Biochimica et Biophysica Acta 1793 (2009) 117–124

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Supramolecular organization of protein complexes in the mitochondrial inner membrane

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ARTICLE INFO

Article history:
Received 28 February 2008
Received in revised form 21 May 2008
Accepted 23 May 2008
Available online 3 June 2008

Keywords:
Respiratory chain
Mitochondria
Supercomplexes
Protein structure
Membrane proteins
Electron transfer

ABSTRACT

The liquid state model that envisions respiratory chain complexes diffusing freely in the membrane is increasingly challenged by reports of supramolecular organization of the complexes in the mitochondrial inner membrane. Supercomplexes of complex III with complex I and/or IV can be isolated after solubilisation with mild detergents like digitonin. Electron microscopic studies have shown that these have a distinct architecture and are not random aggregates. A 3D reconstruction of a $I_1III_2IV_1$ supercomplex shows that the ubiquinone and cytochrome *c* binding sites of the individual complexes are facing each other, suggesting a role in substrate channelling. Formation of supercomplexes plays a role in the assembly and stability of the complexes, suggesting that the supercomplexes are the functional state of the respiratory chain. Furthermore, a supramolecular organisation of ATP synthases has been observed in mitochondria, where ATP synthase is organised in dimer rows. Dimers can be isolated by mild detergent extraction and recent electron microscopic studies have shown that the membrane domains of the two partners in the dimer are at an angle to each other, indicating that in vivo the dimers would cause the membrane to bend. The suggested role in crista formation is supported by the observation of rows of ATP synthase dimers in the most curved parts of the cristae. Together these observations show that the mitochondrial inner membrane is highly organised and that the molecular events leading to ATP synthesis are carefully coordinated.

1. Introduction

A major function of mitochondria is the conversion of energy released by metabolic processes in the mitochondrial matrix into the energy currency of the cell, ATP. Transmembrane protein complexes of the electron transport chain generate an electrochemical gradient across the mitochondrial inner membrane. Electrons are transferred from NADH to complex I (NADH:ubiquinone oxidoreductase) and from there to ubiquinone, and from succinate via complex II (succinate dehydrogenase) to ubiquinone. From ubiquinone they are passed via complex III (ubiquinol:cytochrome *c* oxidoreductase), the peripheral electron carrier cytochrome *c* and complex IV (cytochrome *c* oxidase) to the terminal acceptor, molecular oxygen. Complex I, III and IV are proton pumps. Together they generate an electrochemical proton gradient which is used by complex V (F_1F_o–ATP synthase) to produce ATP (Fig. 1).

The complexes I–V are all large, multisubunit complexes. Protocols to purify the individual complexes have been available for many decades [1]. Complex I, NADH:ubiquinone oxidoreductase, is the largest complex, with a molecular weight ranging from 550 kDa for the bacterial enzyme to ~1000 kDa in bovine heart mitochondria. It has a common core of 14 subunits which are found in bacterial complex I, while eukaryotes have many additional subunits with a total of at least 45 in bovine heart mitochondria [2]. In all organisms, complex I is L-shaped and consists of a membrane arm and a peripheral or matrix arm. High-resolution information for complex I is only available for the 8-subunit peripheral arm of the bacterium *Thermus thermophilus* [3]. 3D models of complex I by electron microscopy have been determined for several organisms (reviewed in [4]), which all share the overall L-shape. A 16.5-Å resolution map in negative stain was obtained for the yeast *Yarrowia lipolytica* [5], and a cryo-EM structure is available for the bovine enzyme [6]. Complex III, the ubiquinol:cytochrome *c* oxidoreductase or cytochrome bc_1 complex, is a homodimeric complex of 11 distinct subunits per monomer with substantial matrix domains. Crystal structures have been determined for complex III of bovine [7,8], chicken [9] and yeast [10] mitochondria. Complex IV consists of four subunits in bacteria and crystal structures have been determined for the enzymes from *Paracoccus denitrificans* [11,12] and *Rhodobacter sphaeroides* [13]. The mammalian enzyme contains the bacterial core of four subunits and has a total of 13 subunits; the structure of bovine complex IV has been determined by X-ray crystallography [14–17]. Mitochondrial ATP

