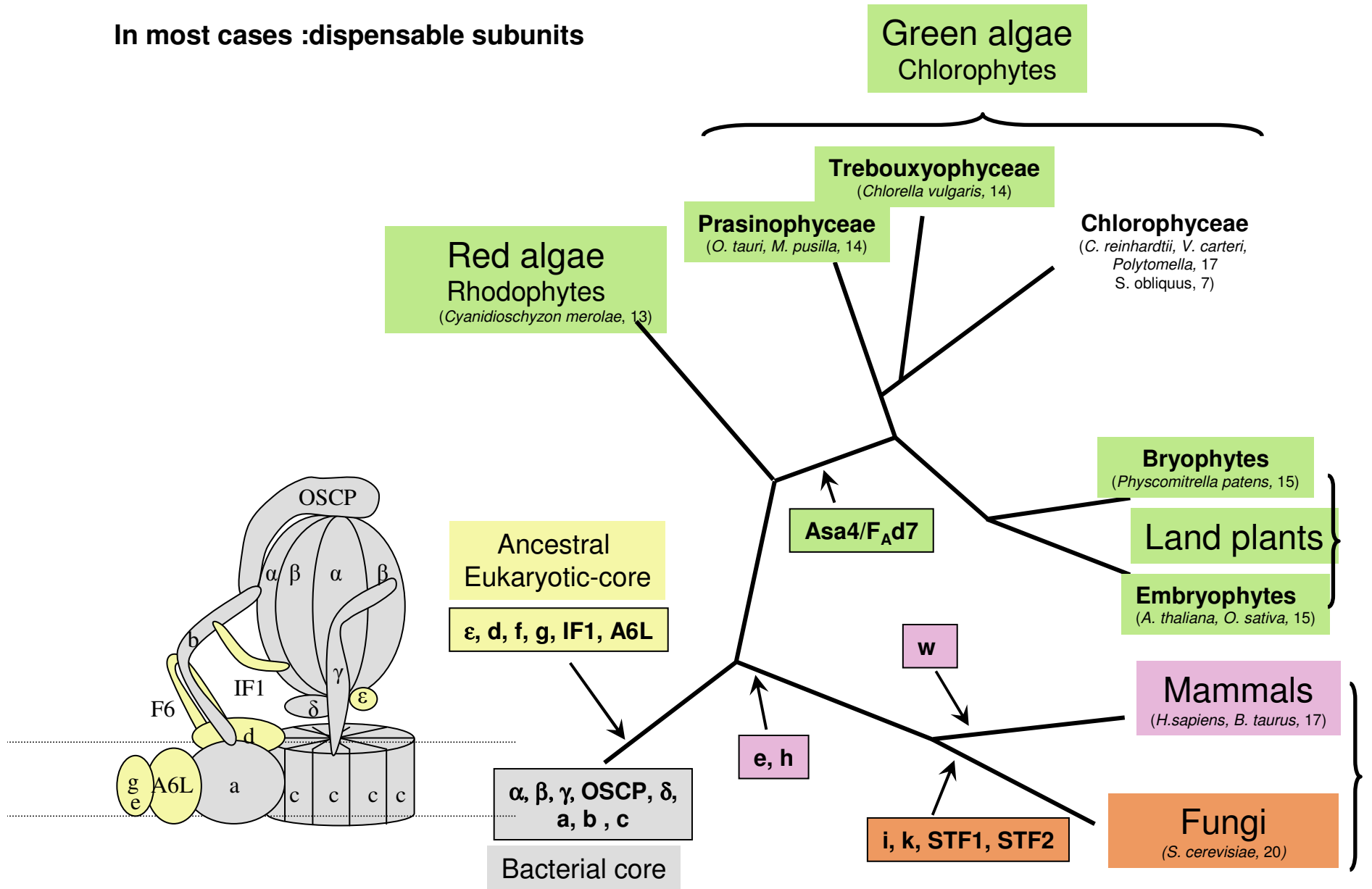


Paul D. Boyer John E. Walker

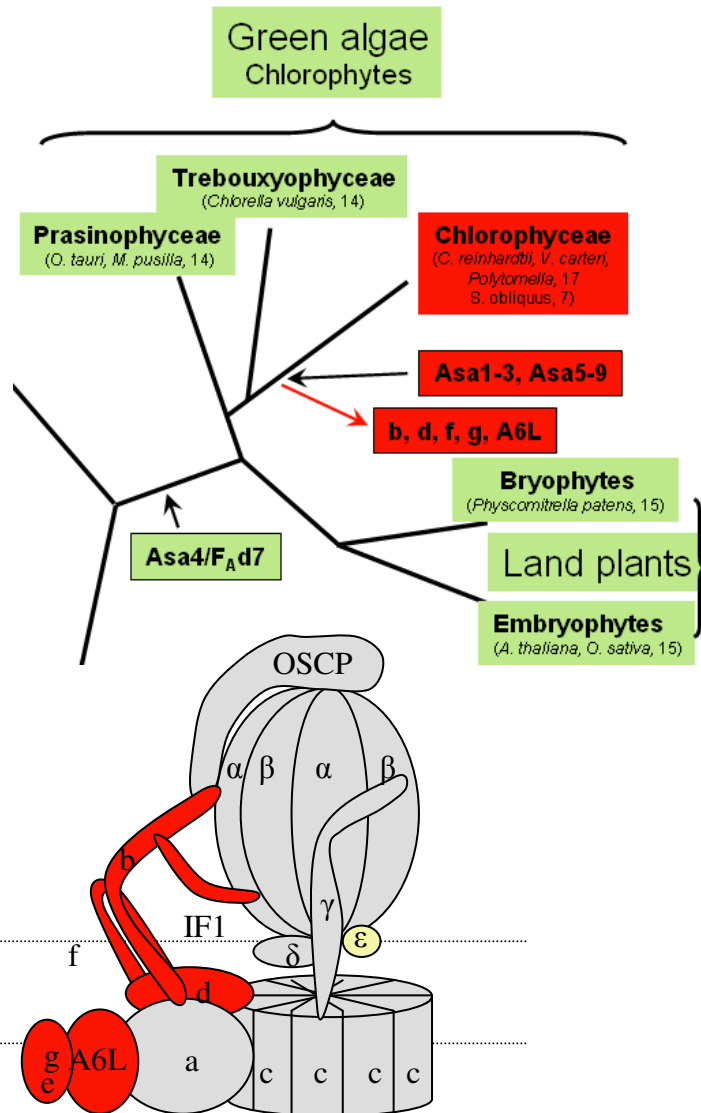


Evolution of Complex V : Complexification in eukaryotes

In most cases :dispensable subunits



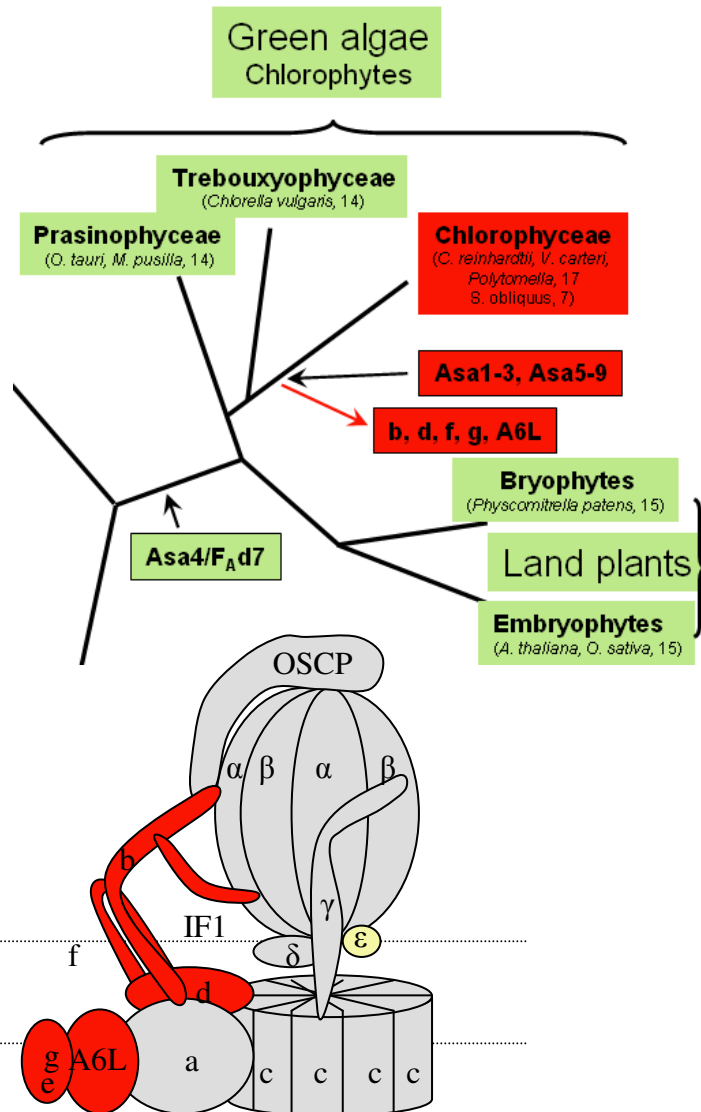
Evolution of Complex V : Chlorophyceae



Complex	R.a.	P.w.	M.p.	A.t.	C.r.	B.t.	N.c.	S.c.
Complexe I								
nd1	+	+	+	+	+	+	+	-
nd2	+	+	+	+	+	+	+	-
nd3	+	+	+	+	-	+	+	-
nd4	+	+	+	+	+	+	+	-
nd4L	+	+	+	+	-	+	+	-
nd5	+	+	+	+	+	+	+	-
nd6	+	+	+	+	+	+	+	-
nd7	+	+	+	+	-	-	-	-
nd8	+	-	-	-	-	-	-	-
nd9	+	+	+	+	-	-	-	-
nd10	+	-	-	-	-	-	-	-
nd11	+	-	-	-	-	-	-	-
Complexe II								
sdh2	+	-	-	-	-	-	-	-
sdh3	+	-	+	-	-	-	-	-
sdh4	+	-	+	+	-	-	-	-
Complexe III								
cob	+	+	+	+	+	+	+	+
Complexe IV								
cox1	+	+	+	+	+	+	+	+
cox2	+	+	+	+	-	+	+	+
cox3	+	+	+	+	-	+	+	+
Complexe V								
atp1	+	+	+	+	-	-	-	-
atp3	+	-	-	-	-	-	-	-
atp6	+	+	+	+	-	+	+	+
atp8	+	-	-	-	-	+	+	+
atp9	+	+	+	+	-	-	+	+

Tableau 1. Constituants de la chaîne respiratoire mitochondriale encodés par le génome mitochondrial chez divers organismes (adapté de Vedel *et al.*, 1999). R.a., *Reclinomonas americana*; P.w., *Prototheca wickerhamii*; M.p., *Marchantia polymorpha*; A.t., *Arabidopsis thaliana*; C.r., *Chlamydomonas reinhardtii*; B.t., *Bos taurus*; N.c., *Neurospora crassa*; S.c., *Saccharomyces cerevisiae*. + ou - indique la présence ou l'absence du gènes mitochondrial.

