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## Biochimica et Biophysica Acta

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## Review

The function of the respiratory supercomplexes:  
The plasticity model☆☆

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## ARTICLE INFO

## Article history:

Received 16 September 2013

Received in revised form 12 December 2013

Accepted 17 December 2013

Available online 22 December 2013

## Keywords:

Mitochondrial supercomplex

Respirasome

OXPHOS

Metabolism

## ABSTRACT

Mitochondria are important organelles not only as efficient ATP generators but also in controlling and regulating many cellular processes. Mitochondria are dynamic compartments that rearrange under stress response and changes in food availability or oxygen concentrations. The mitochondrial electron transport chain parallels these rearrangements to achieve an optimum performance and therefore requires a plastic organization within the inner mitochondrial membrane. This consists in a balanced distribution between free respiratory complexes and supercomplexes. The mechanisms by which the distribution and organization of supercomplexes can be adjusted to the needs of the cells are still poorly understood. The aim of this review is to focus on the functional role of the respiratory supercomplexes and its relevance in physiology. This article is part of a Special Issue entitled: Dynamic and ultrastructure of bioenergetic membranes and their components.

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## 1. Introduction

Mitochondria are fascinating organelles that reside in eukaryotic cells. Although known mostly for their role in meeting the cell's energy requirements in the form of ATP through oxidative phosphorylation (OXPHOS), mitochondria also have functions in several other physiological processes. These include buffering cytoplasmic calcium [1], controlling cellular redox status, generating and releasing reactive oxygen species (ROS), releasing metabolites that regulate critical processes and pathways such as succinate and  $\alpha$ -ketoglutarate [2], regulating apoptosis [3], adapting cells to changes in substrate availability through different signaling pathways [4], and remodeling their structure and dynamics as sensor of their quality control [4]. Mitochondria are thus central to maintaining the delicate balance between life and death and need to be tightly regulated. This regulation occurs both through long-term responses at the level of expression, transcription and translation and through short-term posttranscriptional responses. Recent findings suggest that an additional level of short-term regulation is the dynamic supra-organization of the respiratory complexes in the inner mitochondrial membrane. This review examines the function of the respiratory supercomplex (SC) assembly and its relevance to physiological processes.

## 2. Organization of respiratory complexes in the inner mitochondrial membrane

Mitochondrial respiratory complexes (complexes I to IV) are responsible for the oxidation of the reducing equivalents, in the form of NADH or FADH<sub>2</sub>, originating in different metabolic pathways (glycolysis, fatty acid oxidation or the Krebs cycle). Oxidation of NADH and FADH<sub>2</sub> is coupled to the pumping of protons into the intermembrane space, and the resulting proton gradient is used by the ATPase (complex V) to generate utilizable energy in the form of ATP. NADH reducing equivalents enter the mitochondrial electron transport chain (mtETC) through complex I, whereas FADH<sub>2</sub> reducing equivalents enter the mtETC through complex II or other dehydrogenases such as electron-transferring-flavoprotein (ETF) dehydrogenase. The electrons are then passed to coenzyme (CoQ), and subsequently to complex III, cytochrome c, and complex IV, which passes them to oxygen as the final acceptor.

The organization of respiratory complexes in the inner membrane has been an object of intense debate. The respiratory components were initially proposed to be closely packed to guarantee accessibility and thus high efficiency in electron transport [5,6]. However, this original model was progressively abandoned and replaced by the fluid or random collision model [7]. In the fluid model, the respiratory complexes are viewed as independent entities embedded in the inner membrane, with CoQ and cytochrome c acting as mobile carriers that freely diffuse in the lipid membrane. In a landmark study published in 1986, Hackenbrock and co-workers confirmed that this model offered better explanation for the structural organization of the mtETC [7]. However, new evidence from yeast and mammalian mitochondria demonstrated that it was possible to purify stable associations of respiratory complexes [8,9] and a reformulation of the solid model proposed that respiratory complexes are organized in larger structures (respiratory

☆☆ This article is part of a Special Issue entitled: Dynamic and ultrastructure of bioenergetic membranes and their components.

