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Supercomplex organization of the oxidative phosphorylation enzymes in yeast mitochondria

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Abstract Accumulating evidence indicates that the enzymes involved in mitochondrial oxidative phosphorylation (OXPHOS) are co-assembled into higher-ordered supercomplexes within the mitochondrial inner membrane. This review will focus largely on the OXPHOS supercomplexes of the yeast *Saccharomyces cerevisiae*. The recent evidence to indicate that diversity in the populations of the cytochrome bc_1-COX supercomplexes exist shall be outlined. In addition, the existence of dimeric/oligomeric F_1F_o-ATP synthase complexes and their proposed role in establishment of the cristae architecture of the inner mitochondrial membrane shall also be discussed.

Keywords Mitochondria · Oxidative phosphorylation · Supercomplex · Cytochrome bc_1 · Cytochrome oxidase · F_1F_o-ATP synthase · Cristae morphology

Introduction

Mitochondria are essential organelles, which in addition to the aerobic production of ATP, are required for other cellular processes and events such as lipid metabolism, FeS cluster biosynthesis, metabolite synthesis, apoptosis and calcium signaling. Mitochondria produce ATP through a process termed oxidative phosphorylation (OXPHOS; Saraste 1999). In most eukaryotic organisms the OXPHOS machinery consists of the ATP synthesizing enzyme, the F_1F_o-ATP synthase complex (often referred to as complex V) and four electron transport chain complexes, the NADH: ubiquinone oxidoreductase (complex I), the succinate dehydrogenase (complex II), the ubiquinol cytochrome *c* reductase, also known as and referred to here as the cytochrome bc_1 complex (complex III) and the cytochrome *c* oxidase (complex IV). These OXPHOS complexes are multi-subunit enzymes embedded in the mitochondrial inner membrane and are composed of both nuclearly and mitochondrially encoded subunits (with the exception of complex II, which entirely nuclear in origin). Electrons generated from NADH and $FADH_2$ are passed along the complexes I–IV and ultimately transferred to molecular oxygen, a process which is coupled to the pumping of H^+ from the matrix across the inner membrane to the intermembrane space (Saraste 1999). Electron transfer between the complexes involves ubiquinone, a mobile electron carrier within the inner membrane and cytochrome *c*, a small protein located on the intermembrane space-side of the inner membrane. The proton gradient established through the activity of the OXPHOS complexes drives not only the synthesis of ATP by the F_1F_o-ATP synthase in the matrix, but also supports events such as metabolite and protein transport across the inner membrane. The ADP/ATP carrier (AAC) proteins are integral membrane proteins, which facilitate the equimolar exchange of ATP for ADP across the inner membrane (Pebay-Peyroula et al. 2003). Thus the AAC proteins also form a critical component of the mitochondrial OXPHOS system as they are responsible for the distribution of ATP synthesized in the matrix to the rest of the cell and for replenishing the mitochondrial matrix with ADP, the substrate of the F_1F_o-ATP synthase enzyme.

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Organization of OXPHOS complexes in the mitochondrial inner membrane

The assembly, enzymology and regulation of the mitochondrial OXPHOS complexes have been the subject of intense research for many years. Despite significant advances in our understanding of the workings of the individually purified OXPHOS complexes, at the enzymatic level, and in many cases at the structural level, how these complexes are organized within the mitochondrial inner membrane remains the subject of some debate and novel research directions. Contrasting views on their organization in the lipid bilayer of the inner membrane range from the "fluid state" to the "solid state" models (recently reviewed by Lenaz and Genova 2007). The fluid-state organization model supports that the complexes I–V exist as independent complexes which randomly diffuse within the lipid bilayer. Electron transfer between complexes I–IV is supported though their diffusion-coupled collision, where mobile electron carriers, ubiquinone and cytochrome *c*, would exhibit faster diffusion rates relative to the larger membrane-embedded, multi-subunit complexes. The solid state model on the other hand, favors that a higher-level of organization of the OXPHOS complexes exists within the inner membrane which promotes directed electron channeling between physically associated OXPHOS complexes. The ability to purify detergent-solubilized OXPHOS complexes individually and in their enzymatically active forms, demonstrates that their co-association in the membrane is not necessary for their enzymatic function. However, a number of independent studies have demonstrated that the mitochondrial OXPHOS complexes can be co-purified together when solubilized from the mitochondrial membranes using mild detergent (e.g. digitonin) solubilization conditions. The demonstration of purified "OXPHOS supercomplexes" formed by the physical association of proteins between these complexes and thus uniting them into a higher level of organization, favors that the OXPHOS complexes are not randomly organized as individual complexes in the membrane, as the liquid state model would predict. An extensive analysis of the experimental evidence supporting both the liquid state and solid state models of OXPHOS complex organization, has been recently presented in detail (Lenza and Genova 2007) and therefore is not be discussed further here.