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doi:10.1016/j.bbamcr.2008.05.019

synthase is an intricate, two-domain structure of ~600 kDa consisting of a transmembrane, proton-translocating F_o domain in the mitochondrial inner membrane and a catalytic F_1 domain in the mitochondrial matrix, which are connected by a central and a peripheral stalk (Fig. 2). The complex forms a rotary machine and the enzyme functions via changes in the nucleotide binding pockets in F_1 upon rotation of the central stalk during proton transport [18,19]. Related ATP synthases exist in bacteria and chloroplasts. F_1F_o ATP synthases in bacteria and chloroplasts consist of eight different subunits with a stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$ for F_1 and ab_2c_{11-15} for F_o. The mitochondrial ATP synthase contains up to 9 additional subunits, not all of which are essential for activity; in bovine heart, the peripheral stalk consists of subunits OSCP, d, F_6, and the soluble part of subunit b [20], while F_o contains the additional subunits e, f, g and A6L [19–21]. Since the groundbreaking determination of the bovine F_1 structure [18] the structures of several other subcomplexes have been determined by X-ray crystallography, including a partial bovine peripheral stalk [22]. Structural knowledge of the F_o part is still limited. The structure of a $\alpha_3\beta_3\gamma\varepsilon c_{10}$ complex from yeast has been determined by X-ray crystallography [23], providing the first view of a c-ring. A bacterial c-ring was found to have eleven subunits [24,25] and the structure of this ring was determined at high resolution [26]. Studies by AFM, electron crystallography and mass spectroscopy have shown that the c-ring of F_1F_o ATP synthases can contain different numbers of subunits: c_{11} [24,27–29] and c_{13-15} [30–32] rings have been found in different species of bacteria and c_{14} in chloroplasts [33]. The structure of the other F_o subunits, a and b, is not known, but a cryo-electron microscopy structure of the bovine ATP synthase [34] shows the overall shape of the complete enzyme (Fig. 2).

The mitochondrial inner membrane has a large surface area and is folded into structures called cristae, which can vary in shape from narrow tubes of 30–40 nm diameter to flat lamella [35,36]). The respiratory chain is found in the cristae. The traditional view of interaction between the different complexes is, in its extreme form, the “fluid state” model, which envisions the complexes floating freely in the membrane and electron transfer occurring during random collisions [37]. This was the textbook view until recently. In the “solid state” model on the other hand, which was first proposed more than 50 years ago [38] the substrate is channelled directly from one enzyme to the next, which assumes a high degree of organisation of the complexes. Increasing evidence from many different sources supports the solid state model. This evidence and its implications are the subject of this review.

2. Respiratory chain supercomplexes

Already 30 years ago, it was shown that the electron transfer rates of reconstituted proteins are higher if the different complexes are present at defined stoichiometries [39]. Early purification protocols often led to co-purification of other complexes [1,40,41], but at the time these co-purifications were considered as artefacts and not as indications for an association between the complexes. As a consequence, the idea of supramolecular respiratory chain protein assemblies and a direct substrate channelling was put aside in the scientific community for many years. Respiratory chain supercomplexes isolated from bacteria such as *P. denitrificans* [42] and the thermophilic bacterium PS3 [43], and from the archaeon *Sulfolobus* sp. strain 7 [44] were considered to be special features of these two kingdoms.

However, during the last decade various additional lines of evidence have suggested that individual respiratory chain complexes assemble into supercomplexes, supporting the solid state model. In yeast, it was shown by inhibitor tritations using antimycin that neither ubiquinone nor cytochrome *c* display pool behavior, supporting the idea that the respiratory chain acts as a single functional unit [45]. Flux control analysis in bovine heart mitochondria gave kinetic evidence of the association between complexes I and III, though not IV, while complex II was fully independent [46] (recently reviewed in [47]).

Supercomplexes can be isolated from the membrane if a mild detergent, such as digitonin, is used for solubilisation [48–57]. The methods used to characterize supercomplexes biochemically are blue-native and colorless-native polyacrylamide gel electrophoresis (BN- and CN-PAGE) and density gradient centrifugation. Second dimension BN-PAGE with a stronger detergent, such as Triton, to dissociate the supercomplex is usually used to determine the complex compositions and second dimension SDS-PAGE to show the subunit compositions.

The first supercomplexes characterized biochemically were two supercomplexes from *Saccharomyces cerevisiae*, consisting of a complex III dimer and one or two copies of complex IV [58], and different bovine supercomplexes with one complex I, a complex III dimer and up to four copies of complex IV [48]. Since then, respiratory chain supercomplexes have been found in bacteria, e.g. *P. denitrificans* [55] and in mitochondria from fungi [53], higher plants [50,52,59] and mammals [54,56,60] using CN- and BN-PAGE, gel filtration and immuno-precipitation. In these studies, supercomplexes of various stoichiometries have been detected, such as assemblies of monomeric complex I (I_1) with dimeric complex III (III_2), and complex IV in various

