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Assembly of the Complexes of the Oxidative Phosphorylation System in Land Plant Mitochondria

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Abstract

Plant mitochondria play a major role during respiration by producing the ATP required for metabolism and growth. ATP is produced during oxidative phosphorylation (OXPHOS), a metabolic pathway coupling electron transfer with ADP phosphorylation via the formation and release of a proton gradient across the inner mitochondrial membrane. The OXPHOS system is composed of large, multiprotein complexes coordinating metal-containing cofactors for the transfer of electrons. In this review, we summarize the current state of knowledge about assembly of the OXPHOS complexes in land plants. We present the different steps involved in the formation of functional complexes and the regulatory mechanisms controlling the assembly pathways. Because several assembly steps have been found to be ancestral in plants—compared with those described in fungal and animal models—we discuss the evolutionary dynamics that lead to the conservation of ancestral pathways in land plant mitochondria.

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1. INTRODUCTION

Mitochondria are cellular organelles present in nearly all eukaryotes. Mitochondria originated from the integration of an alphaproteobacterium into an Archaean host cell. It is now clear that all known living eukaryotes are descendent from a single mitochondria-containing ancestor, the Last Eukaryote Common Ancestor (LECA) (32). However, to date, many types of mitochondria or mitochondria-related organelles with variable functions populate the different eukaryotic lineages (59, 115). Therefore, all types of mitochondria and mitochondria-related organelles evolved from the mitochondrial ancestor present in LECA, which was already an established organelle able to perform various biochemical functions (50, 147). Some of these functions were differentially lost in mitochondrial lineages whereas other functions were acquired (115). Despite these differences, the major role shared by all mitochondria is energy production through aerobic respiration.

Aerobic respiration is a catabolic pathway that requires oxygen to produce ATP, the chemical energy used to fuel metabolism and growth. Aerobic respiration is composed of three steps: metabolic reactions oxidizing nutrients such as glycolysis, the tricarboxylic acid (TCA) cycle in the mitochondrial matrix, and the oxidative phosphorylation (OXPHOS) system in the inner mitochondrial membrane (IMM). These mitochondrial reactions are essential for aerobic eukaryotes, including plants. The organization of mitochondrial respiration in plants has been reviewed before (93). Briefly, during glycolysis, sugars are degraded into pyruvate, which is imported in the mitochondrial matrix. Pyruvate is then converted into acetyl-coenzyme A, which is incorporated into the TCA cycle. Many enzymes involved in glycolysis and the TCA cycle produce reduced

cofactors (NADH, $FADH_2$). The OXPHOS system regenerates the oxidized form of these cofactors. The electrons resulting from the oxidation of the cofactors are transferred through a series of large protein complexes (termed complexes I to IV) to molecular oxygen. While electrons are transferred, protons are exported from the matrix to the intermembrane space (IMS), creating an electrochemical gradient across the IMM. This gradient is ultimately used by the ATP synthase to produce ATP.

In this review, we focus on recent progress made in understanding the assembly of the OXPHOS complexes in land plants, mainly in the reference organism *Arabidopsis thaliana*. We summarize the different steps required for the building of these macromolecular complexes and outline the specific assembly pathways. We also present the regulatory processes known to control the assembly pathways. Finally, we discuss these pathways in the context of the evolution of mitochondria.

2. THE OXPHOS SYSTEM IN PLANTS

2.1. Composition of the OXPHOS System

The OXPHOS system is very well conserved from bacteria to higher eukaryotes. It is composed of five complexes termed complexes I to V. Complexes I to IV form the respiratory chain and are responsible for electron transfer and for the building of an electrochemical proton gradient across the IMM. The respiratory chain can be divided in two parts. First, some enzymes transfer electrons to ubiquinone, a mobile electron carrier that diffuses in the membrane (**Figure 1**). Complex I transfers electrons from NADH, and complex II transfers electrons originating from the conversion of succinate to fumarate. The second part of the respiratory chain transfers electrons from ubiquinol to molecular oxygen (**Figure 1**). Complex III passes electrons from ubiquinol onto cytochrome *c*, a small hemoprotein located in the IMS. Cytochrome *c* donates electrons to complex IV, which reduces oxygen.

Besides the main complexes, additional proteins are present in the plant OXPHOS system (**Figure 1**). These proteins form additional routes for electrons and are called alternative pathways. The alternative NAD(P)H dehydrogenases are located on both sides of the IMM. They transfer electrons from NADH or NADPH to ubiquinone and therefore represent a potential bypass of complex I. Alternative oxidase is an ubiquinol-oxygen oxidoreductase that fulfills a function similar to that of the complex III–complex IV system. Importantly, the alternative pathways do not participate in the formation of the proton gradient. In theory, their presence makes the activity of the OXPHOS system more flexible, for example, in cases when cofactor recycling should be uncoupled from ATP production (143). Many other biochemical reactions feed electrons to the respiratory chain at either the ubiquinone level or the cytochrome *c* level (118). None of these reactions contribute to the formation of the proton gradient.

Complexes I, III, and IV are the only enzymes that pump protons from the matrix to the IMS. The energy from this electrochemical gradient is used by complex V, the ATP synthase. It couples the transfer of electrons back into the matrix with ATP synthesis.

The composition of the OXPHOS complexes in plants has been investigated using native gel electrophoresis and mass spectrometry (39, 88, 92). Interestingly, some complexes were found to have an unusual composition when compared with complexes present in fungal and animal mitochondria, the main references for study of the OXPHOS system (**Supplemental Table 1**). Complex I has an extra domain on the matrix side of the membrane arm, most likely containing ancestral subunits (136). Complexes II and IV also contain additional subunits (39, 92). However, for complex IV, the exact set is currently debated (124).

Supplemental Material >

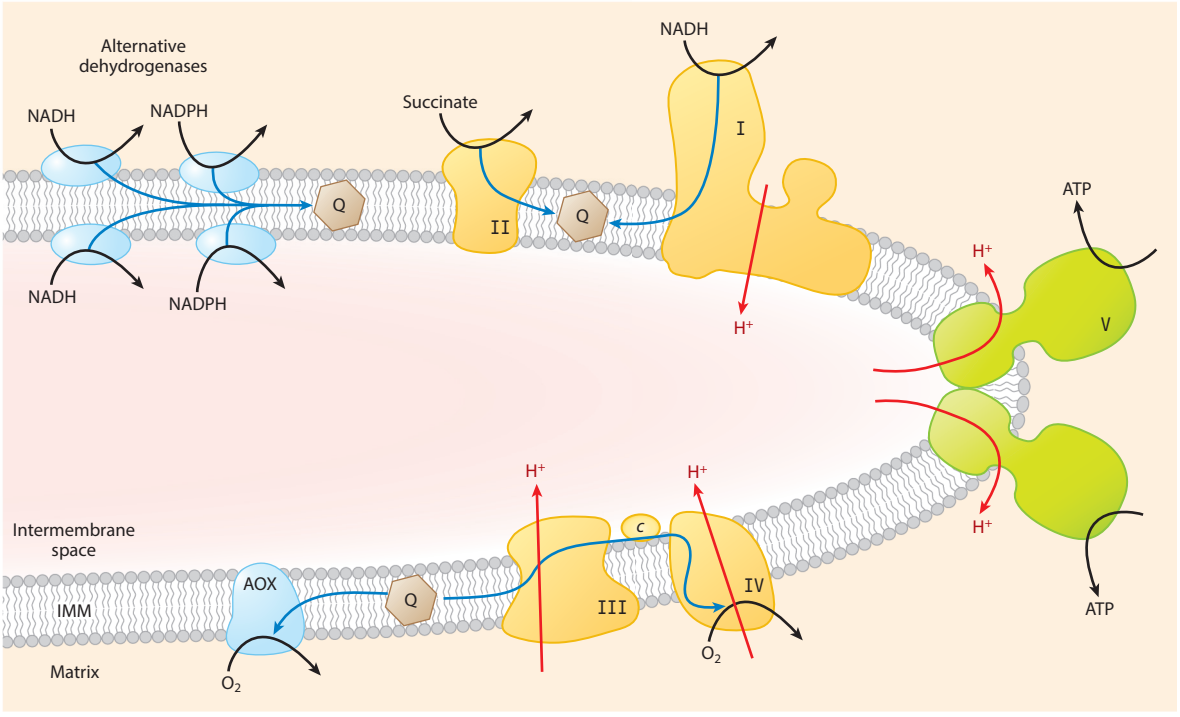

Figure 1

Overview of the OXPHOS system in plants. The OXPHOS system is located within the cristae of the IMM. In the upper part, enzymes reducing the quinone pool are shown. In the lower part, enzymes oxidizing the quinone pool and reducing oxygen are shown. Electron transfers are indicated with blue arrows, and proton movement is indicated with red arrows. The main complexes of the respiratory chain are indicated in orange, and the alternative pathways are indicated in blue. Complexes I to IV are labeled I, II, III, and IV, respectively. The ATP synthase (complex V) is represented in green as a dimer located at the tip of the cristae. Abbreviations: AOX, alternative oxidase; *c*, cytochrome *c*; IMM, inner mitochondrial membrane; OXPHOS, oxidative phosphorylation; Q, ubiquinone.