Evolution of Complex V : Chlorophyceae



Transfer of Mitochondrial genes to the nucleus

Loss of conserved subunits

Recruitment of ASAs

- i) relocation of previously extant proteins to the mitochondria,
- ii) acquisition of novel genes by lateral gene transfer

No sequence or pattern similarities in existing DB

Evolution of Complex V : Chlorophyceae

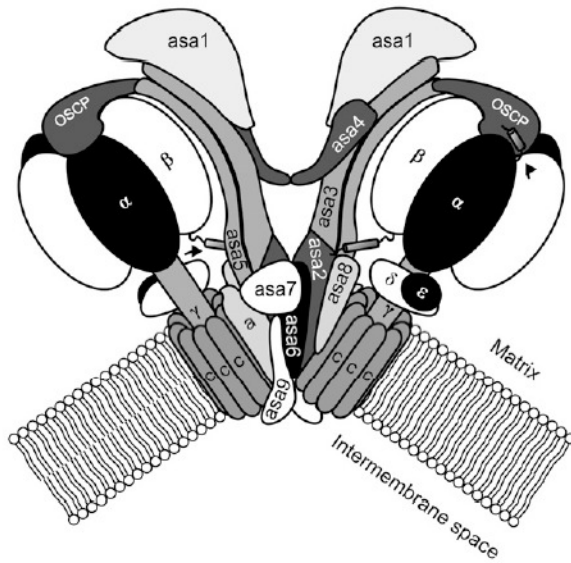
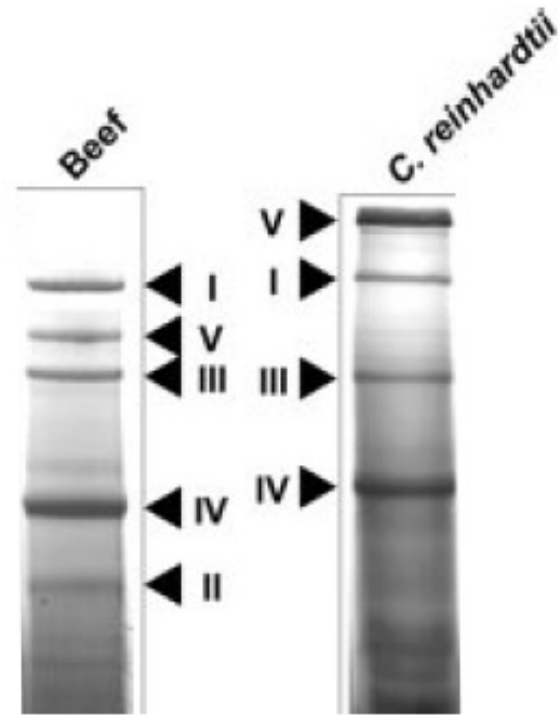


Figure 6. Working model of dimeric mitochondrial ATP synthase in Chlamydomonad algae, based on our current knowledge of the enzyme from both *C. reinhardtii* and *Polytomella* sp. The monomers are shown rotated 180° one from another (around a vertical axis), as proposed previously for the yeast enzyme (Paumard et al., 2002).

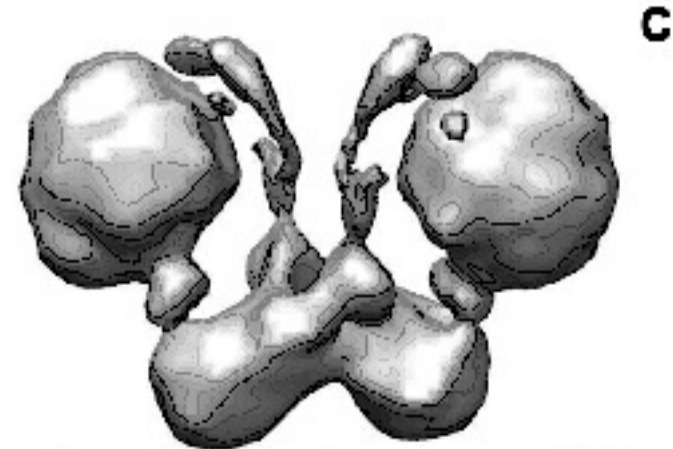
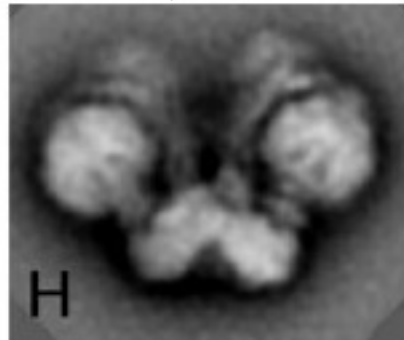
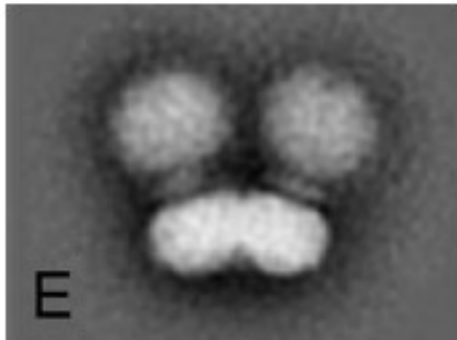


Plant Physiology, May 2003, Vol. 132, pp. 318–330,

N.V. Dudkina et al. / FEBS Letters xxx (2006) xxx–xxx

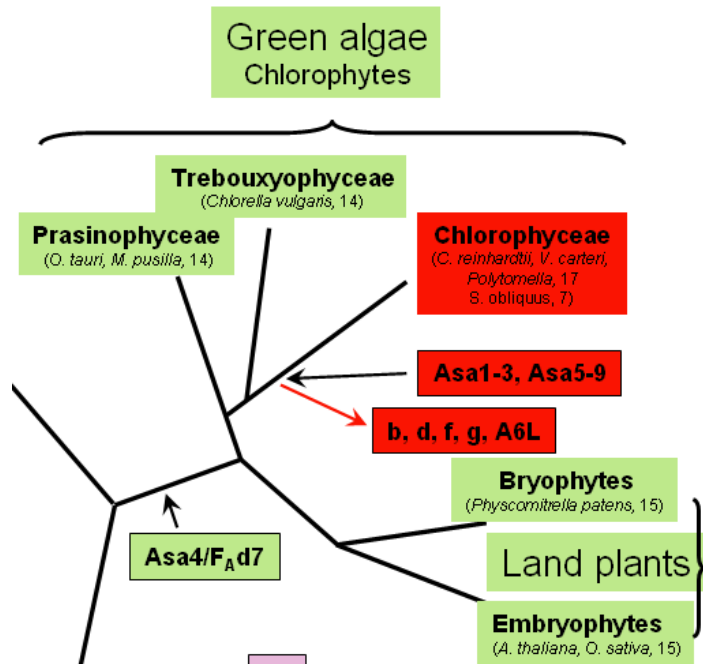
Yeast

Polytomella

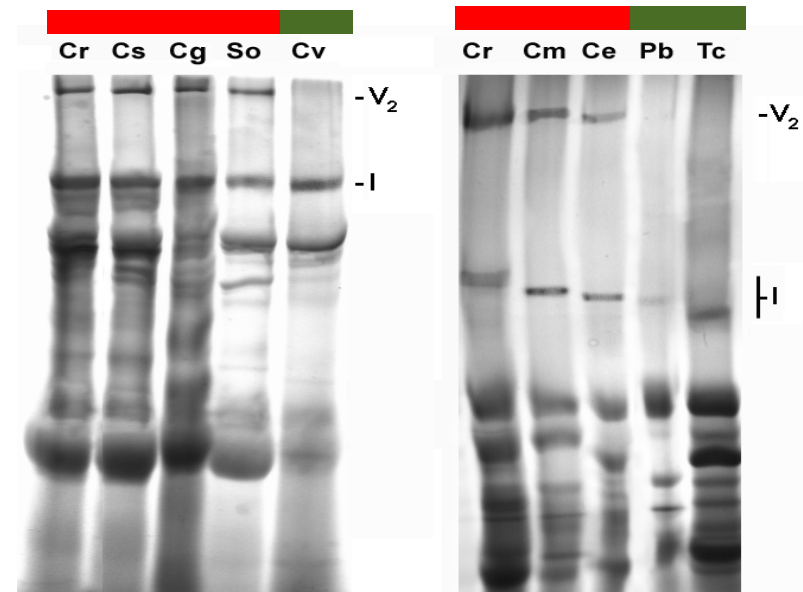
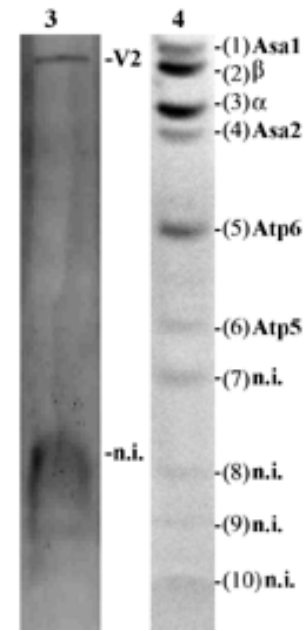