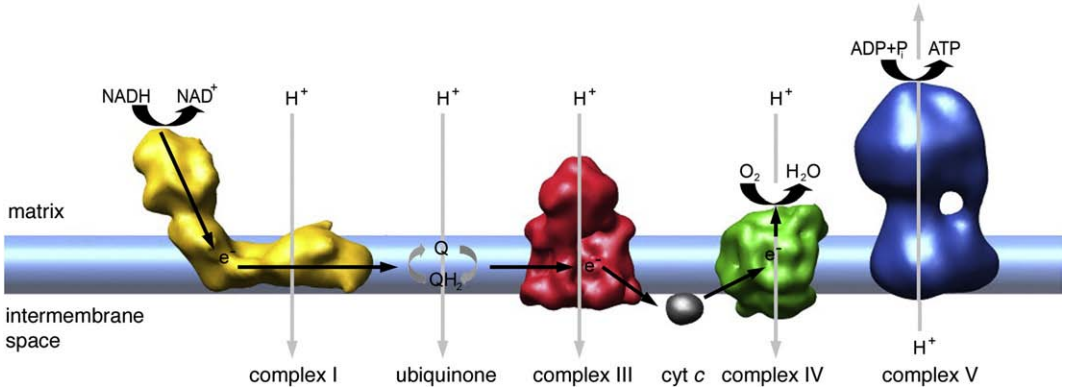

Fig. 1. The mitochondrial respiratory chain. The transmembrane protein complexes of the electron transport chain generate an electrochemical gradient over the mitochondrial inner membrane. NADH is oxidized to NAD^+. The electrons are transferred from NADH via complex I and ubiquinone (Q) to complex III. Afterwards they pass through the peripheral electron carrier cytochrome *c* and complex IV to the terminal acceptor, molecular oxygen, which is reduced to water. The electrochemical proton gradient is used by complex V (F_1F_o ATP synthase) to produce ATP. The X-ray structure of dimeric bovine complex III [77], monomeric complex IV [14] and of cytochrome *c* [78] are filtered to 20 Å. Complex III is shown in red, complex IV in green and cytochrome *c* in grey. The negative stain electron microscopy map of bovine complex I [6] is displayed in yellow and the cryo-EM map of bovine complex V [34] in dark blue. The putative location of the membrane is indicated in blue. The figures were made using the UCSF Chimera package [112] from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR-01081).

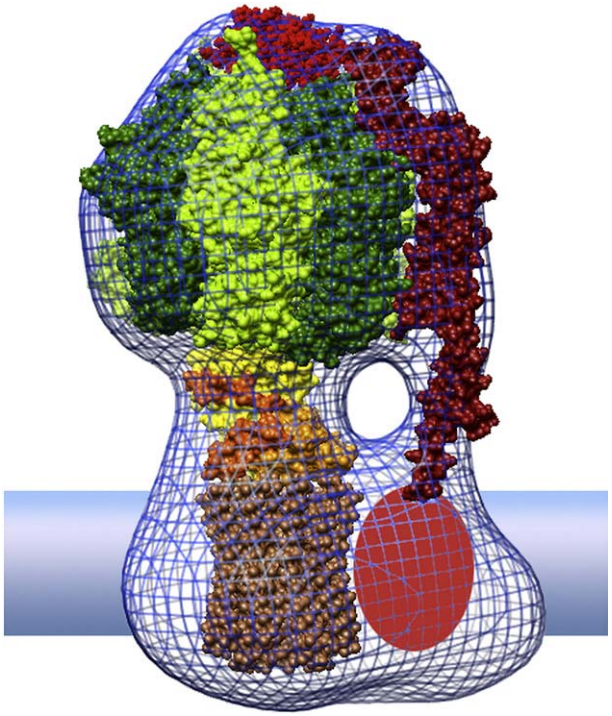

Fig. 2. Subunit and domain composition of mitochondrial ATP synthase. ATP synthase consists of a catalytic head with composition $\alpha_3\beta_3$ (green). The head is penetrated by a long helix from the γ subunit (yellow), which together with δ and ε (orange) forms the central stalk (pdb entry 1h8e). The central stalk is connected to the c_{10} rotor ring (brown) (from pdb entry 1qo1)[23]. The peripheral stalk (red) consists of the OSCP subunit (top) (pdb 2bo5), which attaches to the head, and the mainly alpha-helical b, d and F_6 subunits (pdb entry 2cly) [22]. The membrane domain of the stator (red oval) consists of the a subunit and several small subunits of unknown structure. Proton flow through the a-c_{10} interface causes a rotation of the c-ring and central stalk, synthesizing ATP by conformational changes in the nucleotide binding pockets. The different subunits are shown fitted into the bovine heart mitochondrial cryo-EM map [34].

copy numbers (IV_x). An association of complex II has never been found, in agreement with kinetic evidence of its independence [46].

Although the studies described above gave strong support for a supramolecular organisation of the respiratory chain, the observed supercomplexes could still be interpreted as aggregates, caused by the detergent treatment. In the last few years the first structural insights in these multi-complex assemblies have been obtained by electron microscopy (partially reviewed in [61]). A distinct structure was observed for all supercomplexes that were investigated, giving clear proof for a specific interaction of the respiratory chain complexes. 2D-projection structures from three different organisms have been reported: supercomplexes consisting of a complex III dimer and one or two copies of complex IV (III_2IV_1 and III_2IV_2) from yeast [62], a supercomplex consisting of monomeric complex I and dimeric complex III (I_1III_2) from the plant *Arabidopsis thaliana* [63], and the two supercomplexes I_1III_2 and $I_1III_2IV_1$ from bovine heart mitochondria [57]. Recently the first 3D map of a supercomplex has been determined, for the bovine $I_1III_2IV_1$ [64].