The organization of the mitochondrial OXPHOS complexes into supercomplexes has been described for many organisms, including representatives from fungi, plants and mammals (reviewed in Schägger 2002; Dudkina et al. 2006a, Boekema and Braun 2007; Devenish et al. 2008, Vonck and Schäfer 2008). The predominant supercomplexes described involve complexes I–III–IV and the dimeric/oligomeric forms of the ATP synthase. As the OXPHOS supercomplexes of the yeast *Saccharomyces cerevisiae* are the focus of this review, the higher-organization of complex I (not present in *S. cerevisiae*) shall only be briefly dealt with here. Three OXPHOS supercomplexes have been described to date in *S. cerevisiae* (1) the mitochondrial dehydrogenase supercomplex, (2) the cytochrome bc_1-COX supercomplex (III–IV) and (3) the dimeric/oligomeric F_1F_o-ATP synthase complex.

Complex I-containing supercomplexes

In both plant and mammalian mitochondria, the rotenone-sensitive NADH:ubiquinone oxidoreductase (complex I) has been shown to exist in supercomplex forms with complexes III and IV. Different stoichiometric combinations of these supercomplexes have been reported to exist (Dudinka et al. 2005; McKenzie et al. 2006; Marques et al. 2007; Peters et al. 2008; Vonck and Schäfer 2008). Almost half of the known diseases associated with defects in the mitochondrial OXPHOS system can be attributed to defects in complex I and therefore the understanding of the organization of complex I-containing supercomplexes represents an active area of research currently. Loss of the correct assembly of complex III (due to mutations in the cytochrome *b*, a core subunit of this complex) has been shown to lead to a secondary effect of complex I instability (Acín-Pérez et al. 2004). Organization of complex I into of the complex I–III–IV supercomplexes therefore appears to be important to ensure its stability within the mitochondrial membrane system (Schägger et al. 2004). The lipid cardiolipin, unique to the mitochondrial inner membrane, has also been shown to play an important role in the formation and/or stabilization of the I–III–IV supercomplexes. The absence of the mature cardiolipin was shown to cause destabilization of the I–III–IV supercomplexes and compromised complex I biogenesis in Barth syndrome patients (McKenzie et al. 2006). It is currently not known which subunits of complex I and III are physically localized at the interface of these two complexes within the I–III–IV supercomplex. On-going single particle analysis of complex I-containing supercomplexes from bovine and plant mitochondria by electron microscopy, is addressing this important issue (e.g. Dudkina et al. 2005; Schäfer et al. 2006, 2007). This level of analysis will be crucial for furthering our understanding of the complex I–III and complex I–III–IV interactions. This is true not only for the complex I-containing supercomplexes, but also for the cytochrome bc_1-COX supercomplex (III, IV) and the F_1F_o-ATP synthase dimeric complexes, as will be outlined below later.

In contrast to many mitochondrial functions, the yeast *S. cerevisiae* is not a model organism of choice for complex I

analysis, as unlike most eukaryotic organisms, *S. cerevisiae* does not contain a traditional rotenone-sensitive multi-subunit complex I (see Grandier-Vazeille et al. 2001 for review). Rather, this yeast contains a rotenone-insensitive NADH-ubiquinone oxidoreductase composed of one nuclearly encoded subunit, Ndi1, localized in the matrix-side of the inner membrane. In addition, cytosolic NADH can be oxidized through an intermembrane-space facing NADH-ubiquinone oxidoreductase, which is composed of two subunits encoded by the nuclear genes NDE1 and NDE2. Recent analysis has indicated that the Ndi1, Nde1, Nde2 proteins are organized together into a supercomplex which has been proposed to associate with other NADH dehydrogenases, the glycerol-3-phosphate dehydrogenase (Gut2) and the D- and L-lactate dehydrogenases, D-LD and cytochrome b_2, respectively. The co-organization of these NADH dehydrogenases has been speculated to play a key role in NADH channeling in the yeast OXPHOS system (Grandier-Vazeille et al. 2001).