2.2. Structural Organization of the OXPHOS System

In mitochondria, the OXPHOS system is localized in the cristae of the IMM. Studies on the organization of the OXPHOS system initially led to the conclusion that complexes were randomly distributed in the cristae. However, recent work has discussed this idea and proposed that the OXPHOS system is spatially organized (26, 74). For example, dimerization of ATP synthase imposes a curvature on the membrane. As a result, arrays of complex V dimers are formed at the tip of the cristae (33, 122).

With the development of native electrophoresis techniques, supramolecular assemblies of complexes were detected (37, 75). In addition to the complex V dimer, supercomplexes containing complexes I, III, and IV were identified and termed respirasomes (20, 150). In plants, the main supercomplex appears to contain only complexes I and III (33) but complex IV–containing supercomplexes were also observed (86, 124). However, the relative abundances of the different supercomplexes seem to vary between plant species (38, 86, 123). Overall, while the structures of the supercomplexes are well characterized, the role of this type of organization is still not understood (91).

3. PRODUCTION OF THE COMPONENTS OF THE OXPHOS SYSTEM

The OXPHOS system is an intricate machine. In plants, it is composed of more than 100 proteins and several cofactors (**Table 1**). Assembling all of these components together into five functional complexes requires sophisticated and efficient assembly processes. In the case of failed assembly, the physiological consequences are dramatic. In humans, several diseases are caused by altered OXPHOS function (34, 68) and many others have been linked to assembly defects (40, 141). Similarly, in plants, mutants impaired in OXPHOS assembly exhibit strong phenotypes from growth retardation to embryo lethality (see Section 4 for details).

3.1. Mitochondria-Encoded Subunits

Interestingly, 18 OXPHOS subunits are encoded by the mitochondrial genome (**Table 1**). The reasons why some subunits remain encoded by the mitochondrial genome are currently unclear. It has been proposed that the biophysical properties of the genes (GC content) and the subunits (high hydrophobicity) are key determinants for gene retention in the mitochondrial genome (65). Another hypothesis is that the gene product exerts feedback regulation on its gene and, therefore, genes and gene products must be present within the same compartment (2). These two hypotheses are not exclusive and are rather consistent with one another (3). This suggests that mitochondria retain genes that encode key subunits for the assembly of a complex. Interestingly, mitochondrial gene content varies between plant species (**Supplemental Table 2**). However, reductive evolution of the mitochondrial genome is more marked in some eukaryotic groups such as opisthokonts (115). Therefore, by comparing the gene content of mitochondrial genomes from various eukaryotes, we can propose subunits playing key roles in the assembly pathways. These subunits are Nad1, Nad2, Nad4, Nad5, and Nad6 for complex I; cytochrome *b* (Cob) for complex III; cytochrome *c* oxidase 1 (Cox1) for complex IV; and Atp6 and Atp8 for complex V.

Supplemental Material >

3.2. Different Steps of the Assembly Processes

Before the occurrence of assembly per se, different components of the complexes (subunits, cofactors) must be produced and targeted to the place where assembly occurs (**Figure 2**).

3.2.1. Expression and membrane insertion of mitochondria-encoded subunits. The expression of the mitochondrial genome involves many steps. Large transcripts are found in the mitochondrial matrix and posttranscriptional mechanisms, such as transcript splicing, end processing, and editing, are necessary to produce the mature transcript that will be translated (14). Many mutants in proteins involved in these posttranscriptional processes show alteration in the expression of one or a few mitochondrial genes and can therefore be used to investigate the role and assembly of the encoded gene products (see 27 for a review).

One of the biggest challenges in terms of OXPHOS subunit assembly is the actual insertion into the IMM of the hydrophobic mitochondria-encoded proteins. While these events have not been extensively studied in plant mitochondria, much can be learned from other reference organisms. The major route for insertion into the IMM from the matrix takes place via the conserved protein Oxa1 (56). Oxa1 is part of a large family of proteins now termed the Oxa1 superfamily, all of which are involved in membrane insertion in bacteria, mitochondria, chloroplasts, endoplasmic reticulum, and archaea (23). To overcome the problem of having membrane proteins found in the matrix, Oxa1 interacts directly with the mitochondrial ribosomes and inserts mitochondria-encoded proteins in a cotranslational manner (101). One significant difference between plant

Table 1 Composition of the oxidative phosphorylation (OXPHOS) complexes in the reference plant *Arabidopsis thaliana*

		Complex I	Complex II	Complex III	Cytochrome *c*	Complex IV	Complex V	Alternative pathways	Total
Total number of subunits[a]		52	8	10	1	16	15	9	111
Mitochondria-encoded subunits		9	–	1	–	3	5	–	18
Cofactors	Flavine cofactors	1 FMN	1 FAD	–	–	–	–	1 FAD[b]	9
	Fe-S clusters	8 Fe-S clusters (6 [4Fe-4S], 2 [2Fe-2S])	3 Fe-S clusters ([2Fe-2S], [4Fe-4S], [3Fe-4S])	1 Fe-S cluster ([2Fe-2S])	–	–	–	–	12
	Hemes	–	1 (heme *b*)	3 (two hemes *b* and one heme *c*)	1 (heme *c*)	2 (hemes *a* and a_3)	–	–	7
	Metal centers	–	–	–	–	2 Cu centers (Cu_A and Cu_B)	–	1 di-iron center[c]	3

[a]Subunit numbers do not take into account isoforms and include putative subunits. The list of the subunits of each OXPHOS complex can be found in **Supplemental Table 1**.
[b]For each of the seven alternative NAD(P)H dehydrogenases.
[c]For each of the two alternative oxidases.
Abbreviations: Cu, copper; FAD, flavine adenine dinucleotide; FMN, flavine mononucleotide.