2.1. Structural information on supercomplexes

2.1.1. Supercomplex III_2IV_2 from S. cerevisiae

In *S. cerevisiae*, which lacks complex I, two supercomplexes were found, consisting of a dimeric complex III and one or two copies of complex IV [48]. Based on BN-PAGE, they have molecular masses of ~650 kDa and ~850 kDa, respectively. The interaction of the individual complexes in these supercomplexes was proposed to be mediated by the subunits cytochrome *b* and c_1 from complex III [65]. The lipid cardiolipin, which is abundant in the mitochondrial inner membrane, was shown to be important for their stability [65–67]. Structural information about these supercomplexes was derived from 2D projections [62], and their relative orientations were modelled by comparison with X-ray data. The interaction between complex III and complex IV involves cytochrome *b* and cytochrome c_1 as proposed [65]. Complex IV is present as a monomer on one or both sides of the complex III dimer. In the yeast supercomplexes the concave face of complex IV, which forms the dimer interface in the complex IV crystal structure [14], faces towards the lipid bilayer (Fig. 3a).

2.1.2. Supercomplex I_1III_2 from A. thaliana and Bos taurus

In mammalian as well as plant mitochondria a supercomplex consisting of complex I and complex III (I_1III_2) has been found [48,50,59,68,69], which is the most abundant one in plants [69]. Based on BN-PAGE it has a molecular mass of ~1500 kDa. Structural information, in the form of 2D averages from negative stain electron microscopy data, has been obtained for the supercomplex from *A. thaliana* [63] and *B. taurus* [57]. In both the plant and the mammalian supercomplex, a complex III dimer is associated with the membrane arm of complex I. The apparent interaction between the two complexes is quite different in the two species, however. In the bovine supercomplex the interaction surface is more extensive and complex III is attached to the middle of the complex I membrane arm, whereas in the plant structure complex III attaches to the end of the membrane arm (Fig. 3b, c). There is biochemical evidence that the *A. thaliana* complex I has several additional subunits [70,71]. It has a different appearance in the electron microscope than the *Y. lipolytica* [5] or bovine [6] complex I, displaying two unique protein densities on either side of the membrane arm [63]. Thus, from the limited data available to date, it appears that the interaction of individual complexes in supercomplexes may be species- or kingdom-specific.

2.1.3. Supercomplex $I_1III_2IV_1$ from B. taurus

A supercomplex consisting of one complex I, dimeric complex III and one copy of complex IV ($I_1III_2IV_1$) with a molecular mass of ~1700 kDa was found in the mitochondria of rats [56,60] and cows [48,54,57,60,68] as well as in plants [59] and fungi [53]. Compared to the smaller supercomplex I_1III_2 the bovine $I_1III_2IV_1$ has higher activity and stability [57].

In bovine heart mitochondria, $I_1III_2IV_1$ is the most abundant supercomplex and it provides the only three-dimensional data for a supercomplex so far [64]. The structure was determined in negative stain at a resolution ~36 Å and the orientations and locations of all the individual complexes were determined unambiguously.

Complex III and IV are both associated with the membrane arm of complex I and are also in contact with each other (Fig. 3d). Complex III forms a dimer, whereas complex IV exists as a monomer. Complex IV is associated with the rest of the supercomplex through the concave face, which is the dimer interface in the X-ray structure. This is in contrast to the proposed interaction in yeast [62] where this interface faces the lipid bilayer (Fig. 3a).

The quinone binding site in complex I is supposed to be in the matrix arm near the matrix/membrane arm connection [3,72–74]. In complex III the ubiquinone binding site is at the Rieske iron–sulfur protein and cytochrome *b* [75–77]. These subunits of complex III are attached to complex I close to the matrix/membrane arm connection [64]. Via a cavity in complex III, which is facing the ubiquinone binding site of complex I, the ubiquinone reaches its binding site in complex III (Fig. 4). In complex III the subunit cytochrome c_1 is the cytochrome *c* binding site [78]. Complex IV seems to interact with cytochrome *c* through the globular domain of subunit II [14]; this subunit is facing the cytochrome *c* binding site in complex III (Fig. 4). On the basis of the structural information gained from the 3D map, the putative mobile electron carrier (ubiquinone or cytochrome *c*) binding site of each complex is facing the corresponding binding site of the succeeding complex in the respiratory chain (Fig. 4). In this assembly, both