The cytochrome bc_1-COX supercomplexes

Under mild detergent extraction conditions (digitonin detergent) the yeast cytochrome bc_1 complex (complex III) was shown to co-purify with the cytochrome *c* oxidase (COX, complex IV; Cruciat et al. 2000; Schägger and Pfeiffer 2000; Zara et al. 2007). Co-association of these enzymes was initially demonstrated through their co-migration on a native gel electrophoresis system developed by Schägger, termed the blue native gel electrophoresis system (BN-PAGE; when electrophoresis is performed in the presence of Coomassie blue) or the clear native CN-PAGE system (when Coomassie blue is omitted). In addition, co-immunoprecipitation experiments confirmed the co-migration profiles of complexes III and IV on native gels was due to their physical association together in a supercomplex form (Cruciat et al. 2000). Two predominant forms of the III-IV supercomplex, have been described to exist, the III_2–IV_2 and III_2–IV forms respectively, and which differ in their stoichiometry of complex IV relative to complex III (Schägger and Pfeiffer 2000). The association of cytochrome bc_1 with the COX complex has been proposed to enhance electron flow between the complexes, a step catalyzed by cytochrome *c* (Schägger and Pfeiffer 2000).

As was mentioned previously for the I–III–IV supercomplex in mammals, the lipid cardiolipin plays a critical role in the stabilization of the yeast cytochrome bc_1-COX supercomplex. In the absence of cardiolipin (i.e. in mitochondria isolated from the cardiolipin synthase deficient yeast mutant) the III_2–$IV_{1–2}$ supercomplexes can still form, but they were observed to be very susceptible to the detergent extraction and the BN-PAGE procedure. Thus the presence of cardiolipin appears to be critical for the stability of the assembled cytochrome bc_1-COX supercomplex (Pfeiffer et al. 2003).

An ambitious study designed to map the interface of the complex III and complex IV in the supercomplex has been undertaken by the Braun laboratory (Heinemeyer et al. 2007). Single particle analysis by electron microscopy has revealed the first structural model for the III_2–$IV_{1–2}$ supercomplex. This analysis indicates that the complex III dimer forms a central core which can interact with two COX monomers. The complex III subunits, cytochrome c_1, Qcr6 (the acidic hinge protein), Qcr7 and Qcr8 and the COX subunits Cox1, Cox2, Cox5 (equivalent to CoxIV in mammals) and Cox8 (equivalent to CoxVIIc in mammals), are proposed to form the subunits of contact at the interface between the cytochrome bc_1 and COX complexes.

Diversity in the III_2–$IV_{1–2}$ supercomplex forms

A number of recent observations have highlighted a new level of complexity in the higher-organization state of the cytochrome bc_1-COX (III_2–$IV_{1–2}$) supercomplexes. These findings revealed that organization of the cytochrome bc_1-COX supercomplex is not limited to the proposed III_2–IV_2 and III_2–IV forms, but rather multiple forms of these supercomplexes exist in mitochondria, which differ in their further interaction partners. A subpopulation of the TIM23 protein translocase and its associated import motor, the PAM machinery, has been found in association with a population of the cytochrome bc_1-COX supercomplexes (van der Laan et al. 2006; Wiedemann et al. 2007; Saddar et al. 2008; Dienhart and Stuart 2008). This association is particularly labile to detergent being observed when low digitonin concentrations are used for the membrane lysis step. The function of the association of the TIM23 complex with the cytochrome bc_1-COX is presently unclear but it has been proposed that the OXPHOS supercomplex may bioenergetically support the associated TIM23 protein translocase, a voltage sensitive channel, which facilitates the membrane potential dependent import of nuclearly encoded proteins (van der Laan et al. 2006, Wiedemann et al. 2007; Kutik et al. 2007). In addition, two COX assembly proteins, the Shy1 and Cox14 proteins (Glerum et al. 1995; Mashkevich et al. 1997; Barrientos et al. 2004), have also shown to associate with a population of the cytochrome bc_1-COX supercomplex (Mick et al. 2007). The functional relevance of this interaction is also currently unknown, but most likely is related to the regulation of COX assembly and/or turnover, but awaits further investigation.

