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Review

Structures of mitochondrial oxidative phosphorylation supercomplexes and mechanisms for their stabilisation ☆

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ABSTRACT

Oxidative phosphorylation (OXPHOS) is the main source of energy in eukaryotic cells. This process is performed by means of electron flow between four enzymes, of which three are proton pumps, in the inner mitochondrial membrane. The energy accumulated in the proton gradient over the inner membrane is utilized for ATP synthesis by a fifth OXPHOS complex, ATP synthase. Four of the OXPHOS protein complexes associate into stable entities called respiratory supercomplexes. This review summarises the current view on the arrangement of the electron transport chain in mitochondrial cristae. The functional role of the supramolecular organisation of the OXPHOS system and the factors that stabilise such organisation are highlighted. This article is part of a Special Issue entitled: Dynamic and ultrastructure of bioenergetic membranes and their components. Guest Editors: Ziv Reich and Giorgio Lenaz.

1. Introduction

Mitochondria play a number of vital roles in the eukaryotic cell, among which the most important one is the production of ATP during oxidative phosphorylation (OXPHOS). The heavily folded inner membranes of mitochondria called cristae accommodate many copies of the respiratory chain components, or OXPHOS complexes (I–IV). Together with ATP synthase (complex V) they form the machinery for production of ATP, the energy currency of the cell. Complexes I–IV are multisubunit enzymes that work in concert to create an electrochemical proton gradient across the mitochondrial inner membrane that is used by the F_1F_o ATP synthase (complex V) to produce ATP via oxidative phosphorylation, although complex II is not directly able to pump protons. NADH or succinate, generated during glycolysis, fatty acid oxidation and in the citric acid cycle, form the fuel for the respiratory chain. During catalysis, there is electron transfer between the complexes mediated by two small components: lipid-soluble ubiquinone and water-soluble cytochrome *c*. They diffuse between the respiratory complexes I and III, and III and IV, respectively, and the latter takes them to form water from molecular oxygen (Fig. 1).

For understanding OXPHOS at the molecular level it was all-important that atomic structures of the individual complexes were solved. *Complex I* or NADH dehydrogenase is the largest enzyme of the electron transport chain (ETC) and has a characteristic L shape with an extensive part being embedded in the lipid bilayer and a smaller shoulder protruding into the mitochondrial matrix. The recent X-ray structure of the bacterial complex I suggests the coupling mechanism for NADH dehydrogenase [1]. Complex I binds NADH substrate to the distal end of the hydrophilic arm and transfers two electrons, one at a time, via FMN and seven iron–sulphur clusters to the bound ubiquinone at the interface between two arms [2]. Reduction of the ubiquinone induces conformational changes in the membrane arm resulting in translocation of four protons across the membrane via four channels [1].

Complex II or succinate dehydrogenase is the second independent entrance point of electrons to the respiratory chain. It oxidises succinate and transfers electrons through three iron–sulphur clusters to ubiquinone. Complex II is not a proton pump and it does not contribute directly to the proton gradient formation.

Complex III or cytochrome *c* oxidoreductase, which is known to exist in the membrane as a dimer, oxidises ubiquinone to ubiquinol and as a result it can pump two protons to the intermembrane space. The electrons from ubiquinol are passed to the carrier cytochrome *c* via cytochromes *b* and c_1 of complex III.

The last enzyme of the mitochondrial electron transport chain is *complex IV* or cytochrome *c* oxidase. It accepts electrons from cytochrome *c* and delivers them to an oxygen molecule to convert it to two water molecules. Four protons are pumped to the intermembrane space during this process.