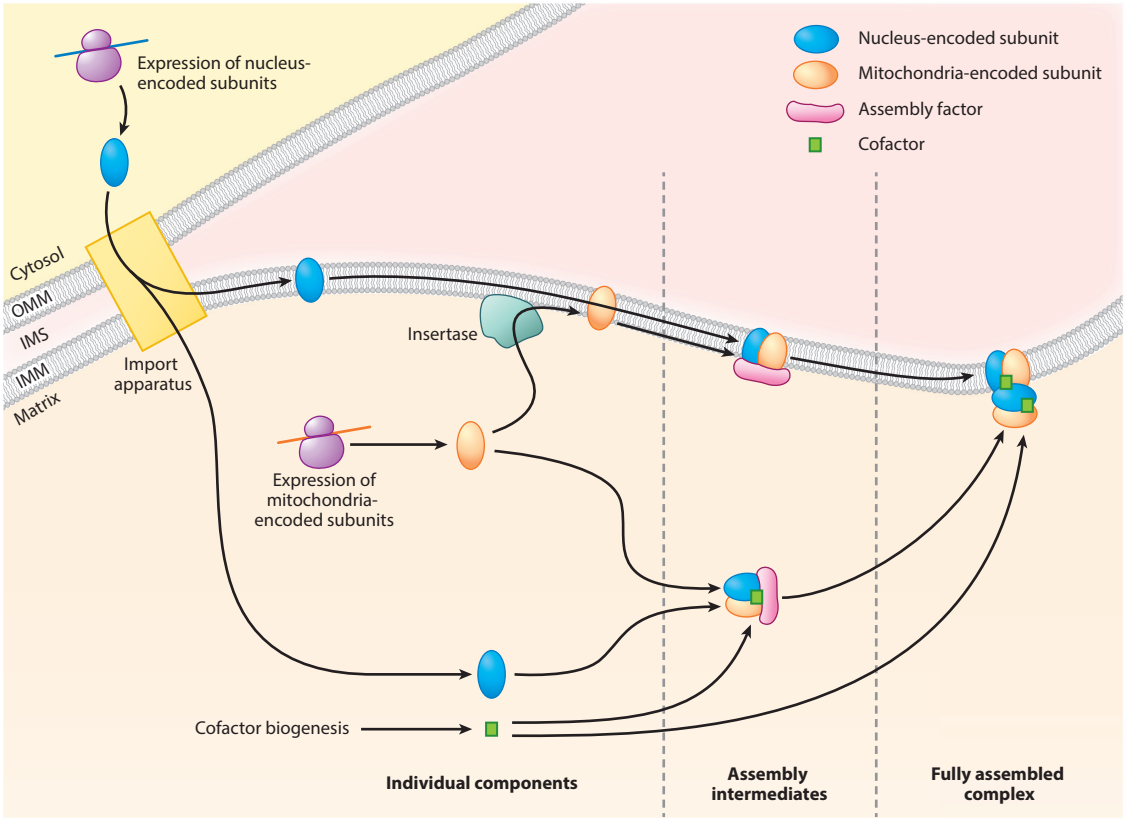

Figure 2

Overview of the assembly of an OXPHOS complex. Different steps in the assembly of an OXPHOS complex are schematically represented. The first step is the production of the individual components of the complex: nucleus-encoded subunits, mitochondria-encoded subunits, and cofactors. Then assembly can occur. Assembly factors are involved in the formation of assembly intermediates, which may contain cofactors. During the last part of the assembly pathway, assembly intermediates merge to form the mature complex. Some cofactors are inserted during this step, and the assembly factors are released. Abbreviations: IMM, inner mitochondrial membrane; IMS, intermembrane space; OMM, outer mitochondrial membrane; OXPHOS, oxidative phosphorylation.

mitochondria and those of yeast and humans is that plant mitochondria contain four Oxa proteins rather than two (12). It has also been observed that two of the plant mitochondrial Oxa proteins contain C-terminal tetratricopeptide repeat (TPR) domains, which are unique to plant mitochondria (12, 67). The function of OXA2b has been recently elucidated (66); however, the exact role of the other plant mitochondrial Oxa proteins remains unknown (67).

3.2.2. Expression and import of nucleus-encoded subunits. Overwhelmingly, the vast majority of plant mitochondrial OXPHOS components are encoded by nuclear genes, translated on cytoplasmic ribosomes, and imported into the mitochondria. Theoretically, nuclear expression of mitochondrial proteins allows more possibilities for regulation of the assembly process, but it also adds a drawback as the expression of the mitochondrial and nuclear genomes needs to be coordinated.

The posttranslational import pathways of mitochondrial proteins are relatively well conserved across species (96). The majority of mitochondrial proteins contain cleavable N-terminal

targeting signals (21). The import of OXPHOS components is thought to be no different than the import of any other mitochondrial protein and occurs through the following pathways: The first point of entry is the translocase of the outer membrane (TOM), through which all mitochondrial proteins must pass (96). Interestingly, the plant TOM20 receptors are not evolutionarily related to other lineages and are an elegant example of convergent evolution (80). After traversing the outer membrane, OXPHOS proteins can be either oxidatively folded in the IMS by the mitochondrial import and assembly pathway (MIA) or chaperoned by the small translocase of the inner membrane (TIM) proteins. If the latter, they move to either the TIM23 complex for passage through to the matrix or the TIM22 complex for insertion into the IMM (96). The TIM23 complex can also insert single membrane–containing proteins using a stop transfer mechanism (96).

3.2.3. Cofactor biogenesis. Respiratory complexes are equipped with metal-containing cofactors, which are essential for the transfer of electrons (**Table 1**). These cofactors are almost all produced within mitochondria. The enzymes responsible for the production of FMN and $FADH_2$ from riboflavin were shown to be present in mitochondria isolated from tobacco cells (44). Twelve iron-sulfur (Fe-S) clusters are found in the respiratory chain. Plant mitochondria contain the machinery for de novo synthesis of Fe-S clusters (9). Another iron-containing cofactor present in abundance in the respiratory chain is heme (**Table 1**). In plants, heme is synthesized in the chloroplasts (138). The last step of this biosynthetic pathway, the chelation of iron, is also proposed to be present in plant mitochondria (24); however, this localization has been disputed (79). Complex IV also contains two copper centers; the mechanism of import of copper in plant mitochondria has not yet been elucidated. Similarly, nothing is known about the biogenesis of the di-iron center present in alternative oxidase.

4. ASSEMBLY OF THE DIFFERENT OXPHOS COMPLEXES

Assembly of a complex is the process by which its different components are put together into a functional unit. This process requires the action of so-called assembly factors, proteins that aid assembly. Assembly factors have a wide range of functions, such as subunit modification, stabilization of assembly intermediates, and cofactor delivery (**Table 2**). If some assembly factors associate with assembly intermediates, they are never present in the mature complex (**Figure 2**).

4.1. Assembly of Complex I

Supplemental Material >

Complex I is the first enzyme of the OXPHOS system; it transfers electrons from NADH to ubiquinone and pumps protons in the IMS (58). Complex I contains two arms: The membrane arm is embedded in the IMM, and the matrix arm protrudes into the matrix. In plants, complex I is composed of at least 47 subunits (**Supplemental Table 1**) and is proposed to contain eight Fe-S clusters (84, 104). Complex I assembly has been extensively studied in animal and fungal mitochondria. Recent work in humans systematically investigated the assembly pathway and proposed the most detailed assembly model for complex I (53, 135). Assembly of complex I is a stepwise process. Building blocks or assembly intermediates are initially formed and later assembled together to form the whole complex (**Figure 3**). Many assembly factors have been identified in humans as playing a role during the assembly process (41). The functions of these assembly factors remain mostly unknown (**Table 2**). NFUFAF5 and NDUFAF7 have been shown to modify arginine residues on complex I subunits (112, 113), and NUBPL was proposed to be involved in the delivery of Fe-S clusters (125).

Table 2 List of *Arabidopsis* proteins that are orthologous to human or yeast assembly factors involved in oxidative phosphorylation (OXPHOS) biogenesis[a]

Complex	Human assembly factor	Yeast assembly factor	Role during assembly	*Arabidopsis* ortholog	Reference(s) confirming the role in plants
Complex I	NDUFAF1	–	Membrane arm assembly	At1g72420/At1g17350	–
	NDUFAF2	–	Matrix arm assembly	At4g26965	–
	NDUFAF3	–	Matrix arm assembly: interacts with NDUFAF4	At3g60150/At2g44525	–
	NDUFAF4	–	Matrix arm assembly: interacts with NDUFAF3	At3g21400	–
	NDUFAF5	–	Matrix arm assembly: hydroxylates NDUFS7	At1g22800	–
	NDUFAF6	–	Unknown function	At1g62730	–
	NDUFAF7	–	Matrix arm assembly: methylates NDUFS2	At3g28700	–
	TIMMDC1	–	Membrane arm assembly	–	–
	ACAD9	–	Membrane arm assembly	–	–
	ECSIT	–	Membrane arm assembly	–	–
	TMEM126B	–	Membrane arm assembly	–	–
	FOXRED1	–	Membrane arm assembly	–	–
	NUBPL	–	Matrix arm assembly: Fe-S cluster delivery and/or mitochondrial translation	At4g19540	151
Complex II	SDHAF1	Sdh6p	Delivery of Fe-S clusters	At2g39725	–
	SDHAF2	Sdh5p	Promotes attachment of FAD on SDH1	At5g51040	62
	SDHAF3	Sdh7p	Supports binding of SDHAF1 to SDH2	–	–
	SDHAF4	Sdh8p	Promotes the formation of the SDH1/SDH2 intermediate	At5g67490	11
Complex III	UQCC1	Cbp3p	Cytochrome *b* translation and/or stability	At5g51220	–
	UQCC2	Cbp6p	Cytochrome *b* translation and/or stability	–	–
	UQCC3	Cbp4	Cytochrome *b* recruitment	At1g79390	–
	CCHL	Cyt2p	Cytochrome c_1 heme lyase	Type I cytochrome *c* maturation pathway; At1g15220, At1g63270, At3g51790, AtMg00110, AtMg00180, AtMg00830, AtMg00900, AtMg00960	45, 85, 110, 111, 130
		Cyc3p	Cytochrome *c* heme lyase		
	–	Cyc2p	Heme reduction		
	BCS1L	Bcs1p	Required for Rieske Fe-S protein	Plant mitochondrial Tat pathway; At5g43680 and AtMg00570	22
	LYRM7	Mzm1p	Required for Rieske Fe-S protein	–	–
	TTC19	–	Involved in the clearance of UQCRFS1 N-terminal fragments	–	–