As the levels of the Shy1 and Cox14 proteins, and the TIM23 translocase, are substoichiometric to the complex III and IV levels, it is most likely that not

every cytochrome bc_1-COX supercomplex can exist in association with the Shy1, Cox14 or TIM23 translocase. These observations therefore suggest that potentially multiple forms of the cytochrome bc_1-COX supercomplex exist within a given mitochondrion (Fig. 1). Elucidation of the functional significance of the diversity of populations of the cytochrome bc_1-COX supercomplex, regulation of their levels and their distribution within the mitochondrial inner membrane (see below), will represent a novel direction of future OXPHOS supercomplex investigation.

Proteomic profiling of the yeast cytochrome bc_1-COX supercomplex solubilized under mild digitonin conditions indicated that the AAC also co-assembles with this OXPHOS supercomplex (Dienhart and Stuart 2008). As was observed for the TIM23 complex, the association of AAC with the cytochrome bc_1-COX supercomplex is susceptible to the detergent extraction process. There are three AAC isoforms in the yeast *S. cerevisiae* and using histidine-tagging and affinity purification approaches it could be demonstrated that the abundant Aac2 isoform physically purifies with the cytochrome bc_1-COX supercomplex and in a manner which also included the associated TIM23-PAM machinery. The presence of Aac2 has been shown to support the assembly of the COX complex and attainment of the III_2–IV_2 stoichiometric form of the supercomplex. In the absence of Aac2, a significant decrease in the mitochondrial content of the COX complex was observed. It is possible that the association of Aac2 with the cytochrome bc_1-COX complex may support the function of the associated Shy1 and Cox14 proteins, COX assembly factors. The AAC proteins, like the cytochrome bc_1 and COX complex, are abundant enzymes within the mitochondria and therefore it is considered likely that the majority of the cytochrome bc_1-COX supercomplexes may exist in close physical association with the AAC proteins within the mitochondrial membrane (Fig. 1).

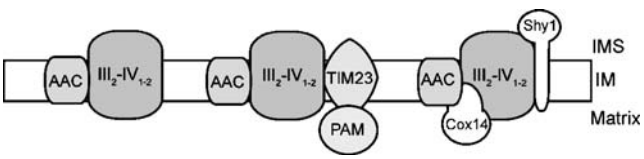

Fig. 1 Diversity of cytochrome bc1-COX supercomplexes exist: Evidence to indicate the cytochrome bc_1-COX supercomplexes (III_2–IV_{1-2}) may exist in physical association with the AAC, TIM23-PAM and the Cox14/Shy1 protein complexes, has been presented recently. See text for further details. *IMS* interembrane space, *IM* inner membrane

The dimeric/oligomeric F_1F_o-ATP synthase complex

The dimeric assembly state of the F_1F_o-ATP synthase has been demonstrated in mitochondria isolated from diverse organisms such as yeast, algae, plants and mammals (Arnold et al. 1998; Eubel et al. 2003; Krause et al. 2005; Vázquez-Acevedo et al. 2006). Current evidence indicates that the ATP synthase dimers can further organize themselves into tetramers and larger oligomeric complexes, which then can form a linear and regular array of oligomers within the mitochondrial inner membrane (Thomas et al. 2008). A number of independent lines of experimental evidence for the ATP synthase dimerization/oligomerization have been presented in the literature and have been recently reviewed (see Devenish et al. 2008, and references therein).

Proteomic analysis of the detergent stable yeast ATP synthase dimers lead to the identification of two novel F_o-sector subunits, the Su e and Su g proteins, the presence of which are not required for the enzyme activity of the ATP synthase, but is required for the stable assembly of the dimeric enzyme (Arnold et al. 1998; Paumard et al. 2002). Subunits Su e and Su g are small proteins, which each span the inner membrane once, whereby a conserved coiled-coil motif present in the C-terminal domain of Su e is exposed to the intermembrane space (Fig. 2; Arnold et al. 1997; Boyle et al. 1999; Vaillier et al. 1999; Brunner et al. 2002; Bustos and Velours 2005; Saddar and Stuart 2005).