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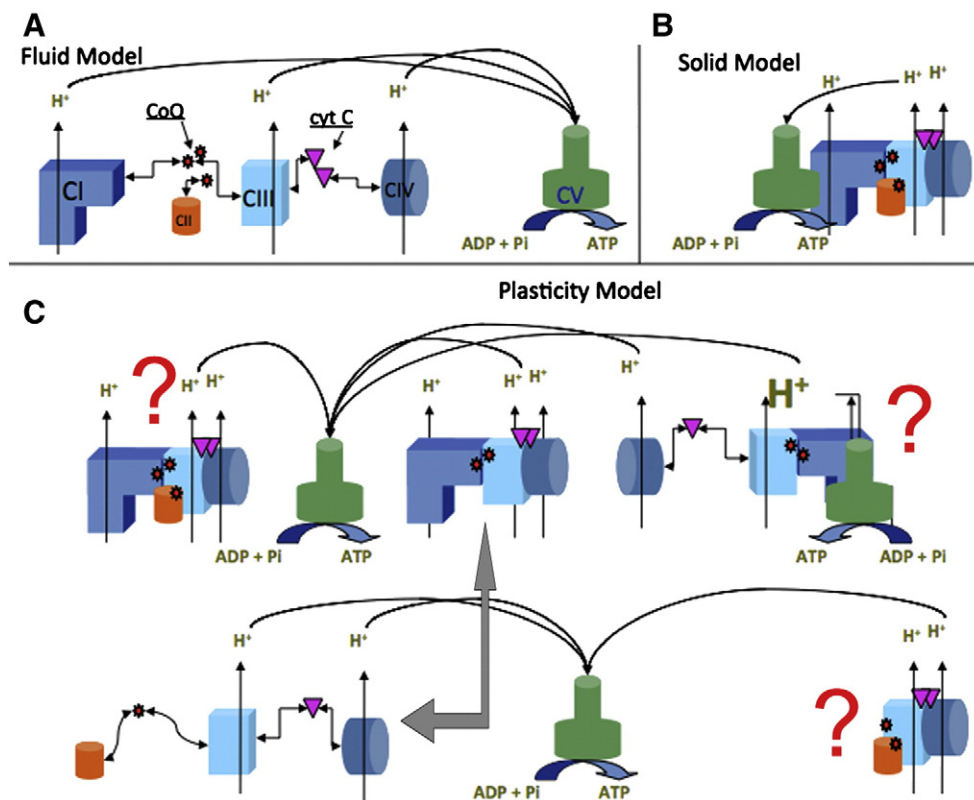
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supercomplexes, SCs), allowing a more efficient transport of electrons (Fig. 1). This ignited debate between the defenders of each of the models, which has revealed that neither model can satisfactorily account for all of the experimental evidence [10], further exacerbates the discrepancy between the two models [11,12].

The main lines of evidence supporting the existence of SCs are the specific co-migration of respiratory complexes on blue native electrophoresis and the co-purification of them by sucrose gradient centrifugation [8,13,14]. However, both these procedures require solubilization of the mitochondrial inner membrane with detergents, and it is therefore reasonable to maintain a skeptical position regarding the reality of SCs as functional *in vivo* entities. One of the main criticisms of the SC theory was the belief that SCs could only be isolated with one detergent (digitonin), and that other detergents yielded only free complexes; however, the opposite turned out to be the case: with all detergents except dodecyl-maltoside revealing the presence of SCs [15]. Aside from the detergent issue, the solid model fails to accommodate the well-supported experimental evidence for the kinetics of the mtETC reactions [16]. This made us reluctant to accept the existence of respiratory SCs as functional mtETC entities: whether or not they were solubilization artifacts, there was no direct evidence that the respirasome, an SC assembled from complexes I, III and IV, was able to respire. We reasoned that if SCs are genuine biological entities, they must satisfy the following conditions: the migration of a particular complex should be dependent on the presence of the other complexes with which it is proposed to interact, and the formation of complexes and SCs should be asynchronous. By using cell lines in which one complex was genetically eliminated, we were able to determine whether the migration of the other complexes in the putative SC assemblies was affected. This genetic

analysis showed that most putative SCs do indeed reveal genuine interaction between complexes. There were, however, exceptions that confirmed the original concern that co-migration on gels or gradients is insufficient evidence of interaction. The dynamic assembly of complexes and supercomplexes in intact cells can also be monitored by metabolic labeling of mtDNA-encoded proteins. Using this approach, we established that there is a gap of several hours between the labeling of free complexes and the incorporation of labeled complexes into SCs. Our interpretation that this shows SC assembly was recently questioned by the proposition from Ugalde's laboratory that respiratory complex I exists in a partially assembled state and that its assembly is completed only through interaction with complex III and complex IV in what they call the pre-respirasome [17]. While this group's analysis was conceptually similar to ours, there were critical differences in the experimental setup. They depleted respiratory complexes from cells in culture by treating them with doxycycline, a specific and reversible inhibitor of mitochondrial ribosomes. They then removed the drug and monitored the re-assembly of complexes and SCs by tracking different respiratory complex subunits by western blot. The key difference from our metabolic labeling approach is that we did not deplete respiratory complexes, but instead tracked their assembly in normally respiring mitochondria.