(*Continued*)

Table 2 (*Continued*)

Complex	Human assembly factor	Yeast assembly factor	Role during assembly	*Arabidopsis* ortholog	Reference(s) confirming the role in plants
Complex IV	COX10	Cox10p	Farnesylation of heme *b*	At2g44520	82
	COX11	Cox11p	Assembly of Cu_B in Cox1	At1g02410	109
	COX14	Cox14p	Avoids Cox1 aggregation before assembly	–	–
	COX15	Cox15p	Hydroxylation of heme *o* to form heme *a*	At5g56090	–
	COX16	Cox16p	Unknown function	At4g14145	–
	COX17	Cox17p	Copper metallochaperone that transfers copper to SCO1 and COX11	At3g15352/At1g53030	8, 42
	COX18	Cox18p	Translocation and export of the Cox2 C-terminal tail into the intermembrane space	At3g44370	66
	COX19	Cox19p	Interacts with COX11 as a reductant; critical for COX11 activity	At1g66590/At1g69750	6
	COX20	Cox20p	Cox2 chaperone for copper metalation	–	–
	COX23	Cox23p	Unknown function	At1g02160/At5g09570	–
	SURF1	Shy1p	Cox1 translation and assembly; proposed to participate in heme *a* delivery	At3g17910/At1g48510	–
	SCO1	Sco1p	Copper chaperone, transporting copper to the Cu_A site on Cox2	At3g08950	5, 131, 132
	SCO2	Sco2p		At4g39740	
	COA3	Coa3p	Stabilizes Cox1 and forms a Cox1–COX14–COA3 complex	–	–
	COA6	Coa6p	Cooperates with SCO2 in the metalation of Cu_A	At5g58005	–
	PET191	Pet191p	Unknown function	At1g10865	–
	CMC1	Cmc1p	Stabilizes the Cox1–COX14–COA3 complex	At5g16060	–
	CMC2	Cmc2p	Unknown function	At4g21192	–
	HIGD1A	Rcf1p	Stabilizes the COX4–COX5A complex	At3g48030	–

(*Continued*)

Table 2 (*Continued*)

Complex	Human assembly factor	Yeast assembly factor	Role during assembly	*Arabidopsis* ortholog	Reference(s) confirming the role in plants
Complex V	ATPAF1	Atp11p	Promotes formation of the F_1 domain	At2g34050	–
	ATPAF2	Atp12p	Promotes formation of the F_1 domain	At5g40660	–
	ATPIF1	Inh1p	Blocks ATP hydrolysis of uncoupled ATP synthase	At5g04750	–
	FMC1	Fmc1p	Formation/stability of F_1 domain	–	–
	ATP23	Atp23p	Required for processing of ATP6	At3g03420	–
	ATP10	Atp10p	Promotes formation of the F_O domain	At1g08220	–
Supercomplexes	HIG2A	Rcf1p	Promotes formation of complex IV–containing supercomplexes	–	–
	RCF2	Rcf2p	Promotes formation of the complex III–complex IV supercomplex	–	–
	UQCC3	Cbp4p	Cardiolipin-binding protein that stabilizes complex III–containing supercomplexes	At1g79390	–
	SLP2	Slp2p	Cardiolipin-binding protein	At4g27585	43
	DnaJC15	Pam18p	Negative regulator of supercomplex formation	At2g35795	–
	COX7A2L	–	Promotes association between complexes III and IV	–	–

[a]Only proteins involved in specific steps of the assembly are listed. Proteins involved in gene expression or proteins involved in import processes are not included.
Abbreviations: COX, cytochrome *c* oxidase; FAD, flavine adenine dinucleotide; SDH, succinate dehydrogenase.

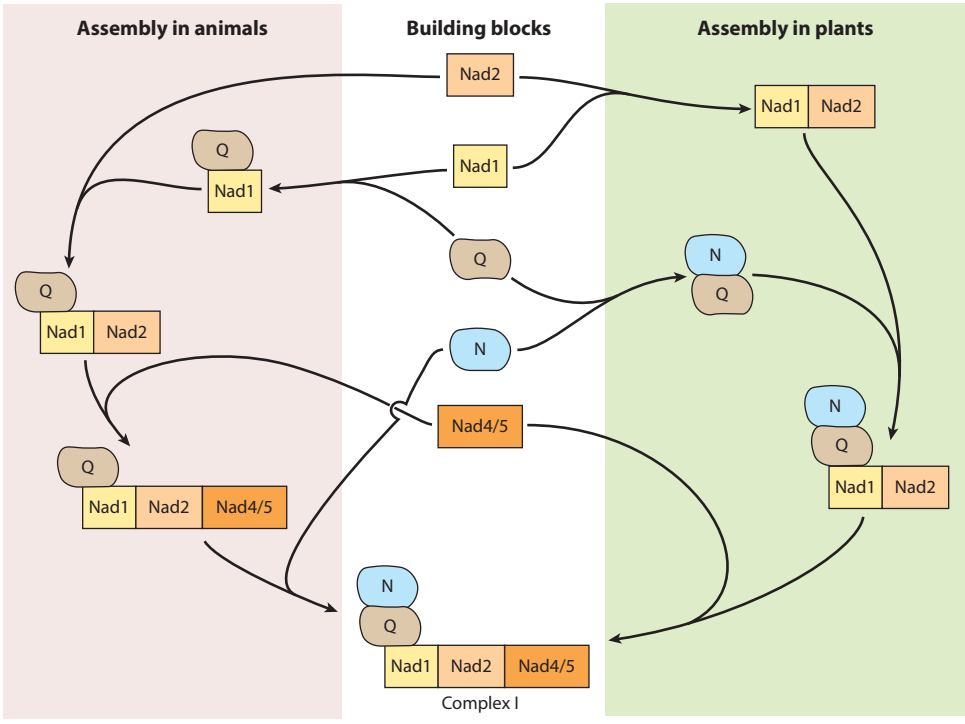

Figure 3

Comparison of the assembly sequences and components of complex I in animals and plants. Shown here are the building blocks (*center*) common to both pathways as well as the assembly intermediates in (*left*) animals and (*right*) plants. The building blocks of the membrane arm are labeled according to the main mitochondria-encoded subunit they contain. The building blocks of the matrix arm are labeled Q for the quinone binding module and N for the NADH binding module.

In plants, the assembly pathway has mostly been investigated using genetic approaches. In mutants impaired in the production of a subunit, assembly intermediates accumulate and can be detected; because of this, some steps of the assembly pathway can be reconstructed. Using reconstruction approaches, assembly of the membrane arm of complex I in *Arabidopsis* has been elucidated (87), and a plant-specific assembly factor was identified (120). A complementary approach based on complexome profiling was performed to determine the complete assembly pathway of complex I in *Arabidopsis* (78). Overall, the assembly of complex I in plants and animals is rather well conserved, and the same building blocks are found in both systems. However, they do not appear to be assembled in the same order (**Figure 3**). The plant pathway also involves ancestral subunits, whereas the animal pathway contains subunits and assembly factors that evolved more recently (36, 77, 78).