Monomer-monomer ATP synthase interactions to form ATP synthase dimers has been proposed to involve core subunits of the F_o-sector, where evidence to support the involvement of the oligomeric Atp9 (equivalent to bacterial subunit c) with Atp6 (equivalent to bacterial subunit a) and the subunits of the peripheral stalk, have been discussed (Wittig et al. 2008; Weimann et al. 2008; Thomas et al. 2008). Formation of ATP synthase oligomers from neighboring ATP synthase dimers involves organized interactions between F_o-ATP synthase subunits and in a manner which is supported by an interface formed by the subunits Su e, Su g and also the Atp4 protein (equivalent to bacterial (and mammalian) ATP synthase subunit b; Fig. 2). The conserved coiled-coil domain of Su e is proposed to support the interactions of the Su e protein from one ATP synthase dimer with a Su e protein from a neighboring dimeric complex, thus building an oligomeric ATP synthase network (Fig. 2; Brunner et al. 2002; Everard-Gigot et al. 2005). The Su e-Su e coiled-coil supported ATP synthase dimers are significantly more stable to detergent extraction than the dimers formed through other F_o–F_o interactions (Fig. 2).

The ATP synthase, and in particular the presence of Su e and Su g, was also found to be important for securing optimal COX activity, and the correct organizational state of the cytochrome bc_1-COX supercomplex and its association with the TIM23 machinery (Boyle et al. 1999; Rak et al.

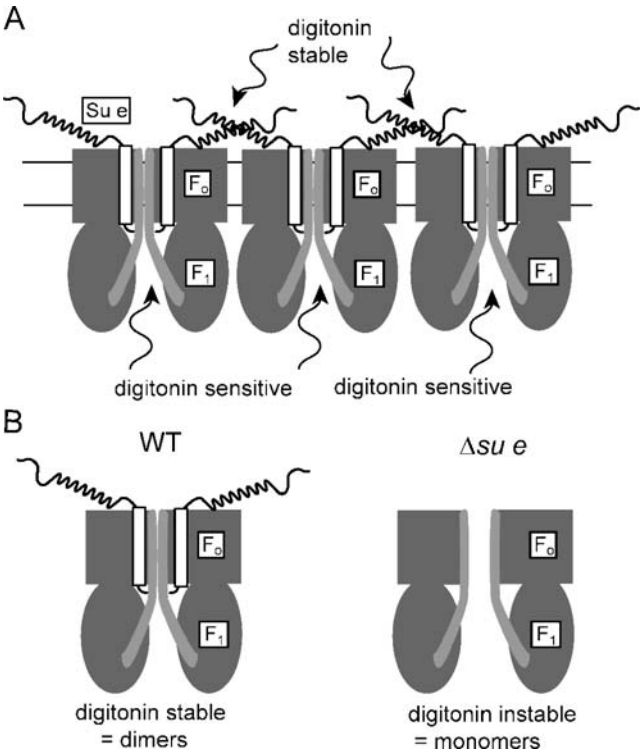

Fig. 2 Coiled-coil interactions of Su e support establishment of ATP synthase oligomers: (**A**) F_1F_o-ATP synthase monomers interact through F_o–F_o contacts in a digitonin-sensitive fashion. Association of neighboring ATP synthase dimers to form oligomeric network involves Su e–Su e dimerization events entailing the conserved coiled-coil domain (*zig-zag line*) of the Su e proteins. (**B**): Following digitonin extraction, solubilized F_1F_o-ATP synthase dimers from wild type (*WT*) mitochondria are maintained in a stable state through Su e-Su e based interactions. In the absence of Su e (Δ*su e* mitochondria), the ATP synthase complexes are largely solubilized as monomers, as the F_o–F_o interactions are not as stable to detergent extraction in the absence of Su e, or more specifically, in the absence of the coiled-coil domain of Su e

2007a, b; Saddar et al. 2008). An altered stoichiometry of the cytochrome bc_1-COX supercomplex and decreased COX activity was monitored in mitochondria isolated from *su e* and/or *su g* null mutant yeast strains. The observation that the organizational state of the ATP synthase complex influences that of the cytochrome bc_1-COX supercomplex may be indicative of the co-organization of these OXPHOS complexes into localized regions or "OXPHOS" platforms" of the inner membrane (Saddar et al. 2008). It is also possible that the alteration in the cytochrome bc_1-COX supercomplex in the absence of Su e and Su g may be due to the dramatically altered cristae membrane morphology which is reported to occur in the *su e* and *su g* null mutants (see below). Loss of cristae membrane architecture may affect the arrangement and stability of the supercomplexes within the inner membrane.