The discrepancy between Ugalde's model and our own is not trivial, and requires some detailed attention. The asynchrony that we detect is compatible with both the random collision model and the solid model since it accommodates the existence of free complexes and supercomplexes without making any assumptions about which would be the functional entity. It is important to consider that in our model there is no obvious necessity to assign SCs a respiratory function. They could instead play structural roles in the inner mitochondrial membrane or



**Fig. 1.** Schematic representation of the different models proposed to explain the organization of the OXPHOS system. (A) Hackenbrock's *random-collision model* or *fluid model*, (B) Schagger's revival of the *Solid model*, and (C) the *plasticity model* [15]. The shape and color code for representing the individual complexes can be seen in panel A; coenzyme Q is represented as small red-filled stars and cytochrome c as red-filled triangles. Only one complex unit of each type is represented in the different supercomplex associations, although the actual stoichiometry may vary. The question mark indicates putative associations or supercomplexes which existence is not fully confirmed. Supercomplexes containing complexes I to IV are also named respirasomes, since they can transfer directly electrons from NADH to oxygen. From [15] with some modifications.

provide an inactive store of respiratory complexes ready for activation when needed. In contrast, Ugalde's model is only compatible with the solid model and obligatorily views the respirasome as the functional respiratory unit. These differences suggest some testable predictions. First, Ugalde's model predicts that in the absence of complex IV complex I cannot be assembled. This was tested in a cell line homoplasmic for a mutation in mitochondrial cytochrome c oxidase 2 (COX2) [18]. The COX2 mutant is unable to assemble complex IV and has no complex IV activity; however, we could detect free complex I in native gels, and more importantly this complex I had NADH dehydrogenase activity. These results provide evidence against the Ugalde's proposal that complex I is an obligate scaffold for complex III and IV assembly and that respiratory activity (including NADH dehydrogenase activity) is acquired only when complexes I, III and IV combine together in SCs.

Two other criteria for functional respiratory SCs are that they should contain CoQ and cytochrome c and be able to transfer electrons among their components and from NADH to O<sub>2</sub>. These conditions have all been demonstrated experimentally, supporting a respiratory function for SCs [15]. These series of experiments led to our proposal of the plasticity model, which accommodates the solid and the fluid models by regarding the organization of the respiratory complexes as a network of different associations as well as individual complexes (Fig. 1C). In agreement with this view, metabolic flux control kinetics studies can also discriminate between the random collision and the solid model. Thus, in the former, each enzyme would be rate limiting while in the latter, the whole system would behave as a single unit where the inhibition of any of its components would affect the whole pathway. Flux control analysis performed in bovine heart mitochondria and submitochondrial particles cannot discard any model and support partially the solid model [19]. Taken together these results, the plasticity model can better accommodate the kinetic evidence.