The different steps of the assembly of complex I are defined. However, many questions remain, particularly concerning insertion of the cofactors. An *Arabidopsis* mutant lacking INDH, which is homologous to human NUBPL, a protein proposed to be involved in the delivery of Fe-S clusters to target proteins, was shown to accumulate the membrane arm of complex I (151). This suggests that INDH may be responsible for the delivery of Fe-S clusters to the matrix arm and that the presence of Fe-S clusters is required for the stability of this arm. However, a translational defect was also detected in *indh*, notably affecting the matrix arm subunit Nad9 (151); therefore,

additional work should be performed to clarify the role of INDH during complex I assembly. Many assembly factors are conserved in plant and animal mitochondria (**Table 2**), but the differences in the assembly pathways observed between plant and animal models suggest that novel assembly factors specific to plants have yet to be discovered.

4.2. Assembly of Complex II

Complex II is the only enzyme of the OXPHOS system that is also part of another metabolic pathway: the TCA cycle. Complex II catalyzes the reversible conversion of succinate to fumarate and transfers electrons via FAD, Fe-S clusters, and a heme to ubiquinone (13). Complex II is composed of four subunits: two membrane subunits, SDH3 and SDH4, which anchor the catalytic subunits, SDH1, which contains the FAD, and SDH2, which harbors three Fe-S clusters (13). In plants, complex II contains additional subunits (61, 92) (**Supplemental Table 1**). Recently, it was demonstrated that two of the additional subunits found in *Arabidopsis* replace the transmembrane helixes lost by SDH3 and SDH4 (119). Complex II assembly has been intensively studied in fungi and animals, and an assembly model was recently proposed (13). The assembly of membrane subunits is currently not understood; however, the assembly of catalytic subunits is better characterized, and four assembly factors were identified as having roles in the formation of the SDH1/SDH2 intermediate. First, the FAD cofactor is incorporated into SDH1 by the assembly factor SDHAF2 (54); second, SDHAF1 and SDHAF3 insert the Fe-S clusters into SDH2 (98); and finally, SDHAF4 interacts with SDH1 to facilitate the association of SDH1 with SDH2 (144).

Supplemental Material >

In plants, recent advances have been made in the understanding of complex II assembly through the identification of proteins homologous to human assembly factors. Similar to its counterpart in opisthokonts, AtSDHAF2 is involved in the initial step of the assembly pathway, the attachment of the FAD cofactor to SDH1 (62). AtSDHAF4 has been shown to play a role in the association of flavinated SDH1 with SDH2 (11). A protein orthologous to another complex II assembly factor, SDHAF1, is encoded by the *Arabidopsis* genome (**Table 2**), but its function has not yet been investigated. The presence of additional subunits suggests that the assembly of complex II in plant mitochondria might be more complex than the corresponding pathway in fungal and animal mitochondria. However, this will have to be experimentally confirmed.

4.3. Assembly of Complex III

Complex III, also known as the bc_1 complex, like complexes I and IV, couples electron transfer to proton shuttling via a mechanism termed the Q cycle. Essentially, the Q cycle results in the oxidation of ubiquinol to ubiquinone, the transfer of electrons via one Fe-S cluster and three hemes on cytochrome *c*, and the transfer of four protons for every two cycles to the IMS (31). In *Arabidopsis*, complex III is composed of 10 subunits, all of which are conserved in yeast and humans (19) (**Supplemental Table 1**). Nine of the subunits are nucleus-encoded, and only one, Cob, is mitochondria-encoded. In comparison to other organisms, plant complex III is unique in that it contains the processing enzymes for mitochondrial targeting sequences (MPPα and MPPβ). This makes complex III a bifunctional enzyme because it catalyzes both the processing of mitochondrial presequences and electron transport (48). Due to the conserved nature of complex III, its assembly is not predicted to differ much between organisms. However, plant mitochondria have two major points of difference with opisthokont mitochondria. First, the assembly of *c*-type cytochromes is performed by a bacterial-type system I rather than system III in yeast and animals (see Section 4.4) (7). Second, plant mitochondria appear to utilize a twin arginine transport (Tat) pathway for insertion and maturation of the Rieske Fe-S protein, similar to bacteria and chloroplasts (22).

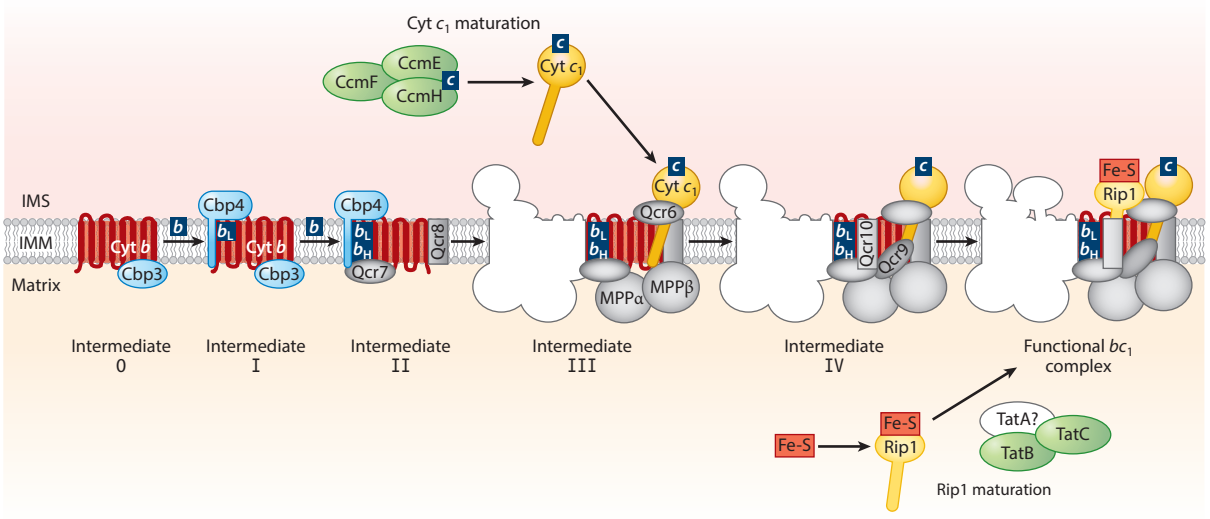

Figure 4

Assembly of complex III in plants. Because the subunit composition of complex III is conserved, its assembly pathway in plants is believed to be similar to the one described for opisthokonts. Therefore, our model shows the intermediates described in yeast (termed intermediates 0–IV). The maturations of Cyt c_1 and Rip1 are shown in green because these steps were found to differ between plants and opisthokonts (7, 22). The only two assembly factors conserved in plants, Cpb3 and Cpb4, are indicated in blue. For each new intermediate, only the names of the newly assembled subunits are indicated. From intermediate III, dimerization is possible; the second monomer of the dimer is represented in white. Hemes are represented with dark blue squares, and the Fe-S clusters are represented by red rectangles. Abbreviations: *b*, heme *b*; *c*, heme *c*; Ccm, cytochrome *c* maturation; Cyt c_1, cytochrome c_1; IMM, inner mitochondrial membrane; IMS, intermembrane space; Rip1, Rieske Fe-S protein; Tat, twin arginine transport.

To date, study of the assembly process of complex III has been almost nonexistent in plants most likely due to the essential nature of this complex. However, much work has been performed in yeast and humans (reviewed in 100), so a potential assembly model for plants can be hypothesized (**Figure 4**). The first step of complex III assembly is the cotranslational membrane insertion of Cob, which is most likely performed by Oxa1. Immediately following translation, Cob is bound by the assembly factor Cbp3, forming assembly intermediate 0 (51). Next, heme *b* is inserted into the b_L site, triggering the recruitment of Cbp4, which stabilizes the acquired heme, and forming intermediate I (57). Then, a second heme *b* is inserted into the b_H site. This is followed by the release of Cbp3 and the attachment of Qcr7 and Qcr8, the first nucleus-encoded subunits, forming intermediate II (57). At this stage, four more subunits—Qcr6, cytochrome c_1 (with an already incorporated heme), MPPα, and MPPβ—are added in a still-unknown order (52). This forms intermediate III, which represents the point at which complex III can dimerize (152). Next, Qcr9 and Qcr10 are incorporated to form intermediate IV. At this point, plant mitochondria most likely use a Tat pathway to insert the fully folded Rieske Fe-S protein from the matrix (where Fe-S cluster biogenesis is performed) into complex III (22), which produces a fully functional bc_1 complex. Several questions remain about complex III biogenesis in plants: How does the proposed Tat pathway function in the absence of an obvious TatA subunit in plants? Are there plant-specific assembly factors? Is assembly order in plants exactly the same as in opisthokonts?