ATP synthase dimers/oligomers are required for normal cristae development

The inner membrane can be divided into two distinct regions, the inner boundary membrane (IBM), and the cristae membrane. The inner boundary membrane runs parallel and in close proximity to the outer mitochondrial membrane. The cristae membrane extends from the IBM, forming invaginations of the inner membrane, where two neighboring inner membrane leaflets are juxtaposed to each other. In the absence of Su g and Su e (and also mutations in mitochondrial specific regions of Atp4), it has been reported that the inner membrane of yeast mitochondria fails to partition into cristae structures but rather accumulates with an "onion-like" morphology (Paumard et al. 2002; Arselin et al. 2004; Weimann et al. 2008; Thomas et al. 2008). In addition, the change in cristae architecture within the mitochondria is correlated with overall collapse of mitochondrial morphology at the fluorescent microscope level. Wild type mitochondria form a large tubular reticular network throughout the cell, whereas those from the Δ*su e* and Δ*su g* cells show an aberrant shape, failing to form the reticulum, but accumulate disc-like mitochondrial membranes (Everard-Gigot et al. 2005). In addition, the Δ*su e* and Δ*su g* mutants exhibit a high frequency of mtDNA loss, a phenotype often associated with a mitochondrial morphology defect.

As the presence of Su e is essential to form stable ATP synthase dimers/oligomers, it was originally proposed that the Su e-mediated dimeric/oligomeric ATP synthase network (Fig. 2) is directly required to form the cristae morphology (Paumard et al. 2002; Giraud et al. 2002). This observation was consistent with an earlier model by Allen (1995), which suggested that oligomerization of neighboring ATP synthase complexes would serve to bend the plane of the membrane surface, forcing it to adopt a tubule curvature required for cristae formation. In agreement with this model, purified detergent stable ATP synthase dimers have been shown to adopt a diverse angular conformations caused by their F_o–F_o interactions, thus indicating the potential of interacting ATP synthase complexes to induce curvature of the lipid bilayer in which they reside (Minauro-Sanmiguel et al. 2005; Dudkina et al. 2006b; Strauss et al. 2008). Electron tomography experiments of yeast mitochondria, however, indicate that the cristae membranes form large lamellar with numerous regular and zipper-like arrays of F_1F_o-dimers, that exhibit little or no local bending at the membrane surface (Thomas et al. 2008). Given this observation, it remains an open question therefore if the curvature of the inner membrane and establishment of the cristae is really directly related to the Su e-mediated oligomerization of ATP synthase complexes. Another observation against the proposal that Su e

acts to promote cristae membrane development by supporting the assembly of the ATP synthase oligomers, came from the observation that the coiled-coil region of Su e, required to support the formation of stable ATP synthase dimers/oligomers, was found not to be required for the establishment of cristae membrane architecture *in vivo* (Everard-Gigot et al. 2005; Bornhövd et al. 2006). Rather, the N-terminal transmembrane domain of Su e (first 36 residues of Su e) was shown to be necessary for the attainment of normal mitochondrial inner membrane morphology, despite the fact that this region of Su e cannot support the stable oligomerization of the ATP synthase complexes. Based on these observations we consider it likely that Su e's essential role in supporting mitochondrial membrane morphology is independent from its role in supporting ATP synthase oligomerization (Everard-Gigot et al. 2005). We favor a model whereby the Su e protein, or more specifically the N-terminal transmembrane region of Su e, may interact with known mitochondrial morphology factors and thus exerts its function on cristae biogenesis through affecting the activity of these proteins (Fig. 3). A number of such morphology factors required for cristae membrane formation have been described in the literature (Hoppins et al. 2007). Indeed preliminary evidence to indicate a functional connection between Su e and Mgm1, an inner membrane GTPase necessary for inner membrane fusion and cristae maintenance, has been presented (Amutha et al. 2004). Thus further experimentation is clearly required to dissect if the role of the Su e protein in modulating the inner membrane morphology is independent from or related to its function in supporting formation of the ATP synthase oligomeric network.