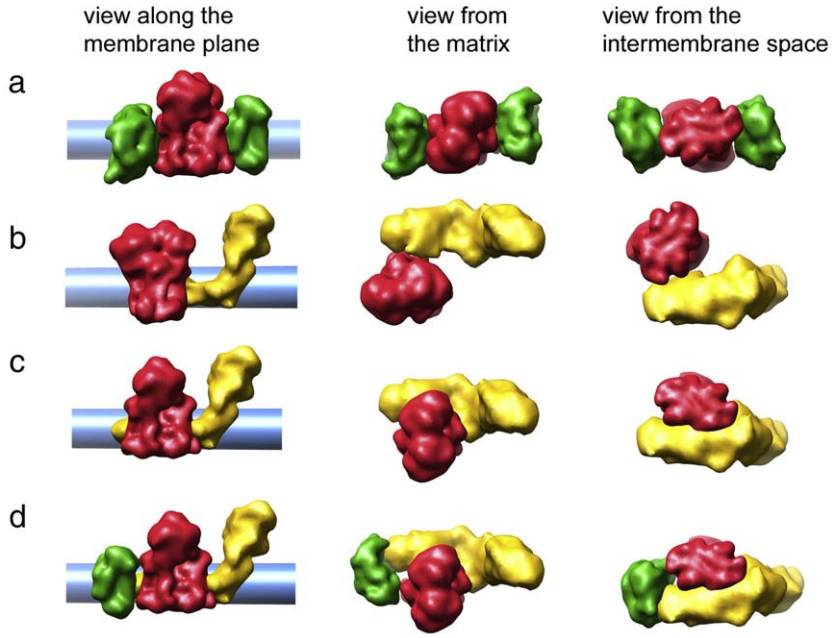

Fig. 3. Models of the respiratory chain supercomplexes. The X-ray structures of dimeric bovine complex III [77] and monomeric complex IV [14] are filtered to 20 Å. Complex III_2 is shown in red and monomeric complex IV in green. The negative stain EM map of bovine complex I [6] is displayed in yellow. The putative location of the membrane is visualized in blue. (a) III_2IV_2 from *S. cerevisiae* [62]. (b) I_1III_2 from *A. thaliana* [63]. (c) I_1III_2 from *B. taurus* [57]. (d) $I_1III_2IV_1$ from *B. taurus* [64].

electron carriers have short diffusion distances, supporting the notion of more efficient electron transfer through supercomplexes as opposed to the fluid-state model where electron transfer depends on the random encounter of the respiratory chain components.

2.2. Role of supercomplexes in complex assembly and stability

The higher efficiency of electron transfer as described above is a likely rationale for the existence of supercomplexes. However, there is evidence that the higher-order organization of the respiratory chain complexes is an essential feature of the mitochondrial architecture. Complex III is only active as a dimer [75,76,78,79], whereas complex IV is also active as a monomer, although it occurs as dimers in the crystal structures [14–17]. In the EM structures of the supercomplexes, complex III is always present as a dimer [57,62,63], but complex IV has so far only been found as monomers [57,62].

There are many indications that the presence of complex III and complex IV is essential for the assembly or stability of complex I [49,51,53–55]. In bovine heart mitochondria, the supercomplex containing complex I, III and IV was more stable and had higher activity than the one missing complex IV [57]. Subunits responsible for supercomplex formation have so far not been identified.

3. Supramolecular structure of ATP synthase

3.1. Evidence for a higher-order organisation of mitochondrial ATP synthase

Conversion of the electrochemical gradient created by the respiratory chain complexes is carried out by complex V, the F_1F_o ATP synthase, which is one of the most abundant proteins in the mitochondrial inner membrane. The F_1 particles are easily recognisable on electron micrographs of submitochondrial particles [80]. A supramolecular organisation of the mitochondrial ATP synthase was first noticed in the tubular cristae of *Paramecium* by rapid-freeze deep-etch techniques [81]. Double rows of F_1 forming a helical array around the cristae could be recognised, giving rise to the hypothesis that the organisation of the ATP synthases was responsible for the formation of cristae [82]. The double rows suggest a lateral organisation of ATP synthase dimers. By cryo-electron tomography of *Neurospora crassa* mitochondria, rows of F_1s were also seen [83]. In addition to supercomplexes consisting of complexes I, III and IV, dimers of ATP synthases were found on BN-PA gels after solubilization