4.4. Assembly of *c*-Type Cytochromes

Cytochromes are heme-containing electron carriers. *c*-Type cytochromes are characterized by the covalent attachment of the heme cofactor onto two cysteines of the apocytochrome. Two

c-type cytochromes are present in the OXPHOS system: Cytochrome c_1 is part of complex III and cytochrome *c* shuttles electrons between complexes III and IV. The maturation of *c*-type cytochromes, meaning the attachment of the heme on the apoprotein, requires dedicated machinery. Up to six maturation systems have been described (4). Mitochondria of opisthokonts and algae utilize system III (7). In land plant mitochondria, however, components of system III are absent. Instead, plant mitochondrial genomes contain several genes encoding proteins homologous to some of the nine cytochrome *c* maturation (Ccm) proteins of the bacterial system I (45, 133) (**Supplemental Table 2**). In the reference plant *Arabidopsis*, three additional Ccm proteins, encoded by nuclear genes, were also characterized (85, 111, 130). These proteins are able to partially complement bacterial *ccm* mutants (85, 130). Knockout mutation of AtCCMH results in embryo lethality (85), and a putative heme lyase complex containing mitochondria and nucleus-encoded proteins was described (110). Altogether, these data demonstrate that an almost-complete system I operates in plant mitochondria for the maturation of cytochrome c_1 and cytochrome *c*. The loss of *ccm* genes from the mitochondrial genomes of opisthokonts and algae was most likely a consequence of the evolution of system III. System III is the most simplified machinery for the maturation of *c*-type cytochromes because it is composed of only two proteins: (*a*) the cytochrome *c* heme lyase (CCHL) (7) and (*b*) the heme reductase Cyc2p (29).

Supplemental Material >

4.5. Assembly of Complex IV

Complex IV, or COX, is embedded in the IMM and catalyzes the transfer of electrons from cytochrome *c* to molecular oxygen. This redox process is coupled to proton translocation. In most eukaryotes, three catalytic subunits, Cox1, Cox2, and Cox3, are encoded by the mitochondrial genome (115). These subunits coordinate four redox cofactors, including two heme groups (hemes *a* and a_3) and two copper metallic centers. A di-copper center (Cu_A) is present in Cox2, and Cox1 carries a single-copper center (Cu_B) (142) (**Table 1**). COX composition has evolved into a sophisticated multimeric complex that incorporates nucleus-encoded subunits acting as protective structural scaffolds to stabilize the catalytic core of the enzyme (105). To date, up to 16 COX subunits have been proposed in *Arabidopsis* (83, 124); 14 are present in mammals (140), and 11 are present in yeast (129) (**Supplemental Table 1**).

The biogenesis of COX is the best-characterized assembly pathway of an OXPHOS complex, and excellent reviews have been published about this process in bacteria (121), yeast (128), mammals (140), and plants (83). In contrast to yeast where mutants lacking COX can be isolated (10), mutants in COX components have not yet been characterized in plants, most likely because they result in embryo lethality (83). Currently, COX assembly is conceived as a linear, modular model where the catalytic subunits of the holoenzyme (Cox1–Cox3) are matured and incorporated as cofactor-protein modules into the growing complex IV (140). The formation of the modules involves a large number of assembly factors, and some of them (COX10, COX15, SURF, SCO, and COX11) are conserved from bacteria to eukaryotes (10). The first step of COX assembly in opisthokonts is the cotranslational insertion of Cox1 into the IMM with the help of Oxa1, COX14, COA3, and CMC1, which forms the Cox1 module (16, 25, 140). Once Cox1 is in the membrane, the heme *a* cofactor synthesized by the integral inner membrane proteins COX10 and COX15 is incorporated by SURF1 (139). Then, after the release of CMC1, a small cascade of proteins delivers copper to Cox1 for the assembly of the Cu_B center. Copper is exported from the matrix, coordinated by COX17 in the IMS, and then transferred from COX17 to COX11. COX11, together with COX19, delivers the copper to Cox1 (15). The insertion of Cox2 in the membrane is performed by Oxa1, COX20, and COX18 (17, 140). The release of COX18 from this module coincides with the incorporation of Cu_A. Copper-loaded COX17 delivers the metal ion to SCO1 and

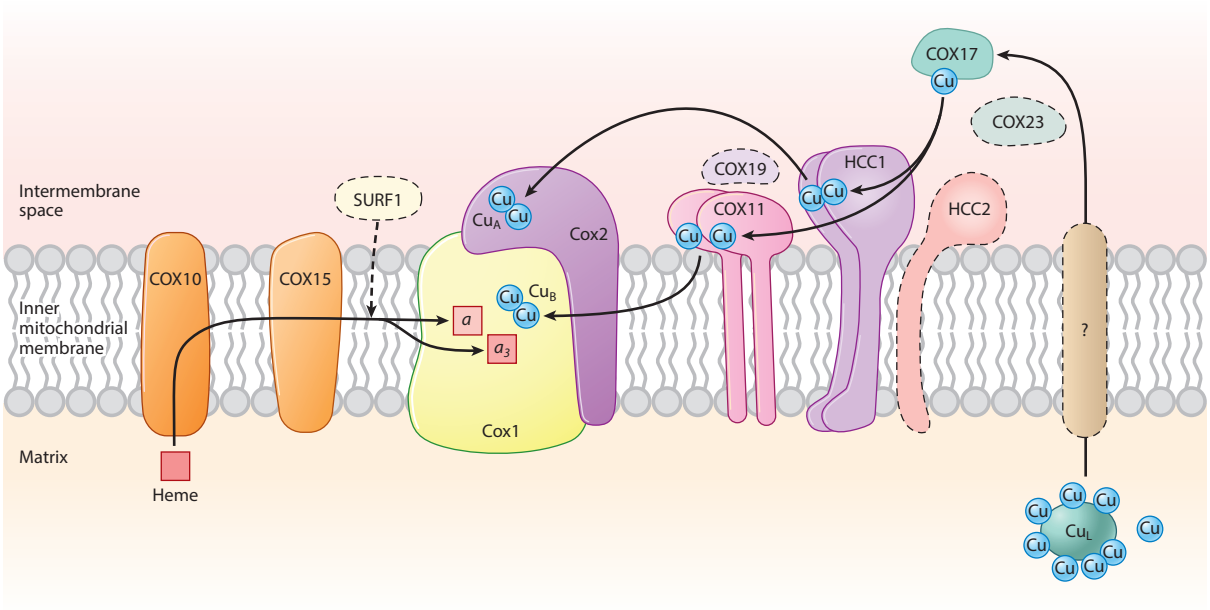

Figure 5

The pathways for heme *a* and metal cofactor delivery into cytochrome *c* oxidase 1 (Cox1) and Cox2 catalytic centers in land plants. Copper (Cu) is bound on the metal chaperone COX17 in the intermembrane space. COX17 transfers copper to COX11 and Homolog of Copper Chaperone SCO1 member 1 (HCC1). HCC1 delivers copper to Cox2, forming the Cu_A center, and COX11 delivers the copper to Cox1, forming the Cu_B center. Heme is modified by COX10 and COX15 to form heme *a* (*a*) and heme a_3 (a_3), which are subsequently delivered to Cox1. Conserved assembly factors whose functions have not yet been confirmed in plants are indicated with dashed outlines. The question mark indicates that the transporter responsible for the transfer of copper from the matrix to the intermembrane space is still unknown. Cu_L represents an unidentified copper ligand.

SCO2 via transient ligand exchange, and SCO1 delivers the copper to the Cox2/COX20 complex (64). Holo-Cox2 is then associated with the Cox1 module, and the other subunits are sequentially associated with the core Cox1–Cox2 module (140). A recent proteomic analysis of COX assembly challenged this view and proposed the formation of more complex assembly intermediates (145).