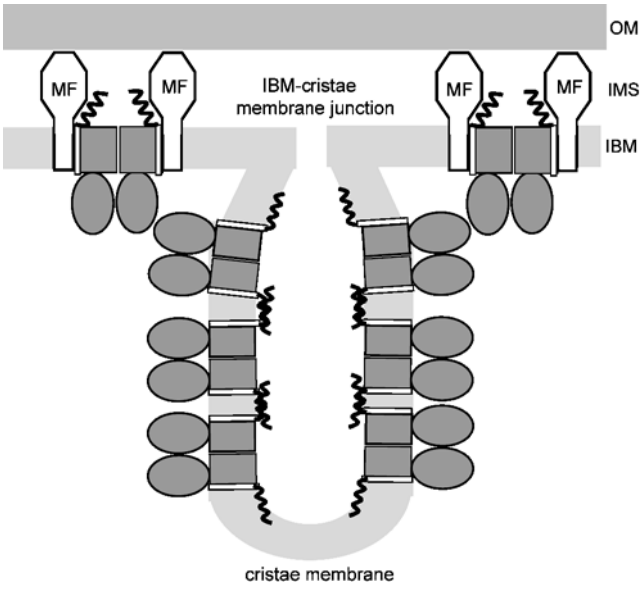

Fig. 3 ATP synthase oligomers are localized in the cristae membrane: Su e plays an essential role in the establishment and/or maintenance of cristae architecture and in the establishment of the oligomeric ATP synthase network. The latter function (and not the former), requires Su e-based coiled-coil (*zig-zag lines*) interactions between complexes. We propose that these two functions of Su e may be independent from each other. A population of Su e may exist in the vicinity of the IBM-cristae membrane junction which differs in its molecular environment from the Su e protein located in the ATP synthase oligomer of the cristae membrane. The Su e protein based in the IBM-cristae junction may interact, through its N-terminal transmembrane domain, with proteins involved in cristae membrane formation, and are indicated here as morphology factors (*MF*). *IBM* inner boundary membrane, *OM* outer membrane, *IMS* intermembrane space

Distribution of OXPHOS supercomplexes within the microenvironments of the inner membrane

The cristae membranes have long been considered to represent areas of high OXPHOS activity (Gilkerson et al. 2003). The existence of cytochrome bc_1-COX supercomplexes in association with the TIM23 translocase, however, opens up new questions with regards to the distribution of the OXPHOS supercomplexes within different microenvironments of the inner membrane, i.e. the IBM and the cristae. As they are undergoing import into the mitochondria via the TIM23 translocase, nuclearly encoded proteins can span both the outer and inner membranes at the same time, indicating the closeness of the TIM23 machinery to the translocase of the outer membrane (the TOM complex) (Schleyer and Neupert 1985). Furthermore, subunits of the TIM23 complex, the Tim21 and Tim23 proteins, have been shown to make physical contact with the TOM machinery and outer membrane, respectively, and serve to tether the TIM23 complex in the environment of the outer membrane, i.e. in the IBM or possibly in the vicinity of cristae junctions (see Kutik et al. 2007 for review). Taking this together, it is conceivable that the cytochrome bc_1-COX-TIM23 supercomplexes are localized within the IBM rather than in the cristae membrane. To ensure localization to a specific microenvironment of the inner membrane, IBM-based OXPHOS supercomplexes may differ from those enriched in the cristae membranes in their protein composition (or possibly post-translational modifications of their components) and function to produce a localized membrane potential that drives IBM-based processes such as protein import by the TIM23 complexes. Formation of these diverse higher-ordered assembly states of the OXPHOS complexes, and their regulated association with other proteins such as the TIM23 machinery, may thus play an important function in ensuring the distribution of these complexes within different microenvironments of the inner membrane. The physiological significance of the diversity of cytochrome bc_1-COX supercomplexes, and also possibly of the ATP synthase dimers/oligomers, represents a new

and exciting direction for future studies of the higher-ordered organizational state of the mitochondrial OXPHOS machinery.

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