Gonzalez-Halphen D., pers. comm. 2009

Evolution of ATP synthase by genomic and proteomic approaches



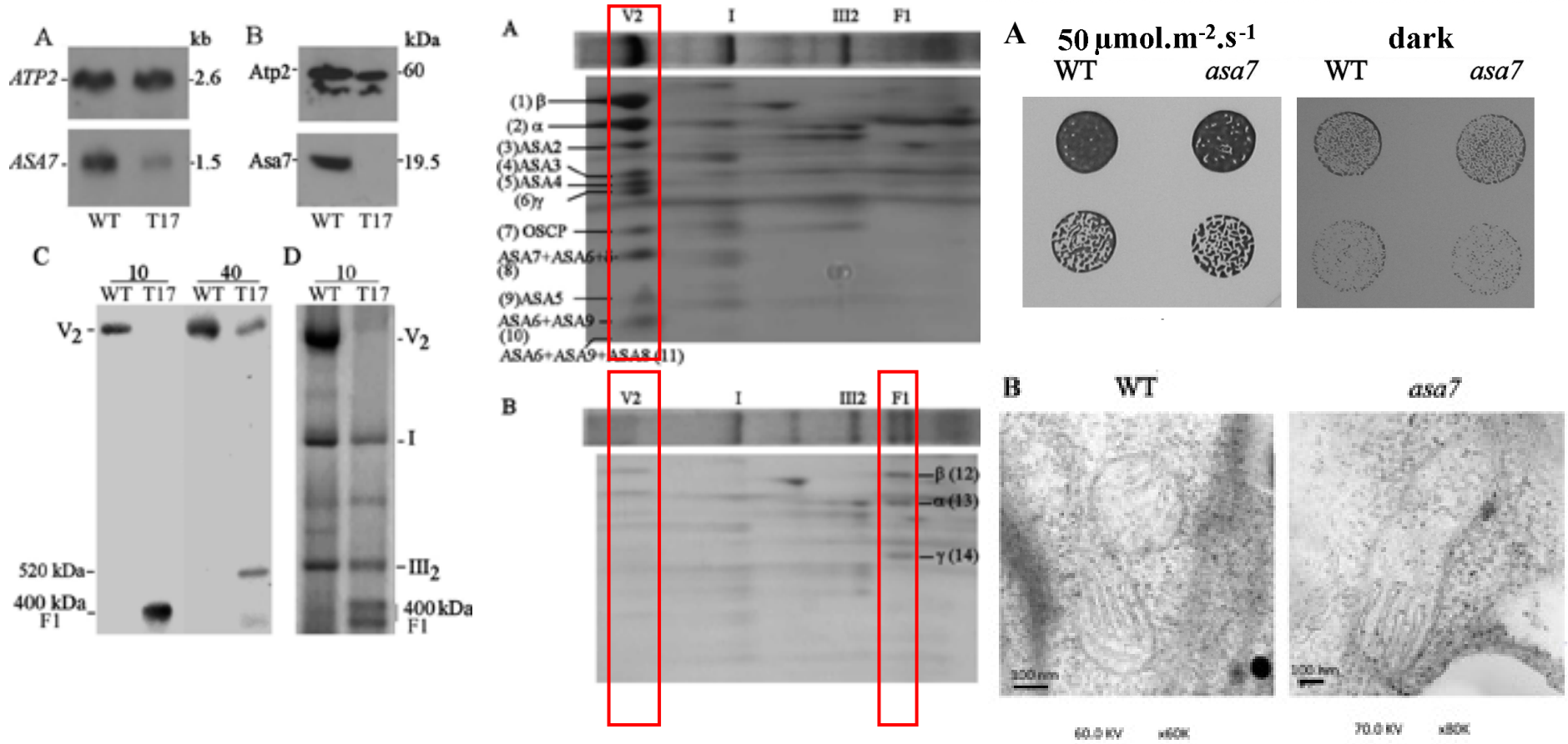
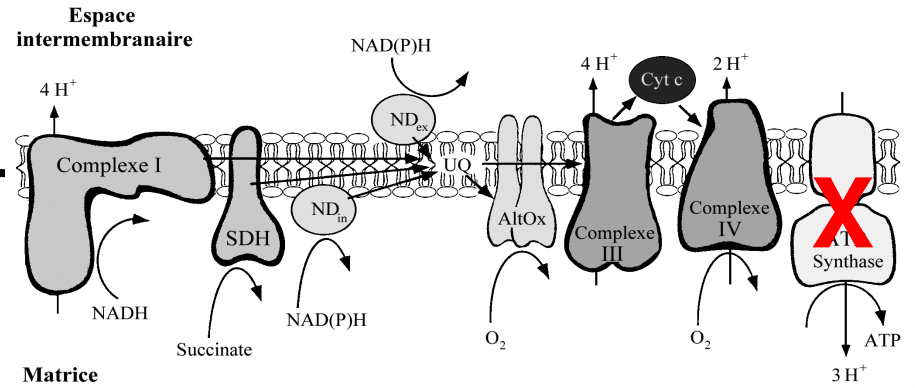
Scenedesmus obliquus
Chlorophyceae



Lapaille et al., 2010, Mol. Biol. Evol.
Vázquez-Acevedo et al., 2006, J. Bioen. Biomembrane

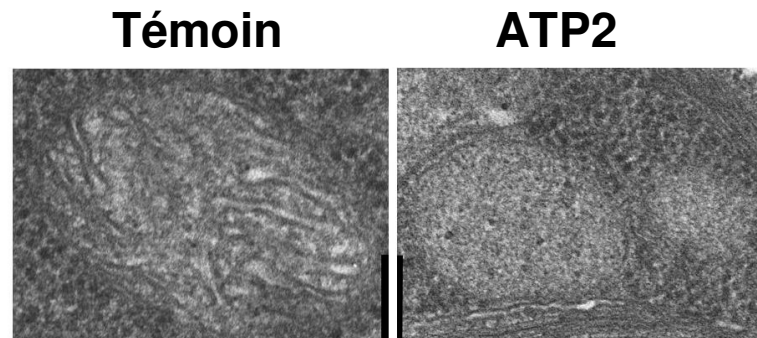
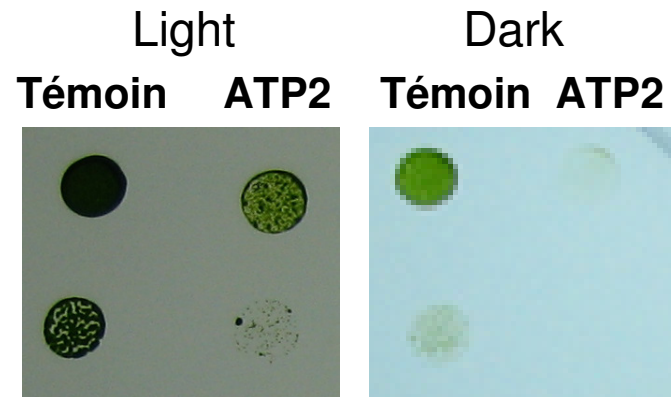
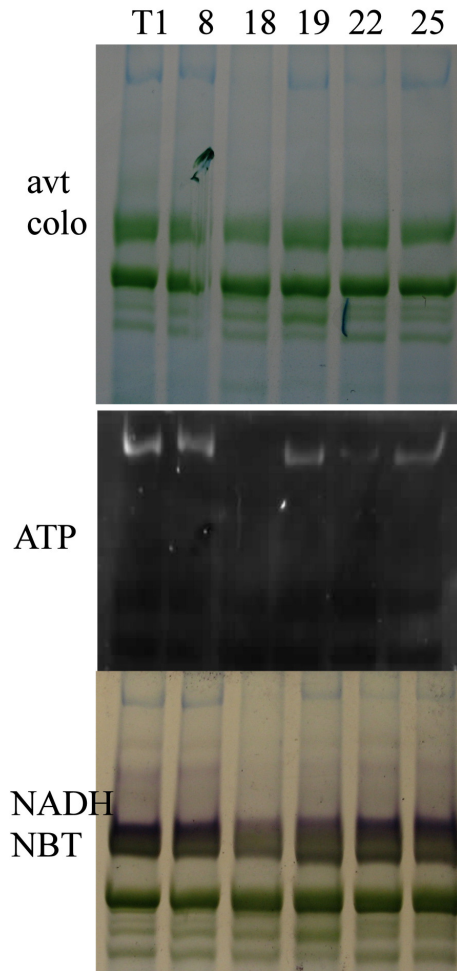
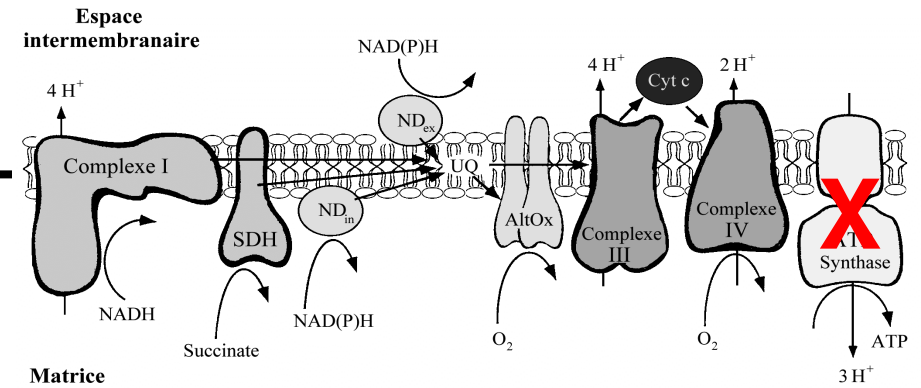
Chlamydomonas mutants