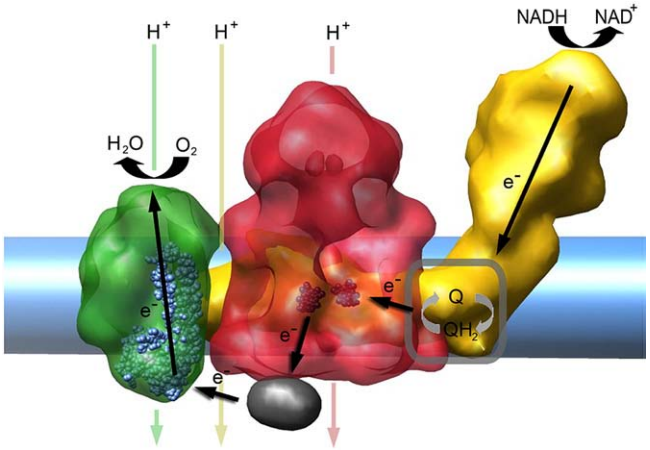

Fig. 4. Hypothetical electron transfer within supercomplex $I_1III_2IV_1$. The X-ray structure of dimeric bovine complex III [77] and monomeric complex IV [14] and of cytochrome *c* [61] are filtered to 20 Å. Complex III is shown in red and complex IV in green. The negative stain EM map of bovine complex I [6] is displayed in yellow. The putative location of the membrane is indicated in blue. NADH is oxidized to NAD^+, the electrons are transferred within complex I to ubiquinone. The putative ubiquinone binding sites in complex I and III_2 are circled in grey. From ubiquinone the electrons are transferred to complex III where some are transferred back into the ubiquinone cycle and others are transferred (see arrow) over the heme b_L (shown in black) to cytochrome *c*. Cytochrome *c* bound to complex III_2 [78] is shown in grey. Cytochrome *c* transfers the electrons to complex IV. The electron binding site in complex IV is in subunit II (shown in light blue). Within complex IV the electrons are transferred to the end acceptor oxygen to produce water. During electron transport along the respiratory chain, protons are translocated in each complex (shown in coloured arrows for each complex) from the matrix into the inner membrane space, thus creating an electrochemical proton gradient which is used by complex V (not shown) to produce ATP. Based on the 3D structure and interpretation from [64].

of mitochondrial membranes with mild detergents, first in yeast [84] and later in mammalian mitochondria [48]. Higher-order oligomers were also found [60,85]; a very mild separation by CN-PAGE, without the use of Coomassie stain, promotes the retention of even-numbered oligomers (4, 6 or 8) only [86], suggesting an association of dimers.

3.2. Dimer-specific subunits of ATP synthase

Separation of the subunits of ATP synthase dimers by 2D gel electrophoresis revealed the presence of three dimer-specific subunits, *e*, *g* and *k*, which were not essential for catalysis [84]. Deletion mutants of *e* and *g* not only lacked ATP synthase dimers but also had altered inner membrane morphology with the inner membranes forming onion-like structures [85,87].

Association between the two ATP synthases in a dimer occurs via the F_o part. Both the *e* and the *g* subunit are F_o components with a single transmembrane span [88]. The *e* subunit has an N-terminal transmembrane helix and a C-terminal hydrophilic domain with high predicted coiled-coil propensity, which would be located in the intermembrane space [88,89]. It was later shown that only the transmembrane domain, not the coiled-coil region is essential for dimerization of the ATP synthase [90]. Homodimers of *e* are associated with the formation of higher oligomers [91] whereas *e*–*g* heterodimers are responsible for the formation of ATP synthase dimers [92]. In a study of yeast ATP synthase, two subunits 4, the equivalent of the bacterial peripheral stalk subunit *b* of which there is only one copy in yeast, could be cross-linked, thus placing this subunit in the dimer interface [85]. In yeast, the peripheral stalk subunit *h* has also been inferred in dimer formation [93].

When there is no transmembrane proton gradient generated by the respiratory chain, F_1F_o will act as an ATPase and pump protons out of the matrix. In mitochondria, the regulatory subunit IF_1 prevents ATP hydrolysis under these conditions by binding to F_1. The C-terminus of IF_1 is a dimerization domain and an IF_1 dimer binds simultaneously to two F_1s. The crystal structures of an IF_1 dimer [94] and of a bovine F_1 dimer in complex with IF_1 [95] have been determined. Thus, a regulatory role for dimer formation based on inhibitor binding was proposed. However, both in yeast [96] and in bovine heart mitochondria [97] dimerization of ATP synthase was shown to be independent of the inhibitor protein and to be mediated by the F_0 domain. A more likely explanation of the function of IF_1 in vivo is binding to pre-existing ATP synthase dimers.

3.3. Electron microscopy of ATP synthase dimers

Recently, electron microscope structures of isolated ATP synthase dimers were obtained for bovine heart [98], yeast [99] and the alga *Polytomella* [100]. In all cases the dimers were found to be associated through their F_o domains, which are at angles of 35–90° to each other, thus bending the membrane. A hypothetical arrangement of the two ATP synthases forming a dimer is shown in Fig. 5. The angled arrangement of the F_o parts strongly supports the notion that this association between the ATP synthases is responsible for inner membrane morphology and crista formation. Molecular details of the dimer interactions are still unclear, however.