In *Arabidopsis*, homologous proteins for many of the known COX assembly factors are encoded by nuclear genes (**Table 2**), although only a few of these have been characterized in plants (**Figure 5**; **Table 2**). Among the factors involved in heme *a* synthesis and insertion, *Arabidopsis* COX10 has been characterized as an essential protein for plant embryogenesis, suggesting its role as a COX assembly factor (82). Regarding the accessory proteins involved in copper delivery and insertion, COX11 was also characterized as an essential protein playing an important role in plant growth and pollen germination (109). SCO proteins are encoded by two genes, Homolog of Copper Chaperone SCO1 (*HCC1*) and Homolog of Copper Chaperone SCO2 (*HCC2*), in *Arabidopsis*. HCC1 seems to be required for COX assembly (5, 132). HCC2 has lost the conserved cysteines and histidines required for copper binding, and current evidence suggests that HCC2 function is related to stress responses in plants (131). Putative homologs of other eukaryotic proteins involved in copper trafficking and insertion are also encoded in the *Arabidopsis* genome. *Arabidopsis* genes encoding COX17 and COX19 are able to complement the corresponding yeast null mutants, confirming that these proteins act as COX assembly factors (6, 8, 42). A recent study also demonstrated that the Oxa superfamily protein OXA2b, which is one of two plant-specific TPR-containing Oxa

proteins, is important for Cox2 membrane insertion (66). While OXA2b appears to play a role similar to COX18 from opisthokonts, it was demonstrated that its function was dependent upon the TPR domain, which is absent from COX18 (66). Interestingly, knockout mutations for all of the assembly factors characterized in plants trigger embryo-lethal phenotypes, highlighting the importance of COX for plant development. This also indicates that these assembly factors are highly specialized and that no alternative pathway is present in plant mitochondria to fulfill their functions. Some assembly factors characterized in opisthokonts do not have orthologs in plants (COX14, COX20, COA3) (**Table 2**), suggesting that plants possess an alternative apparatus to perform the insertion of Cox1 and Cox2 into the inner membrane. Because of these differences, alternative strategies need to be developed for the characterization of COX assembly in plants.

4.6. Assembly of Complex V

Complex V or ATP synthase is the last of the OXPHOS complexes. It uses the proton motive force generated by complexes I, III, and IV to produce ATP. Plant mitochondrial ATP synthase is composed of 15 subunits, which are organized into two domains: the IMM-bound F_O domain and the matrix-exposed F_1 domain. These two domains are linked by two stalks, the F_1 central stalk and the F_O peripheral stalk (18). The assembly of complex V has been intensively investigated in opisthokonts. In particular, one recent study used cells lacking individual ATP synthase subunits to define the exact molecular composition of distinct, vestigial ATP synthase complexes and identify the key assembly intermediates (55). Both the soluble F_1 domain and the membrane-bound F_O domain are assembled separately and then joined together to form the functional complex (55).

In plants, two compartments assemble F_1F_O–ATP synthases, and the assembly of the chloroplastic ATP synthase is well understood (116). In contrast, little is known about how complex V is assembled in plant mitochondria. Two putative assembly factors for the F_1 domain (Atp11/ATPAF1 and Atp12/ATPAF2) (1) appear to have homologs in plants, but, as yet, no functional information is available for them (**Table 2**). Using a combination of in vitro protein imports and a ^{15}N-incorporation strategy, it could be demonstrated that three populations of the F_1 domain are present in plant mitochondria (matrix F_1, inner membrane F_1, and intact F_1F_O) and have different ^{15}N-incorporation rates. This suggests that the matrix F_1 domain is an assembly intermediate (76). Therefore, like opisthokonts, ATP synthase in plants appears to first assemble the F_1 domain independently of the membrane-bound F_O domain.

Interestingly, assembly of the F_O domain and the joining together of the F_1 and F_O domains are performed in slightly different orders in fungi and humans (55, 99). Also, while many assembly factors have been identified in fungi, several are not found in plants (116). This opens up further exciting research on how plant mitochondrial ATP synthase is assembled, in terms of identifying the assembly factors and pathway as well as their regulation. For example, are assembly factors shared between chloroplasts and mitochondria to assemble their respective F_1F_O–ATP synthase?

4.7. Assembly of Supercomplexes

Use of mild detergents to extract the OXPHOS complexes from the IMM and subsequent analysis of these complexes on native gels revealed their association into supercomplexes. These supercomplexes have a defined stoichiometry (117). A recent structural study compared the architecture of the main supercomplex composed of complexes I and III in mammals, yeast, and plants and found that the arrangement of these two complexes within the supercomplex is conserved across species (33). However, the stoichiometry and structure of supercomplexes in different organisms vary; in particular, the supercomplex formed by complexes I, III, and IV occurs infrequently in plants (33).

The formation of supercomplexes occurs only after complete assembly of the individual complexes (53, 135). A few factors (Rcf1/Hig2a, Rcf2, MCJ/DnaJC15) have been proposed to play key roles during the assembly of supercomplexes in opisthokonts. However, the effects of these proteins on supercomplex formation are probably indirect (91). Similarly, cardiolipin-binding proteins (UQCC3, SLP2) have an indirect effect on supercomplex assembly through their role in inner membrane compartmentalization and maintenance (94). COX7A2L, also termed supercomplex assembly factor 1 (SCAF1), was described as important for the association between complexes III and IV (63). However, recent studies indicate that SCAF1 is a supercomplex stability factor rather than an assembly factor (95, 103).

In plants, most of the putative supercomplex assembly factors are not conserved (**Table 2**). In mutants where cardiolipin synthesis or cardiolipin-binding proteins are impaired, lower amounts of supercomplexes are detected, confirming the role of cardiolipins in the assembly or stability of the supercomplexes (43, 106). To date, the exact role of supercomplexes is mostly unknown, and future work will have to elucidate their role as well as how they are formed.

5. REGULATION OF THE ASSEMBLY PROCESS

To efficiently build the macromolecular OXPHOS complexes and avoid the production of deleterious intermediates, the assembly process must be tightly controlled. The establishment of a functional complex involves the expression of genes encoded in two different genomes, the translocation of proteins to their final destination, protein assembly, and cofactor synthesis and insertion (**Figure 2**). All of these steps are potentially regulated to ensure the correct and timely assembly of functional complexes for the successful operation of mitochondrial respiration (148).

5.1. Regulation of Gene Expression

To date, there is no evidence for regulation of transcription in plant mitochondria. A mutant in one of the two mitochondrial RNA polymerases shows deficiencies in complexes I and IV (69). This indicates that putative regulation of mitochondrial RNA polymerase activity could control OXPHOS biogenesis. However, conditions inducing such regulation have not yet been described. Because no clear evidence has been found of the regulation of transcript editing, splicing, or end processing in plant mitochondria (14), regulation of mitochondrial gene expression, if it occurs, happens at the level of translation. Supporting this hypothesis, mRNA levels and protein synthesis do not appear to correlate in plant mitochondria (70, 151). In addition, a recent analysis of ribosome footprints in plant mitochondria indicates that translational control plays an important role in mitochondrial gene expression (107). The importance of this regulation for the assembly process remains to be assessed. Interestingly it was recently shown in fungi that both nucleus-encoded and mitochondria-encoded OXPHOS subunits were more closely correlated at a translational level rather than at a transcriptional level (30). This introduces an interesting point of coordinated translational regulation between the cytosol and mitochondria.

In contrast to the mitochondria-encoded genes, nuclear genes encoding OXPHOS proteins appear to be under much greater transcriptional control. The components of the OXPHOS system display high levels of correlation between transcript and protein levels during germination (71) or across tissues (73). It has also been demonstrated that there is a coordinated expression of nuclear genes encoding different OXPHOS complex subunits in response to changes in carbohydrate supply (46, 49). In addition, expression characteristics of different isoforms that fulfill the same role are related to the presence of different *cis*-regulatory elements (28). Some regulatory elements have been identified. Within their promoter regions, the majority of nuclear OXPHOS

genes contain site II elements, which are the binding sites for TCP transcription factors (149). Furthermore, TCP transcription factors were shown to regulate diurnal changes in promoter activity and transcript and protein abundance of nucleus-encoded OXPHOS proteins (47).