RNA interference : ASA7



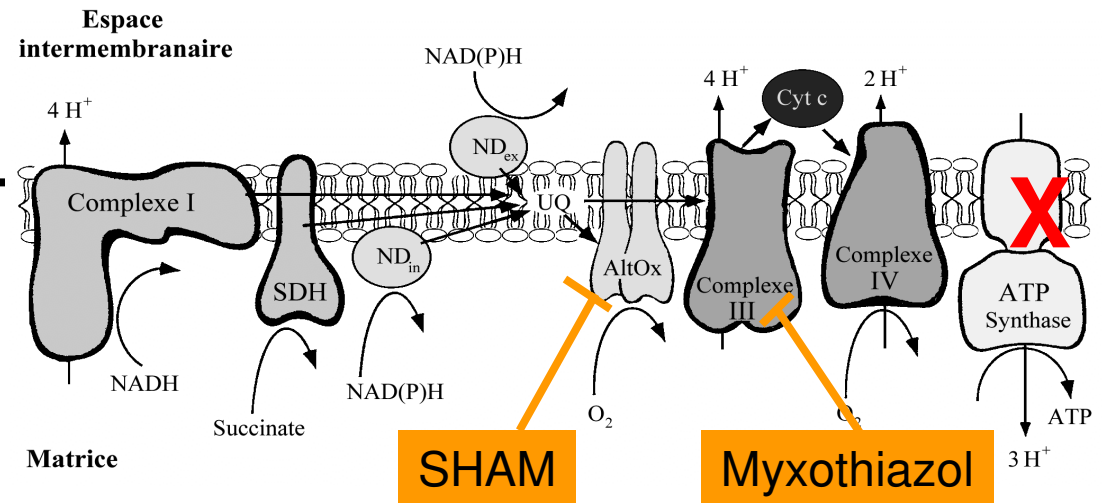
Chlamydomonas mutants

RNA interference : *ATP2* (subunit β)



Chlamydomonas mutants

RNA interference : ASA7



Control Asa7

Respiration μmoles O ₂ · h ⁻¹ · mg chl ⁻¹	No addition	45 ± 3	37 ± 4
	+ Myxothiazol (5 μM)	15 ± 4	18 ± 3
	+ SHAM (1 mM)	45 ± 1	36 ± 6
	+ Myxothiazol (5 μM) + SHAM (1 mM)	2 ± 2	3 ± 2
	+ CCCP (10 μM)	46 ± 2	48 ± 4
	+ Oligomycin (10 μM) + SHAM (1 mM)	46 ± 4	23 ± 3
ATP level nmol ATP · mg chl ⁻¹	No addition	73 ± 2	77 ± 5
	+ Myxothiazol (5 μM)	28 ± 4	23 ± 5
	+ Oligomycin (10 μM)	78 ± 3	57 ± 5

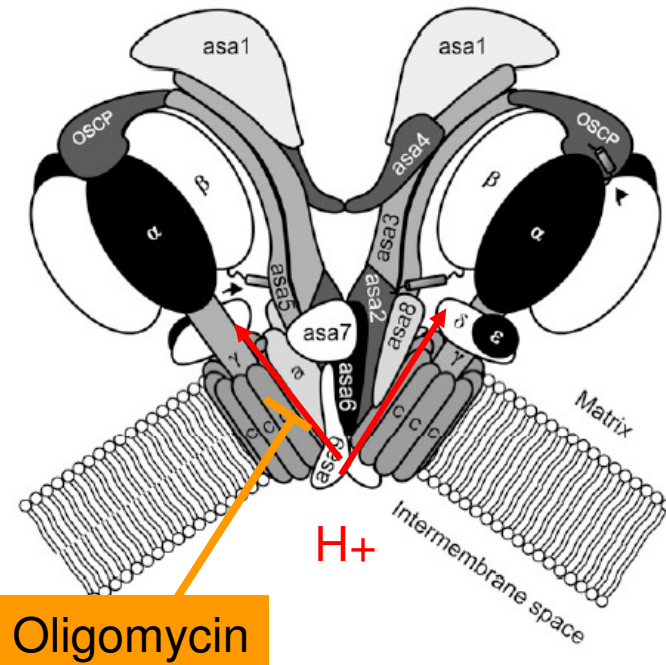


Figure 6. Working model of dimeric mitochondrial ATP synthase in *Chlamydomonas* algae, based on our current knowledge of the enzyme from both *C. reinhardtii* and *Polytomella* sp. The monomers are shown rotated 180° one from another (around a vertical axis), as proposed previously for the yeast enzyme (Paumard et al., 2002).