The *Polytomella* dimer is unusual in its high stability, which may be due to the presence of a unique, 60 kDa, dimer-specific subunit in algal ATP synthase [101]. In the EM average of the *Polytomella* dimer clear peripheral stalks are visible adjacent to each of the F_1 units (Fig. 5), together with an additional mass suggested to be the 60 kDa subunit [100]. In most of the yeast and bovine images, the peripheral stalks are not visible and may indeed be absent, as suggested by the absence of the easily recognisable OSCP subunit on top of the F_1 [99] (Fig. 2). The bovine heart dimers include a smaller angle and their F_1s are close together. A bridging structure between them is visible connecting the bottom of the F_1s [98]. The location of the bridge coincides with the position of the inhibitor protein IF_1 in an F_1 dimer [95], suggesting that the dimer is stabilized by this protein [98]. Also in the bovine heart dimer, an additional structure is visible on the intermembrane space side of F_o [98], which is not seen in monomer preparations [34]. This suggests that it may represent one of the dimer-specific subunits. A likely candidate would be the hydrophilic region of subunit *e*, which is predicted to form a coiled coil on the intermembrane space side [88].

The yeast dimers [99] show relatively few structural details. However, they form two distinct classes with angles between the F_o parts of 90° and 35°, respectively. Both angles also occur for dimers missing one or even two F_1 parts, clearly indicating that the transmembrane domains alone determine the dimer structure. The 90° dimers resemble the *Polytomella* and the 35° dimers the bovine heart dimers. This has led to the suggestion that the two represent different associations within a double row: the 90° structure a "true dimer" across both rows and the 35° structure a "pseudo-dimer" made up of neighbours within a row [99].

Further confirmation of the existence of a supramolecular arrangement of ATP synthase is provided by an AFM study of inner membranes from yeast [102]. It reveals staggered double rows of circular features, interpreted as a view of the *c*-rings from the intermembrane space, in a pattern similar to that seen by freeze-etching of *Paramecium cristae* [81]. The rings are flanked by three-domain features interpreted as the subunits *a*, *b*, *c* and *g*. The pattern is consistent with the arrangement shown in Fig. 5.

A convincing three-dimensional view of the shaping of the cristae by ATP synthase comes from a cryo-electron tomography study of

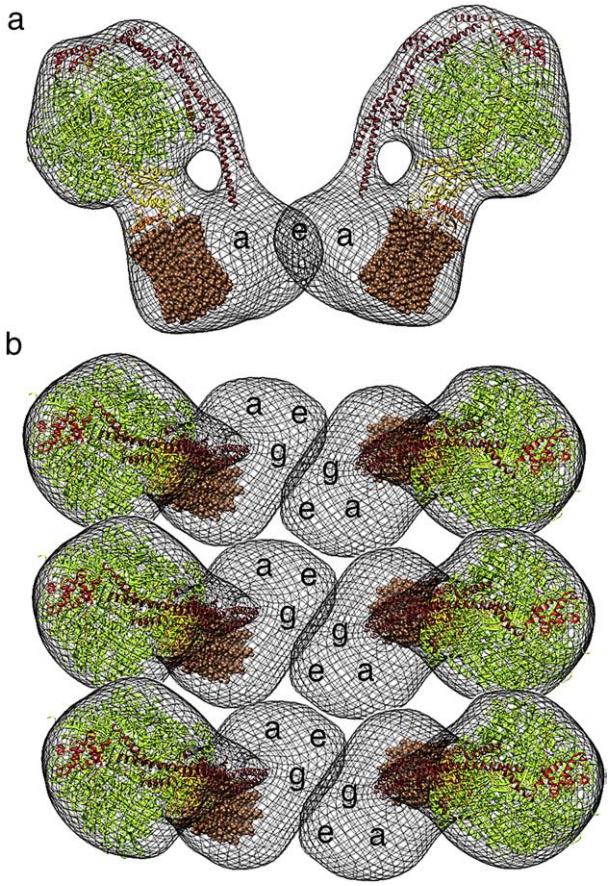

Fig. 5. Hypothetical model of the supramolecular organisation of ATP synthase, based on data in [85,91,92,98–100]. (a) dimer seen from the membrane plane, (b) view of a dimer row perpendicular to the membrane.