5.2. Regulation Through Protein Import

An interesting point of assembly regulation is the import of the nucleus-encoded OXPHOS subunits. It has been shown that during germination when mitochondrial biogenesis is at its highest the level of import components is also at its highest and more abundant than the OXPHOS components (60, 72). Once biogenesis is completed, the level of import components decreases to a maintenance level, demonstrating that import plays a key role in the biogenesis of the OXPHOS complexes. Links between import components and respiratory complexes have also been demonstrated. It was shown for example that the TIM23 protein from the TIM23 import complex is also partially located within complex I (146). This suggests that interactions of import components with the OXPHOS system provide a way for mitochondria to coordinate both biogenesis and activity (97). Recently, it was also demonstrated in yeast that mitochondrial translation can adapt to the import of nucleus-encoded subunits (114), introducing an interesting form of translational plasticity that can react and adapt to the influx of nucleus-encoded proteins.

5.3. Regulation Through Proteolysis

An analysis of the regulation of mitochondrial biogenesis under stress conditions suggests that availability of nucleus-encoded subunits of the OXPHOS complexes is a limiting factor (46). Therefore, control over the amounts of unassembled subunits in the mitochondria by proteases should play an important role in OXPHOS assembly. This hypothesis is supported by several observations. In mutants accumulating partially assembled complex I, unassembled subunits do not accumulate (78, 89). Mitochondrial proteases are upregulated in a mutant with altered mitochondrial translation (70). Disturbance of the mitochondrial protease network also affects OXPHOS biogenesis (90, 126, 127). Such a posttranslational regulation mechanism (removal of unassembled subunits) would allow tight control of the assembly process even if expression of the mitochondrial and nuclear genomes is not coordinated. Additional work is required to confirm if this mechanism is the key regulatory step in assembly of the OXPHOS system.

5.4. Other Putative Regulatory Steps

Most of the steps of OXPHOS assembly occur within the IMM. This membrane is highly structured in all mitochondria (26). A recent study in yeast showed that mitochondria-encoded subunits of complexes III and IV are inserted at sites that differ from those of complex V subunits. Therefore, assembly of complexes III and IV does not occur in the same location as assembly of complex V (134). The mechanism behind this spatial regulation is currently unknown.

Using quantitative proteomics, the half-life of mitochondrial proteins can be calculated. Surprisingly, the degradation rate of subunits assembled within one complex was found to vary; in particular, the matrix arm subunits of complex I have a faster degradation rate than the membrane arm subunits (77). This observation could suggest that when de novo assembly occurs the membrane arm is recycled whereas the matrix arm is not. Alternatively, it could indicate that a repair mechanism exists to replace the subunits or domains that were damaged. In plant mitochondria, the copy number of the genome is very low and variable (108). Therefore, in a single mitochondrion, if a complex is damaged, the machinery to repair or replace it might not be present (if the

gene encoding a subunit is absent from this particular mitochondrion). This could explain why fusion and fission of mitochondria are very active mechanisms in plants (102). Exchanges of genes, transcripts, de novo synthesized subunits, partially assembled or degraded complexes, and fully assembled complexes could occur during these events. Such mechanisms remain to be described.

6. EVOLUTION OF THE OXPHOS ASSEMBLY MACHINERY

Supplemental Material >

The composition of several OXPHOS complexes in plants differs from composition in opisthokonts (**Supplemental Table 1**). This suggests that several aspects of the assembly of these complexes differ between plants and opisthokonts. Some of the divergent steps have already been identified (the initial assembly step of the membrane arm of complex I, *c*-type cytochrome maturation, and Rieske Fe-S protein insertion into complex III). Interestingly, these steps are not plant specific but most likely represent the ancestral assembly pathway present in the mitochondrial ancestor (22, 45, 77, 78). As mitochondria evolved independently in the various eukaryotic lineages, these ancestral steps have been replaced in opisthokonts but kept in plants.

We propose two scenarios to explain the slower evolution of the OXPHOS assembly pathway in plant mitochondria. First, the mutation pressure is high in animal mitochondria but very low in plant mitochondria (81). As a consequence, mutations may have accumulated in animals that made the ancestral assembly machinery inefficient. An example of this possibility is apparent in the first step of the assembly of the membrane arm of complex I. During this step, the mitochondria-encoded protein Nad2 is inserted into the inner membrane. Two pathways have been described: an ancestral pathway in plants and a more recent pathway involving Metazoa-specific assembly factors in humans (36, 78). Interestingly, Nad2 has lost three N-terminal transmembrane helices in humans when compared with plants or the yeast *Yarrowia lipolytica*. When comparing the structures of complex I in *Y. lipolytica* (35) and in mammals (153), there is a hole at the place of these three helices in the human structure (E.H. Meyer, unpublished data). The reduction in the number of transmembrane domains of Nad2 in Metazoa is certainly linked with the replacement of the machinery responsible for its insertion in the membrane. A second scenario to explain slower evolution of the OXPHOS assembly pathway in plants is that because of the presence of photosynthesis, which is an ATP-generating pathway, respiratory ATP production in leaves is likely low. In fact, during the day when photosynthesis is running, the TCA cycle is running in a non-cyclic mode, bypassing complex II (137). As a consequence, plant respiratory complexes are potentially less damaged, and the need to assemble new complexes might be lower in plants than in opisthokonts. Hence, the ancestral pathway may not have needed to be replaced by a more efficient pathway.

Other assembly steps are conserved across all eukaryotes investigated, suggesting that they are ancestral. Either these steps are efficient enough or no alternative, more efficient pathways have evolved so far. Overall, the maintenance of ancestral pathways in plant mitochondria indicates that evolution of the assembly of the OXPHOS system in plant mitochondria was slower than in animal mitochondria and that plant mitochondria are more closely related to the mitochondria present in LECA.

7. CONCLUSION: OPEN QUESTIONS

In this review, we present the current state of knowledge about assembly of the OXPHOS complexes in land plant mitochondria. Although recent advances have been made, the path to a complete understanding of these pathways is still very long. The main approaches developed so far

utilize the knowledge obtained in other systems—in particular, yeast and mammalian mitochondria. That way, many assembly factors with functions conserved across eukaryotes have been identified and characterized in plants as well, and clearer pictures of the assembly pathway of most complexes are emerging. Even so, many candidate assembly factors have not yet been investigated (**Table 2**). Many aspects of the OXPHOS assembly machinery remain to be discovered in plant mitochondria. In particular, plant-specific mechanisms (likely ancestral) should exist for the insertion of mitochondria-encoded proteins in the inner membrane (some likely candidates are Nad2, Cox1, and Cox2) because the assembly factors performing these steps, as described in opisthokonts, are not conserved in plants. Furthermore, evidence for transcriptional, translational, and posttranslational regulation of the assembly process has been obtained. However, the overall regulatory network controlling the biogenesis, stability, and repair of a functional OXPHOS system is not yet understood.

SUMMARY POINTS

1. The OXPHOS system in plants is formed by more than 100 proteins encoded by the mitochondrial or nuclear genomes and more than two dozen cofactors.
2. Assembly of the OXPHOS complexes involves many assembly factors, which are mostly uncharacterized in plants.
3. Many assembly steps in plants are ancestral; these steps involve alternative, recently evolved pathways in fungal and animal mitochondria.
4. Evidence for transcriptional, translational, and posttranslational regulation of the assembly process has been obtained.

FUTURE ISSUES

1. There is still a need to determine the complete assembly pathways. Future studies should characterize candidate assembly factors and identify the machineries involved in assembly steps, which differ between plants and opisthokonts.
2. The regulatory network controlling the assembly pathway must be better understood. At which step(s) is assembly regulated? Is OXPHOS biogenesis linked to respiratory demand, and, if so, how?
3. Is OXPHOS assembly spatially organized within the cristae?
4. Can the OXPHOS complexes be repaired in plants? If so, how is this repair performed given the low genome copy numbers per organelle?
5. Future work should elucidate the forces for differential evolution of the OXPHOS assembly pathway across eukaryotic lineages.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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