Chlamydomonas mutants

RNA interference : ASA7

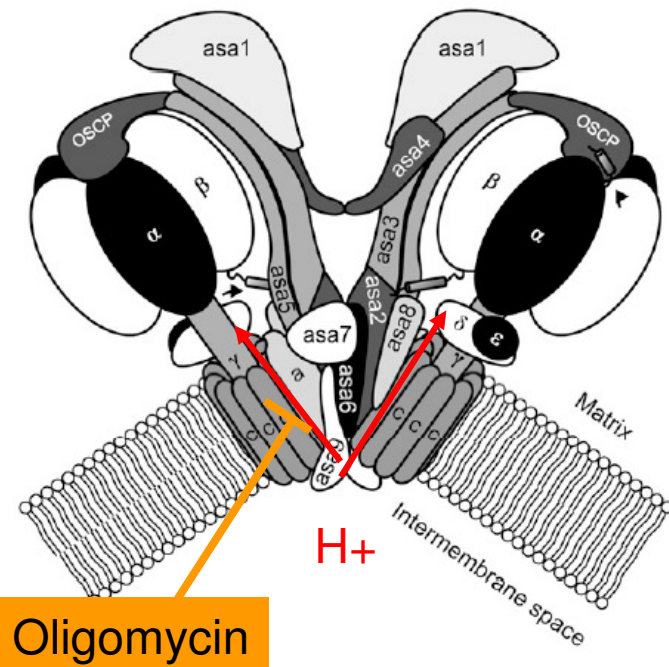
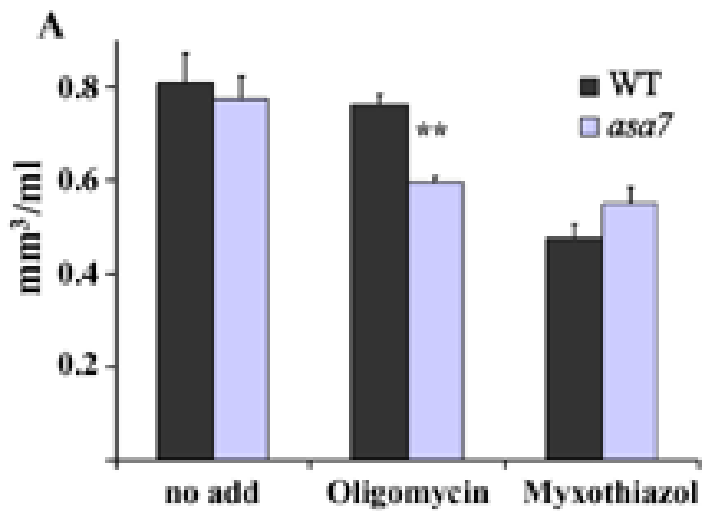
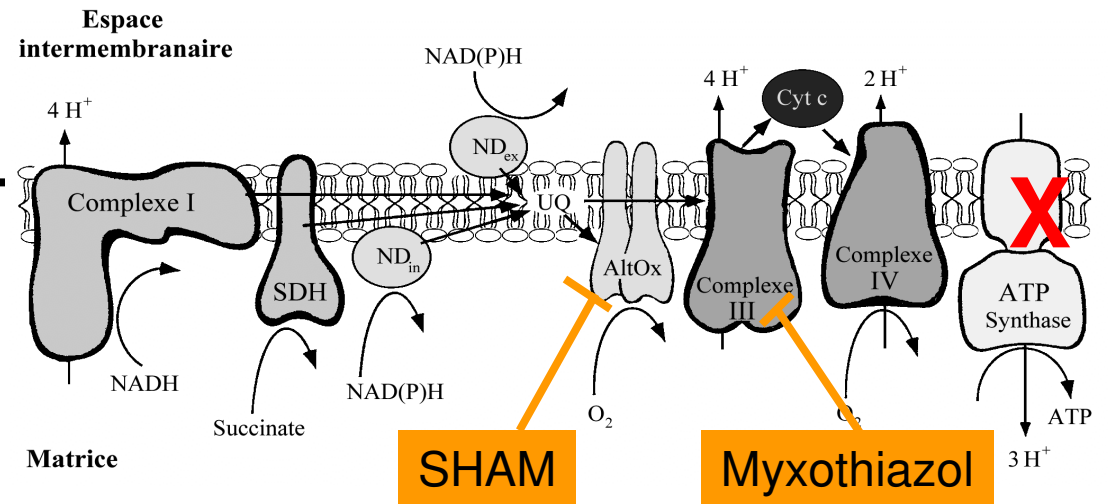
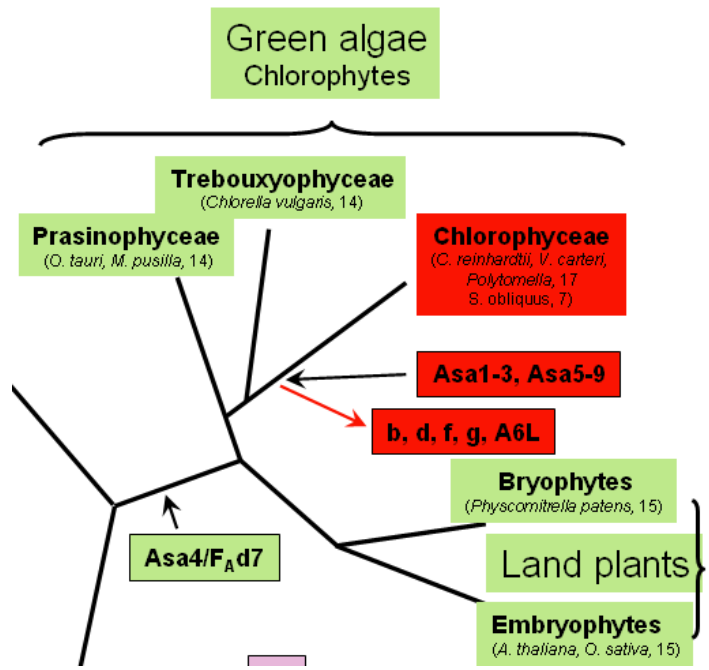
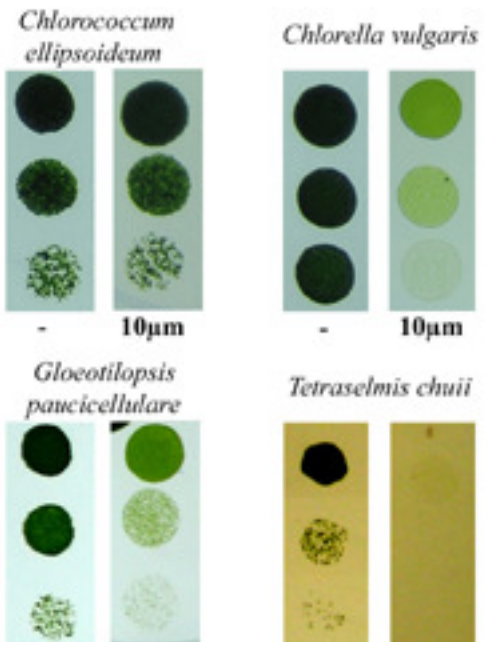
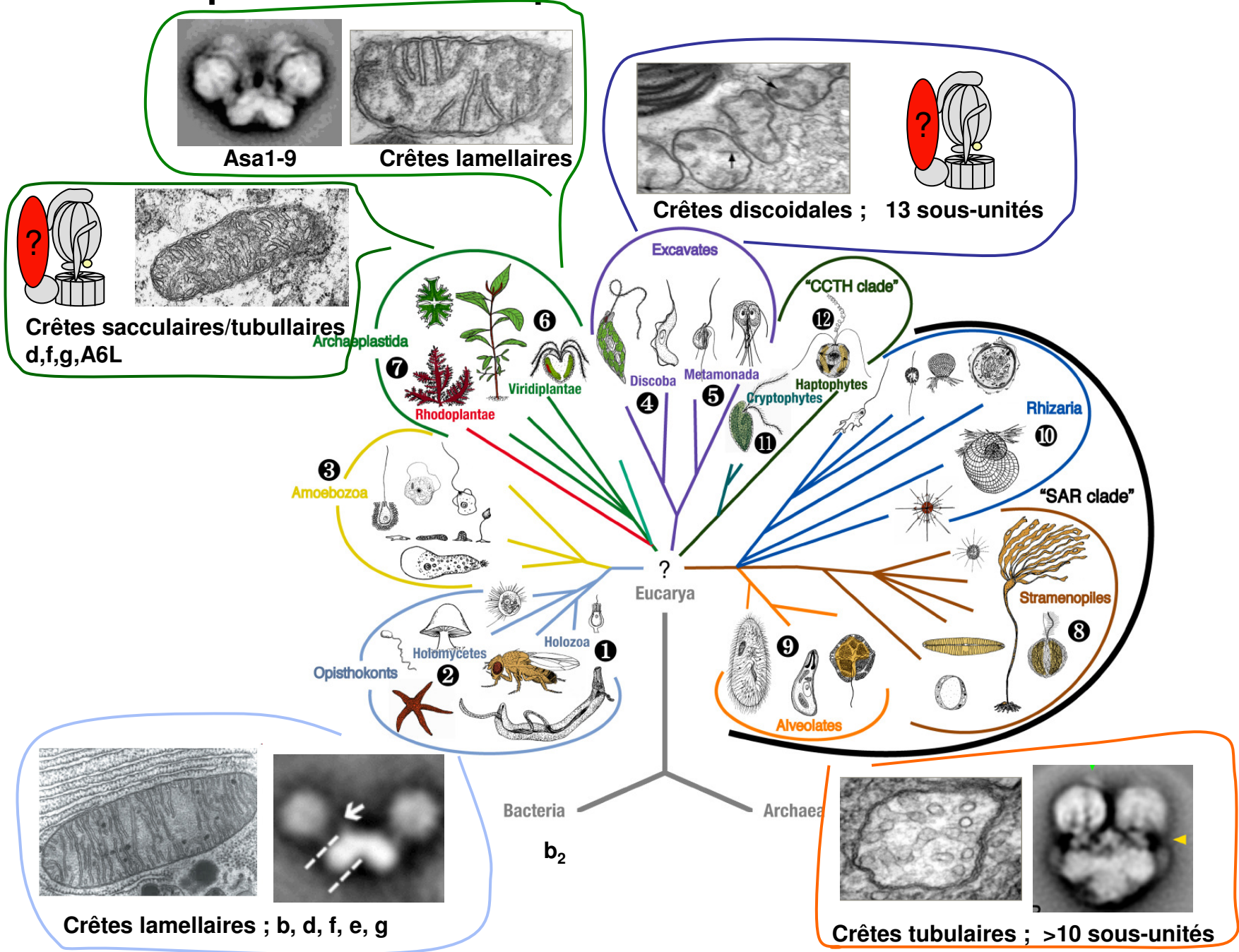


Figure 6. Working model of dimeric mitochondrial ATP synthase in *Chlamydomonas* algae, based on our current knowledge of the enzyme from both *C. reinhardtii* and *Polytomella* sp. The monomers are shown rotated 180° one from another (around a vertical axis), as proposed previously for the yeast enzyme (Paumard et al., 2002).

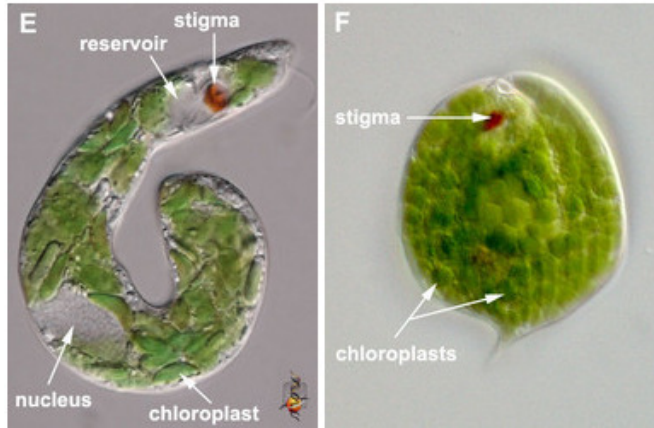


	Oligomycin (µM)	Phototrophic growth				Heterotrophic growth				Dark respiratory rate 10	ATP level 10
		0	0.2	1	10	0	0.2	1	10		
Chlorophyceae											
Chlorococcales	<i>Chlorococcum ellipsoideum</i>									98%	98%
Chaetophorales	<i>Uronema acuminata</i>									nd	nd
Chlamydomonadales	<i>Chlamydomonas reinhardtii</i>									nd	nd
	<i>Chlamydomonas moewusii</i>					■	■	■	■	nd	nd
	<i>Chlorogonium elongatum</i>									nd	nd
	<i>Scenedesmus obliquus</i>									98%	100%
Sphaeropleales											
Trebouxiophyceae											
Chlorellales	<i>Chlorella sorokinia</i>		■	■	■		■	■	■	43%	37%
	<i>Chlorella vulgaris</i>						■	■	■	40%	65%
	<i>Nannochloris</i> sp.	■	■	■	■					nd	nd
	<i>Coccomyxa pringsheimii</i>	■	■	■	■					nd	nd
Coccomyxaceae	<i>Leptospira obovata</i>		■	■	■					nd	nd
Ctenocladales											
Ulvoiphyceae											
Ulvales	<i>Pseudendoclonium basillense</i>		■	■	■		■	■	■	nd	nd
Ulotrichales	<i>Gloeotilopsis paucicellulare</i>		■	■	■					68%	46%
	<i>Ulothrix Fimbriata</i>									nd	nd
Prasinophyceae											
Mamiellales	<i>Micromonas pusilla</i>	■	■	■	■	nd	nd	nd	nd	nd	nd
Chlorodendrales	<i>Tetraselmis chuii</i>	■	■	■	■	nd	nd	nd	nd	39%	44%

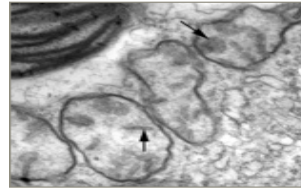
Impact of stator composition on mitochondrial structure