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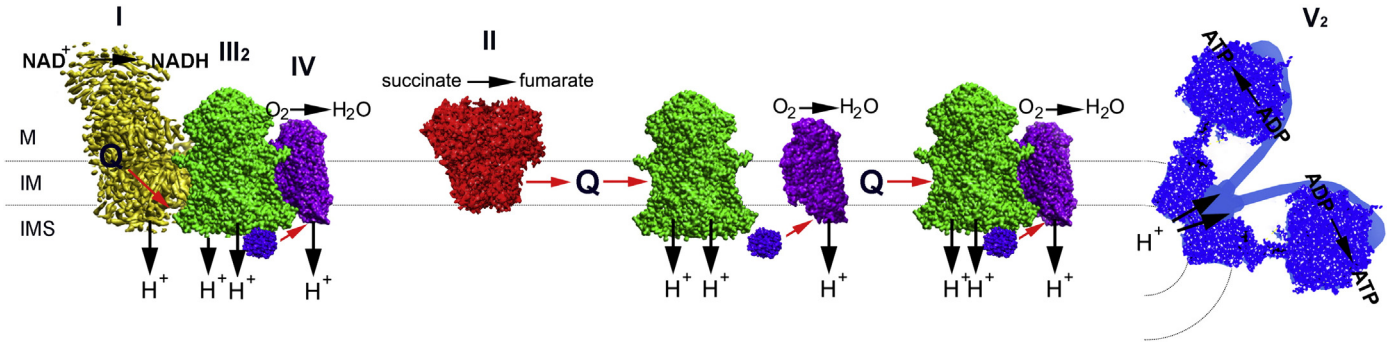

Fig. 1. Plasticity model of the mitochondrial electron transport chain in which complexes I–IV are partly organised into supercomplexes, except for complex II, which feeds electrons via ubiquinone to complex III non-bound to complex I. Red arrows show electron pathways. In yellow, the complex I, marked as I; in red the complex II, marked as II; in green the complex III_2, marked as III_2; in purple the complex IV, marked as IV; in blue the dimeric ATP synthase, marked as V_2; in violet the cytochrome *c*; Q, ubiquinol. The positions of the matrix (M), the intermembrane space (IMS) and cristae or inner membrane (IM) are indicated.

ATP synthase or complex V uses the energy stored in a proton gradient to turn ADP into ATP. It is a complex formed by 15–18 subunits with a total mass of 600 kDa [3]. The water-soluble F_1-part consists of three α and three β catalytic subunits. The F_1-part is connected to the membrane-embedded ring-like subunit c oligomer of the F_o-part by a central and a peripheral stalk. The F_o-part is composed of subunits a (Su 6), A6L (Su 8), e, f, g, the central stalk consists of the γ, δ and ε subunits and the peripheral stalk is made from subunits OSCP (Su 5), b, d, F6 (h) (the nomenclature of subunits in yeast is indicated in brackets, reviewed in [4]). The yeast enzyme has two specific additional subunits, i and k, which belong to the membrane part. Protons cross the membrane via the proton channel in the membrane part rotating the multi-subunit c ring and the central stalk. The αβ trimer of F_1-part is prevented from rotation by the peripheral stalk and catalyses ATP synthesis via the binding change mechanism [5].

2. Supramolecular organisation of the respiratory chain

The most important experimental strategy to characterise the organisation of the OXPHOS system has been blue-native polyacrylamide gel electrophoresis (BN-PAGE), first introduced by Hermann Schägger and colleagues [6,7]. BN-PAGE is based on the mild solubilisation of mitochondrial membranes with non-ionic detergents and is able to separate the largest stable protein complexes that can withstand solubilisation. This technique allowed the separation of respiratory supercomplexes on gels and marked the beginning of the study of the higher level of structural organisation of the OXPHOS system. Electron microscopy single particle analysis provided the first structural evidence for the respiratory supercomplexes [8,9]. In the last two decades, BN-PAGE and other biochemical techniques have triggered a considerable change in the view on the organisation of the electron transport chain in mitochondrial membranes. The idea of random diffusion of complexes in cristae was ruled out by a number of experiments. Flux-control measurements combined with inhibitor titration revealed that the yeast electron transport chain behaves as a single functional unit [10,11].