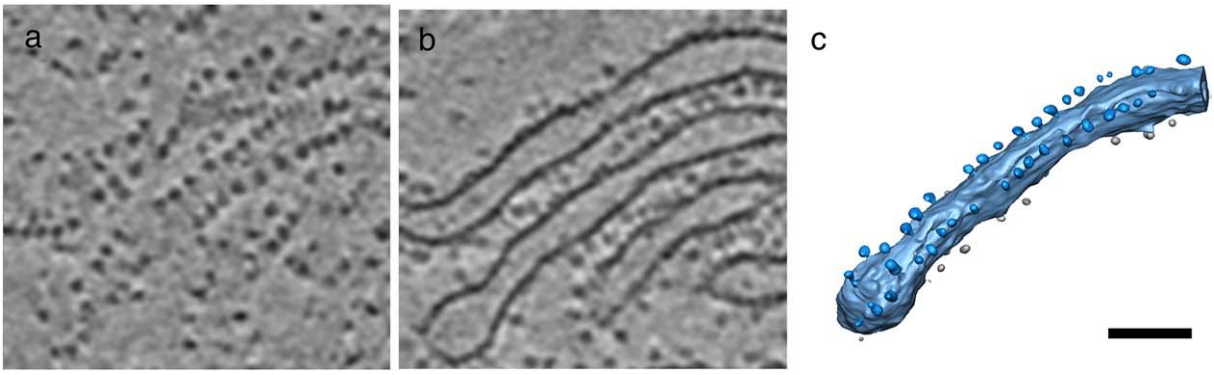

Fig. 6. Tubular vesicles from rat liver mitochondria imaged by cryo-electron tomography. Slices of the tomogram show F_1 ATP synthase dimer rows (a) and a cross section of the tubes (b). A surface rendering of the middle tube (c) displays a dimer row (blue); another row is present on the back of the tube (grey). The scale bar represents 50 nm. Figure courtesy of Mike Strauss and Götz Hofhaus.

bovine heart and rat liver mitochondrial vesicles [103]. The F_1 heads form double rows, which are exclusively located in a helical pattern around tubular vesicles (Fig. 6) and on the rim of flat discs. The angles between monomers are close to 90°, as was found for the isolated dimers of algal and yeast ATP synthase [99,100].

4. Conclusions and outlook

It is becoming clear that the proteins in the mitochondrial inner membrane do not float passively in the lipid bilayer, but are highly organised and are at least partly responsible for shaping the inner membrane. The difficulty of isolating higher-order structures from the membrane has long hindered this realisation, but increasingly sophisticated biochemical methods have shown the existence of well-defined supercomplexes, which have now been confirmed by electron microscopy, both in the case of ATP synthase dimers [98–100] and respiratory chain supercomplexes [57,62,64,104]. It was shown in bovine heart mitochondria that most of complex I occurs in supercomplexes [68]. The role of complex III and IV in assembly and stability of complex I [49,51,53,54] as well as the higher stability and activity of a supercomplex including complex IV compared to I_1III_2 [57] suggests that supercomplex formation is an essential step in mitochondrial respiratory chain assembly.

Is there organisation at a higher level than supercomplexes? In the case of ATP synthase it is now clear that there is, and there are good indications that the bends in membranes induced by ATP synthase dimers are responsible for shaping the inner membranes and forming the cristae. Thus, the organisation of complex V appears to play a structural role. Regarding the respiratory chain complexes, early work by Allen et al. showed not only the ordering of ATP synthase in double rows, but also gave an indication of ordered structures between these rows, which were interpreted as complex I dimers [81,82]. A model by Schägger and coworkers [68,105] elaborates on this observation and suggests the occurrence of strings of supercomplexes, so-called respiratory strings.

Mitochondria are highly dynamic structures, subject to continuous fusion and fission events, which may also have an effect on or be affected by crista morphology. In order to understand the role of supercomplexes, comparisons of the electron transfer rate and efficiency between the individual complexes and supercomplexes must be obtained, and 3D structures of other supercomplexes will be important to understand the pathways of assembly. Higher resolution structures will also be crucial to obtain more detailed information about the specific complex interactions and possible conformational changes due to the supercomplex formation. Up to now it is unclear if, once a supercomplex is formed, it persists until degradation or if there is a permanent transition between individual complexes floating in the membrane and supercomplexes. Detailed information about the arrangement and interaction of the respiratory chain complexes within supercomplexes could lead to the elucidation of causes of mitochondrial diseases caused by deficiency of the complexes. Patients with peripheral arteriosclerotic vascular diseases show a combined decrease in complex I and III activity [106], while dual deficiencies of complex I and IV are observed in idiopathic Parkinson patients [107–109]. In patients with lactic acidosis syndrome decreased complex I and IV activities have been found [110] and toxicity studies on neuroblastoma cells show a simultaneous inhibition of complex I and IV [111]. In the future it may be possible to treat some of these dual deficiencies and their symptoms on the basis of information gained about the structure and function of respiratory chain supercomplexes.

Advances in isolation of larger structures combined with single particle electron microscopy on the one hand, and studies of intact mitochondria by antibody-labelling and cryo-electron tomography on the other hand, as well as a better understanding of mitochondrial dynamics will be needed for a full picture of the structure of the electron transport chain in mitochondria.

Acknowledgements

We thank Niko Grigorieff for the negative stain EM map of bovine complex I, Mike Strauss for providing Fig. 6, and Werner Kühlbrandt and Carolyn Moores for critically reading the manuscript.

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