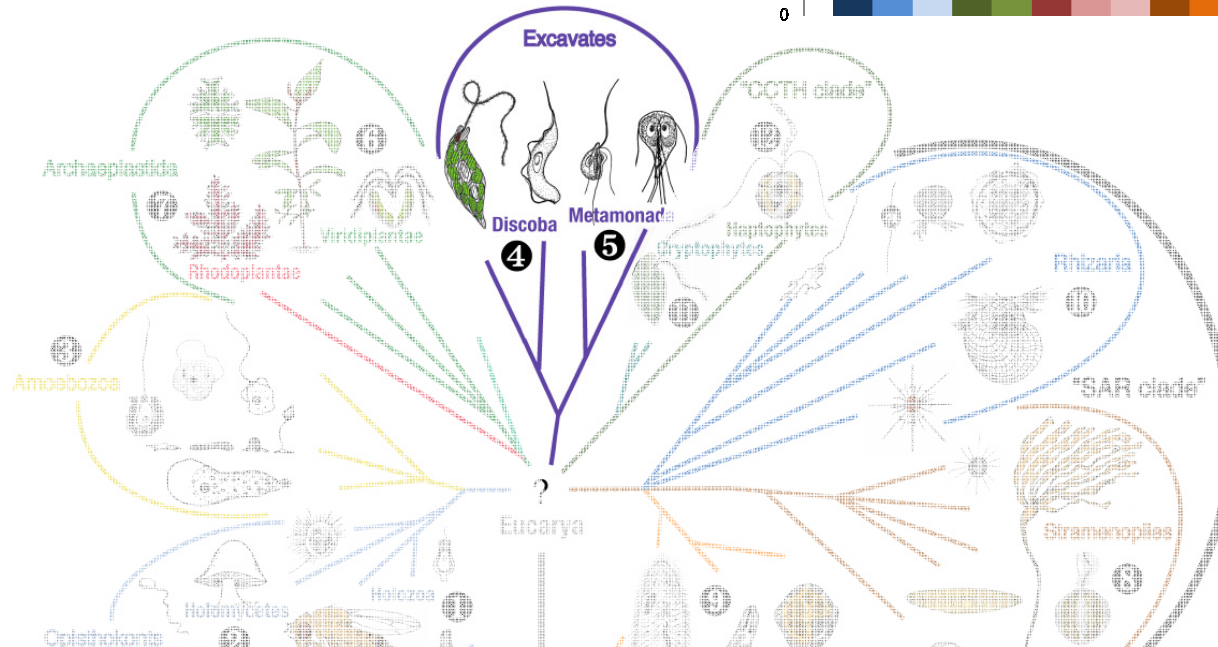
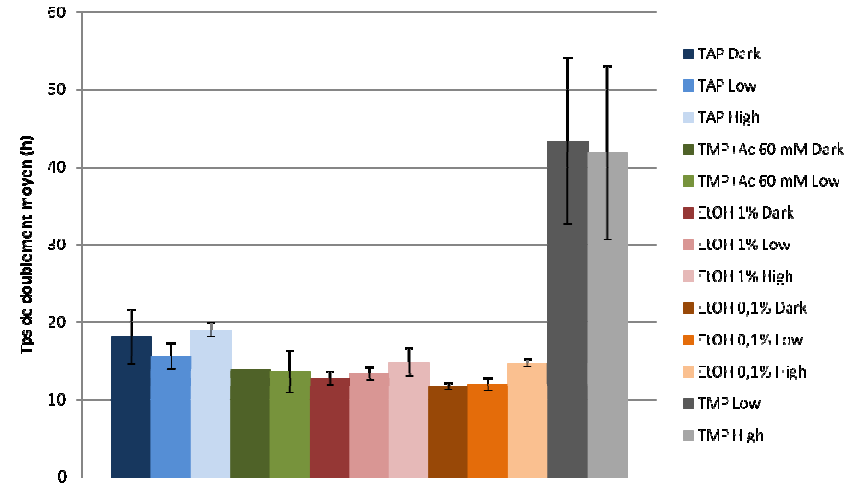
Euglena gracilis as a model system for Excavates



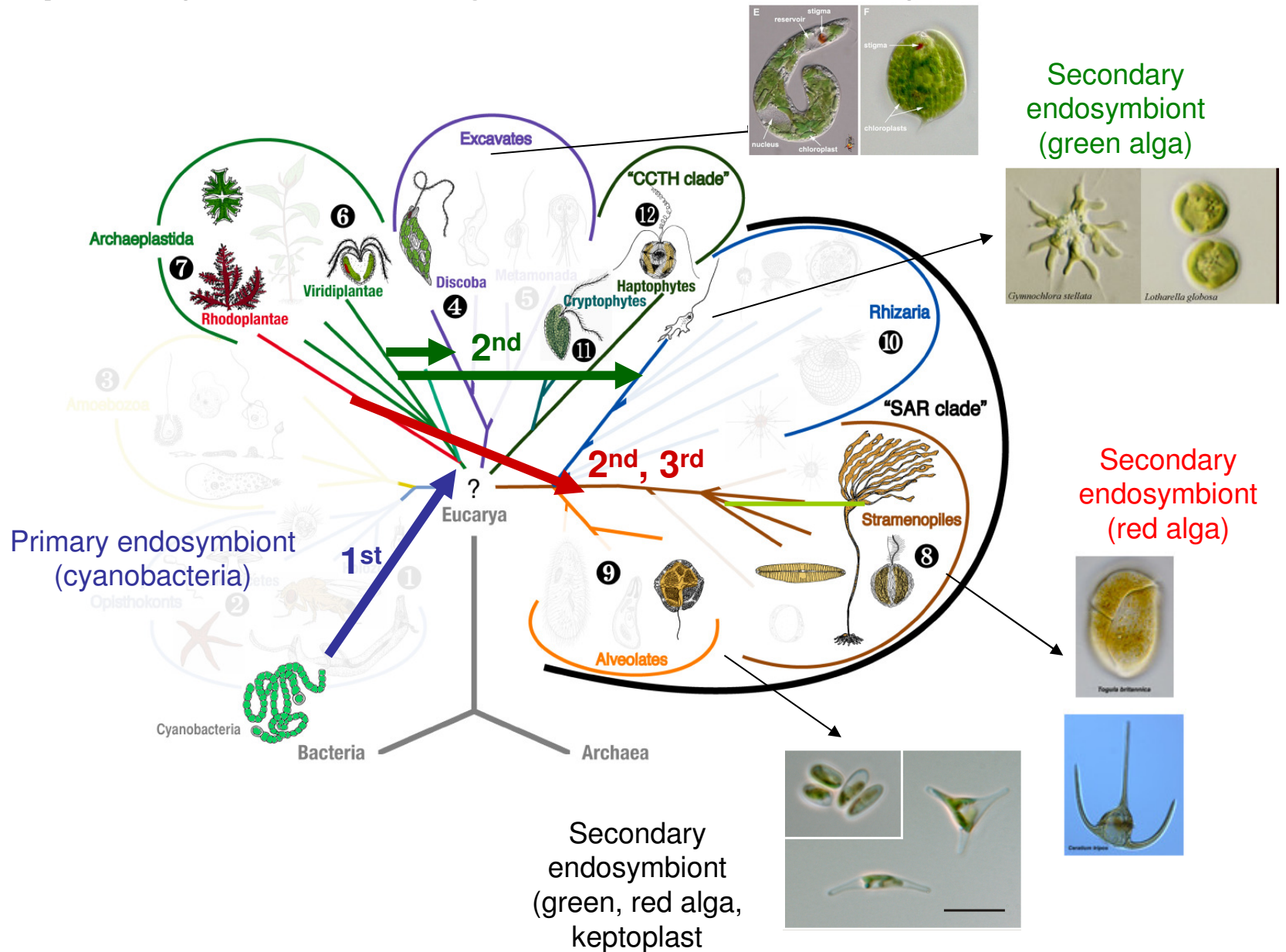
Secondary endosymbiont (green alga)



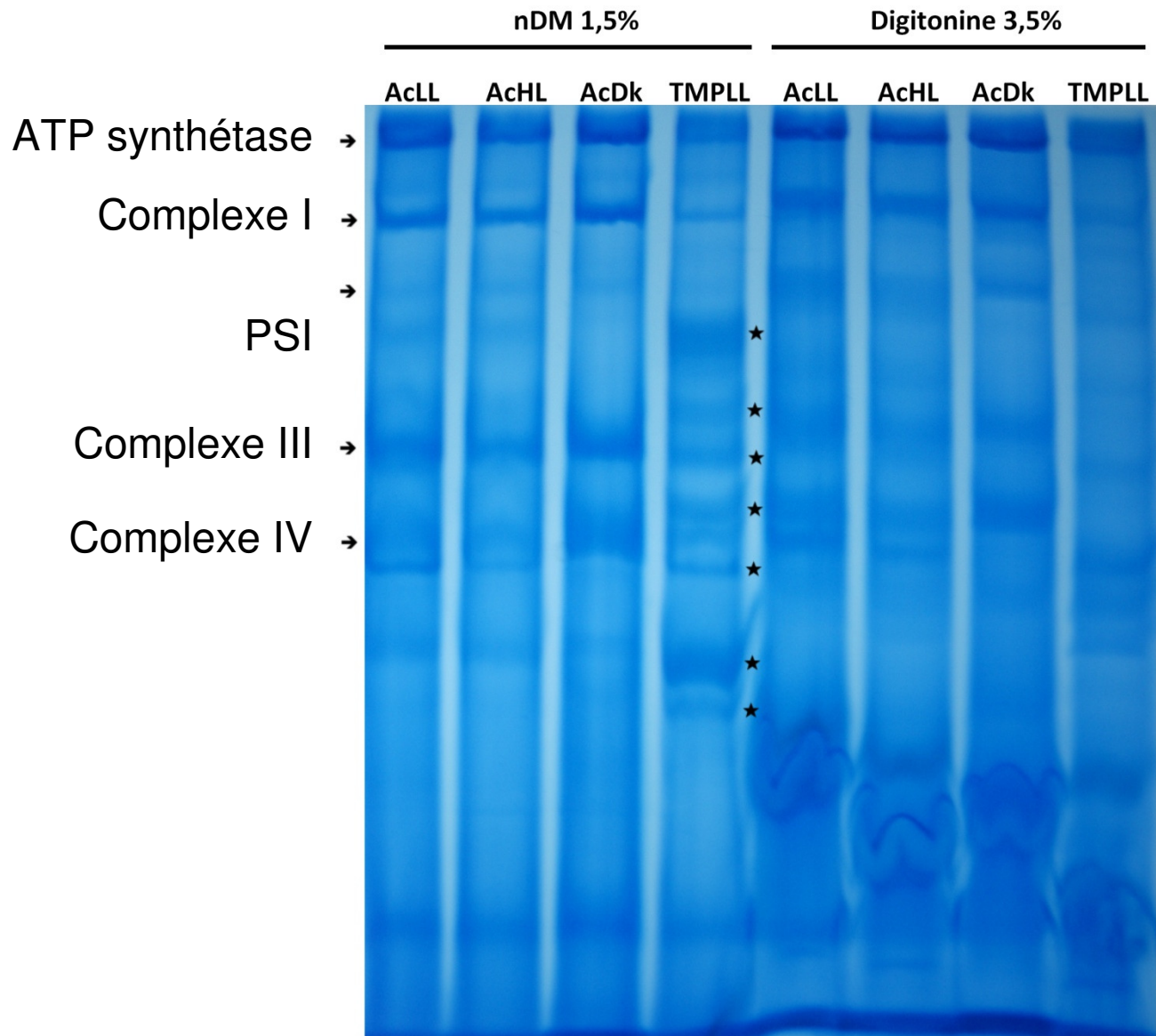
Temps de doublement moyen chez *Euglena gracilis*