Respiratory supercomplexes have been found in organisms belonging to different kingdoms of eukaryotes (Table 1). Despite their phylogenetic distances from each other, all of them have in common that their OXPHOS systems have supramolecular organisation. Based on composition, all supercomplexes can be divided into four main groups. Complexes I, III_2 and IV were found to assemble into I + III_2, III_2 + IV_{1-2} or I + III_2 + IV_{1-4} supercomplexes. ATP synthases form dimers, which constitute oligomeric chains in cristae. Complex II is the only enzyme of the respiratory chain, which does not associate with the other respiratory complexes. Although Acin-Perez et al. reported complex II to be a part of the mouse respirasome, which is able to transfer electrons from succinate [12], this was not supported by any other studies. Unlike the other enzymes of the OXPHOS system, complex II is not only involved into respiration but also directly participates in the citric acid cycle. This could explain why complex II was not found to be part of the respiratory supercomplexes. Interestingly, a recent study revealed that the electron flow from the FAD substrate to complex III is independent from the electron pathway that originates from NAD^+ and takes place within the I + III_2 supercomplex ([13] Fig. 1). These two pathways use two different populations of quinone [13]. The abundance of the supercomplexes with different compositions varies from the organism to the organism. Thus, the supercomplex with composition I + III_2 is the most abundant in plants ([14], Table 1), where 90–100% of complex IV is found in the monomeric form [15]. I + III_2 + IV_{1-4} supercomplex is higher in abundance in mammals [7] and III_2 + IV_2 in fungi [7,16].

Structural studies of mitochondrial membranes revealed supramolecular assemblies of the OXPHOS system in situ. Rapid-freeze deep-etch electron microscopy provided the first demonstration of oligomeric rows of ATP synthase dimers in mitochondria of *Paramecium* in 1989 [17]. This work also suggested ordered linear arrays of the complex I in cristae [17]. The emergence of cryo-electron tomography (ET) has given rise to the study of supramolecular organisation of the respiratory

Table 1
[a]Representation of respiratory supercomplexes in the various kingdoms of living organisms.

Organism		I+III_2	III_2+IV_{1-2}	I+III_2+IV_{1-4}	V_2	Refs
Plants	*Arabidopsis thaliana*	x			x	[14]
	Hordeum vulgare	x				[14]
	Phaseolus vulgaris	x				[14]
	Solanum tuberosum	x[b]	x	x	x	[14,15,28]
	Spinacia oleracea	x	x	x	x	[29]
	Nicotiana sylvestris	x				[87]
	Pisum sativum	x				[88]
	Helianthus annuus			(x)[c]		[89]
	Zea mays	x			x	[27,66]
	Asparagus officinalis	x	x			[30]
Algae	*Chlamydomonas reinhardtii*				x	[90]
	Polytomella sp.	x			x	[8,91]
Fungi	*S. cerevisiae*	-[d]	x	-[d]	x	[6,7]
	Yarrowia lipolytica	x[e]	x	x[e]	x	[20,92]
	Podospora anserina	x	x	x	x	[93,96]
	Neurospora crassa		x	x[e]	x	[97]
Protozoa	*Tetrahymena thermophila*	x			x	[63]
	Plasmodium falciparum				x	[94]
Animals	*Bos taurus*	x	x	x	x	[7]
	Homo sapiens	x	x	x	x	[95,98]

[a] Empty cells in the table indicate that the corresponding supercomplexes have not been discovered.
[b] In potato, two forms of I + III supercomplexes occur, which have I + III_2 and I_2 + III_4 composition.
[c] In sunflower, a complex IV containing supercomplex of >1000 kDa was described, which probably has I + III_2 + IV_{1-4} composition.
[d] The OXPHOS system of *S. cerevisiae* does not have complex I and, therefore, contains no I + III_2 and I + III_2 + IV_{1-4} supercomplexes.
[e] In *Neurospora crassa* and *Yarrowia lipolytica*, a I_2 supercomplex was described.

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