Spread of photosynthesis through 1st/2nd/3rd/etc endosymbiotic events

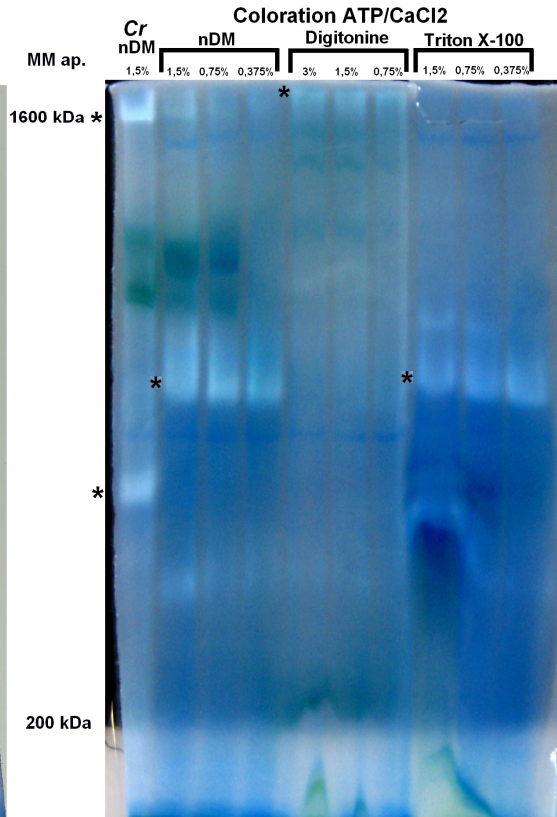


Euglena gracilis : native respiratory complexes

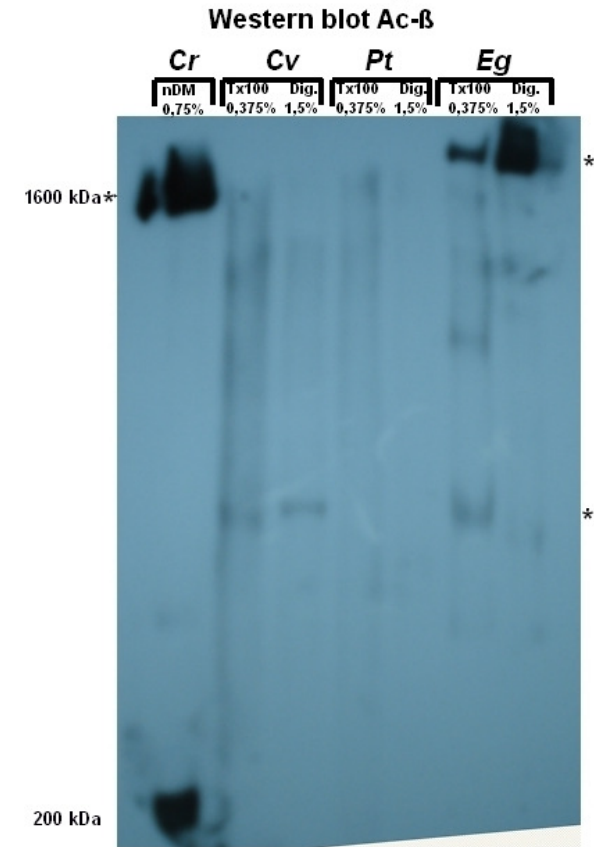


Euglena gracilis : native respiratory complexes

BN-PAGE - *Euglena gracilis* mb



BN-PAGE - *Pt* et *Eg* mb



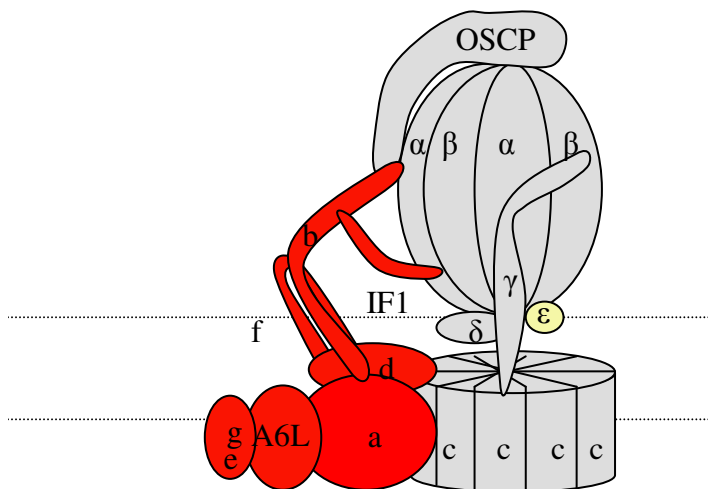
)

activité ATPase ($\mu\text{moles ATP min}^{-1} \text{ mg prot}^{-1}$)

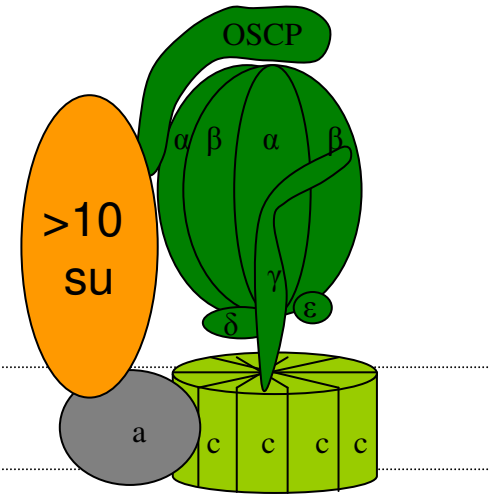
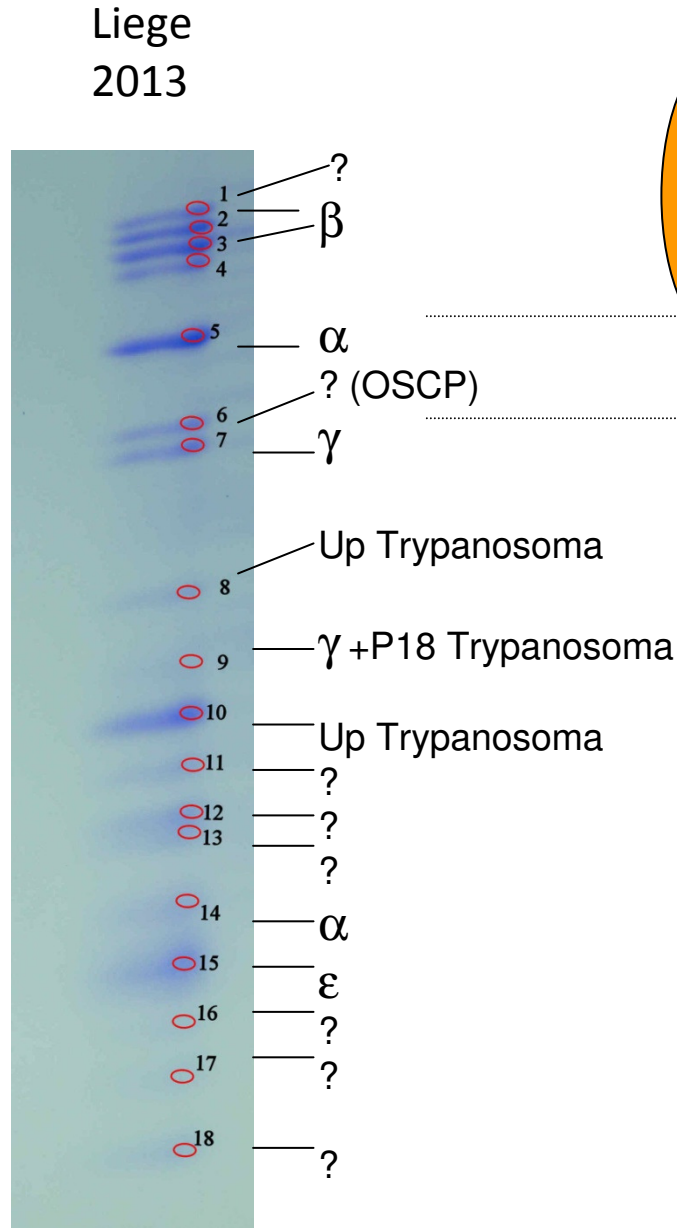
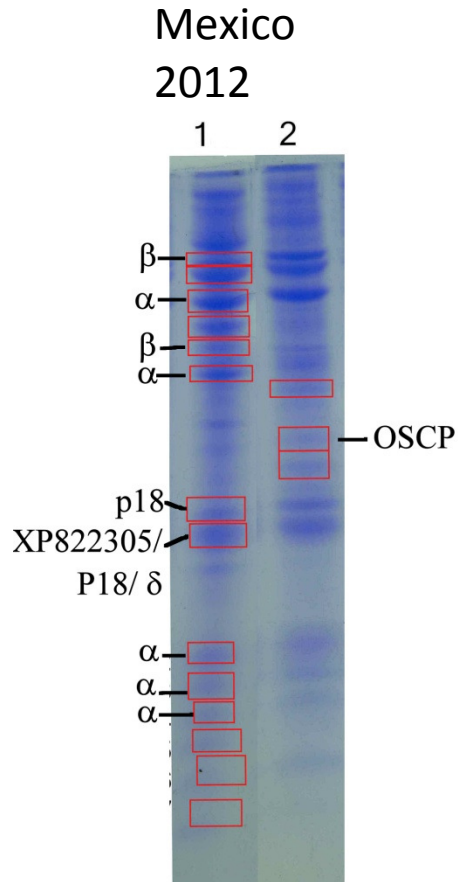
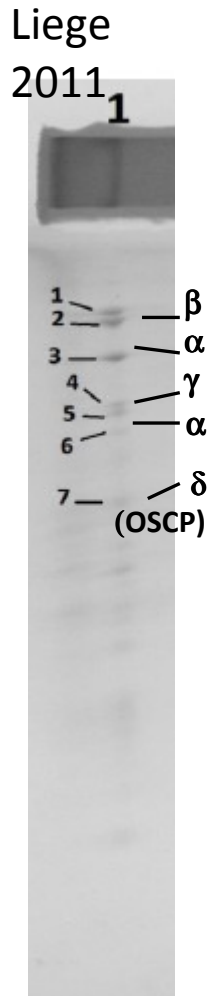
mito Acetate Light +SHAM+KCN	0.19 +/- 0.08
+Oli+SHAM+KCN	0.09 +/- 0.04
mito Ethanol Dark +SHAM+KCN	0.15 +/- 0.06
+Oli+SHAM+KCN	0.08 +/- 0.05

Search for ATP synthase subunits in available ESTs dataset

Eco α ba Cya <u>Ath</u> <u>Chl</u> Hsa Atu Dp Tth Ngr Kin Egr															Eco α ba Cya <u>Ath</u> <u>Chl</u> Hsa Atu Dp Tth Ngr Kin Egr														
Name	l	c.	n.	a	o.	p	b	ur	P	tri	e	u	é.	a	Name	l	c.	n.	a	o.	p	b	ur	P	tri	e	u	é.	a
alpha															ASA4														
beta															ASA1														
gamma															ASA2														
epsilon															ASA3														
delta															ASA5														
OSCP															ASA6														
ATPC															ASA7														
ATPA															ASA8														
ATPB															ASA9														
ATPD															ASP1														
ATPE															ASP2														
ATPF															ASP3														
ATPG															ASP4														
ATPF6															ASP5														
ATPA6L															ASP6														
STF1															ASP7														
															ASP8														
															ASP9														
															ASP10														
															ASP11														
															ASP12														
															ASP13														
															ASP14														



ATP synthase subunit composition in Euglena



Conclusions

Study of the function of typically mt-DNA encoded Complex I subunit

Current works : Introduction and characterization of mitochondrial mutations thought to be responsible for pathologies

Identification of Assembly factors

Combined proteic and genomic approaches leads to identify shared or specific subunits in respiratory-complexes

Current works : Characterization of enzymes in Various eukaryotes

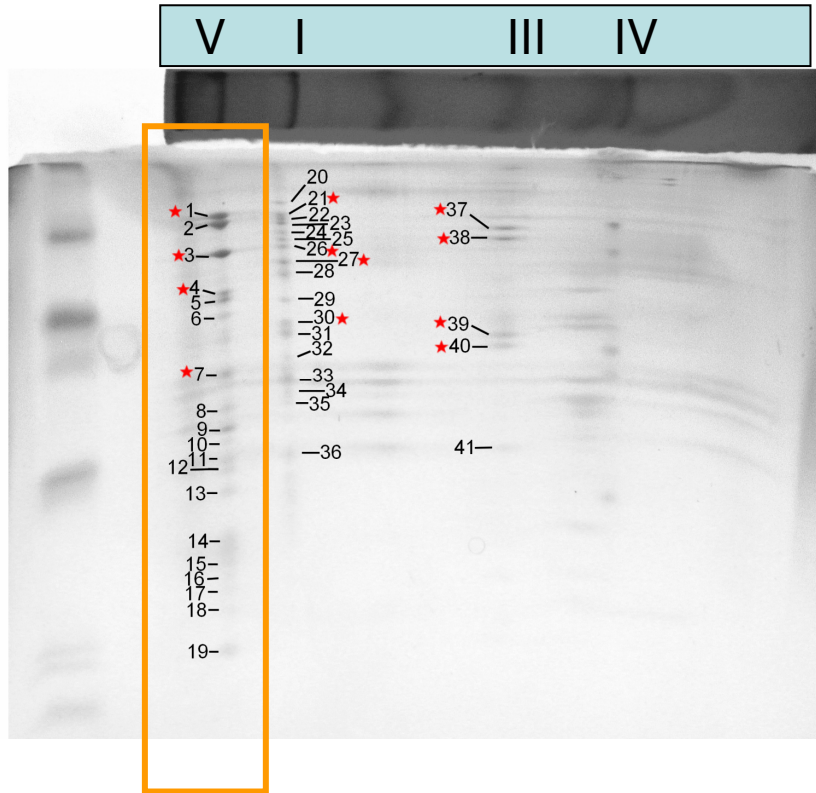
Eukaryotes have evolved different ATP synthase stator structure that confers high stability to the complex

protect the active site (proton channel)

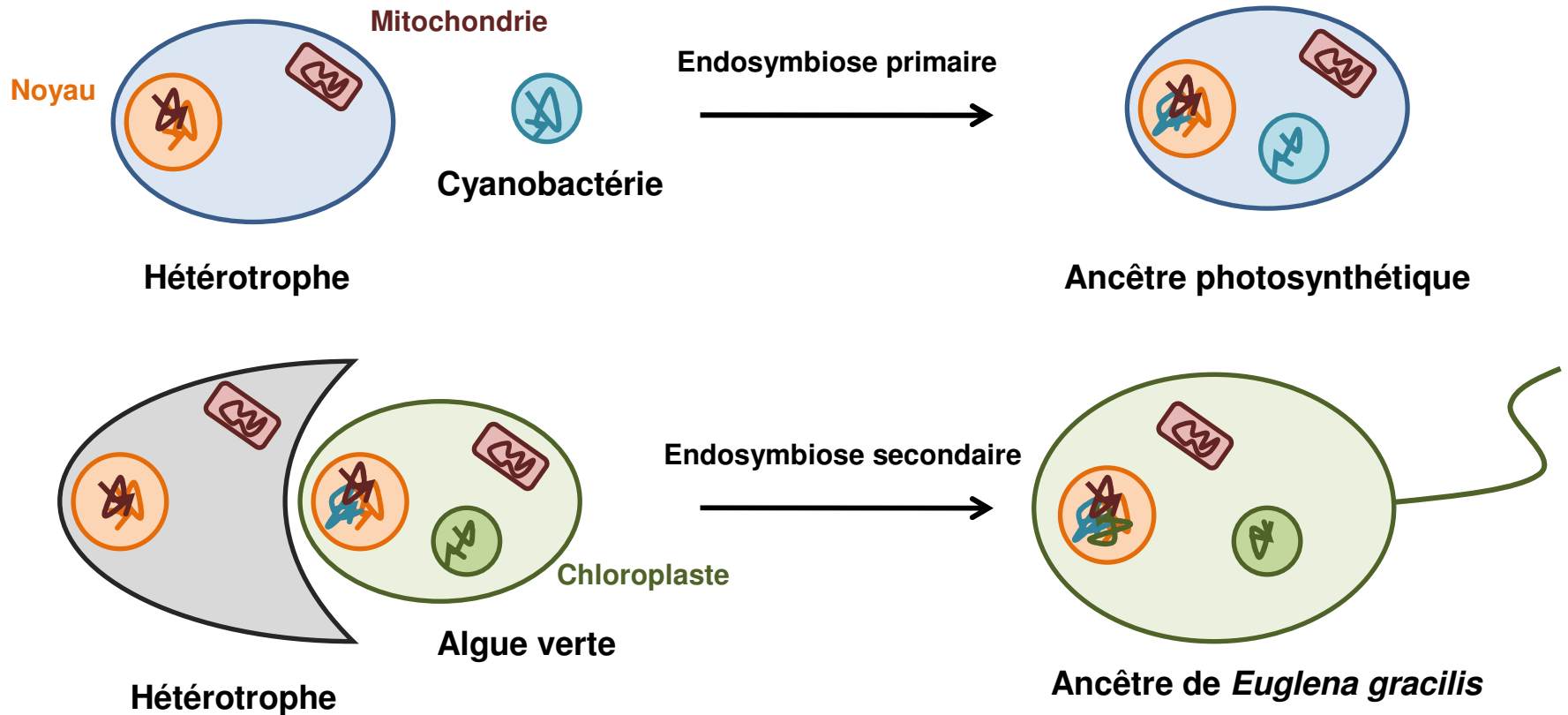
modify the shape of membrane and control pmf utilisation

Current works : Relation structure stator/cristae

Search for ATP synthase subunits by proteomic analysis



- Acquisition du chloroplaste par endosymbiose



Problématique n°1: Quelles interactions mitochondrie-chloroplaste chez *Euglena gracilis* ?