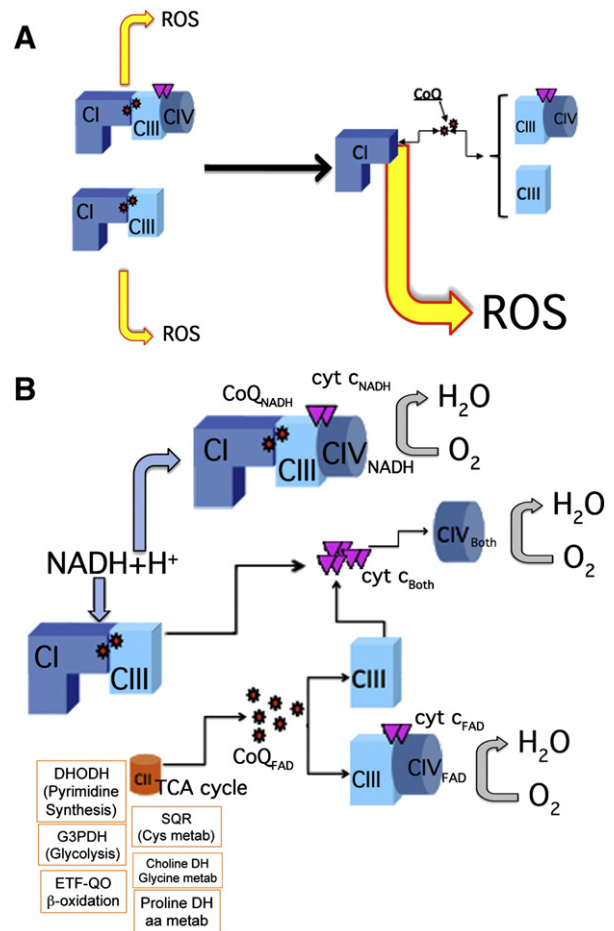
SC formation, stabilization and function are also critically influenced by the lipid composition of the inner mitochondrial membrane. In patients with Barth syndrome, where cardiolipin remodeling is impaired, SCs are unstable, leading to the mitochondrial dysfunction underlying the disease [20–23]. The importance of protein–lipid interaction has been demonstrated in cardiolipin- and phospholipid-deficient yeast strains, in which SC formation and complex activities are defective [22,23]. The importance of lipid composition in SC stabilization is supported by studies with reconstituted proteoliposomes [24–26].

### 3. Roles of respiratory supercomplexes

The interdependency of SC formation and complex stability has been shown in several genetic models, in which low SC levels are detected in the absence of complex III [27], complex IV [28], or cytochrome c [29]. However, we recently found that this dependency, consistently observed in cultured cells grown at 20% oxygen, might not occur at more physiological oxygen concentrations [30], indicating that its physiological significance remains to be determined. By the same token, the assembly of functional complex I in the absence of SC formation again questions Ugalde's proposal [17,18] (author's unpublished results). It has been postulated that SCs would increase the efficiency of electron flux through substrate channeling or enhanced catalysis [19,31]. However, the individual complexes of the respiratory chain can be isolated in relatively pure form and retain functionality [7]. Moreover, the kinetic behavior of CoQ as a homogeneous pool freely diffusing between the dehydrogenases and complex III argues against a role of SCs in respiratory function [16]. The pool behavior of CoQ and the existence of SCs are reconciled by our recent demonstration of two distinct CoQ pools: one dedicated to reducing equivalents coming from NADH and a second, independent pool coming from FADH<sub>2</sub>. By modulating the relative levels of complexes I and III, we have shown that these two CoQ pools compete for the delivery of electrons to complex III [32].

A third role proposed for SCs is an acceleration of electron transport concomitant with sequestration of reactive intermediates to prevent generation of reactive oxygen species [25,33]. Ghelli et al. [33] showed that mitochondria with a missense mutation in cytochrome b, which drastically impairs complex III activity and associated ATP synthesis causing high superoxide production, show almost a normal activity when complexes I + III or II + III were measured. Also the levels of SCs containing complex III were slightly increased [33], suggesting that SCs maintain mitochondrial function by promoting the sequestration of reactive oxygen species (ROS) despite the mutation. In the study by Maranzana et al. [24], complex-I-containing SCs were either disrupted with detergents or their assembly was prevented by blocking complex I + III interactions using reconstituted proteoliposomes at a high lipid/protein ratio; these independent approaches demonstrated that ROS generation by complex I was increased in the absence of SC formation (Fig. 2A) [25].

Although the assembly of individual respiratory complexes in the inner mitochondrial membrane into distinct SCs is established, the factors required for this assembly are unknown. Recently, reports from three independent laboratories have described two related *Saccharomyces cerevisiae* proteins (renamed by the three groups as rcf1 and rcf2) that may be relevant to assembly between complexes III and IV [34–36]. However, the ablation of these proteins impairs overall



**Fig. 2.** Confirmed roles for supercomplexes. (A) Scheme illustrating that detachment of complexes I and III significantly increases ROS production [24]. (B) Plasticity model of mETC organization, showing CI associations with a dedicated CoQ pool coexisting with CIII + CIV associations and free CII, CIII, and CIV. SCAF1 modulates CIV-containing supercomplexes, thereby regulating the proportions of free CIII and CIV and generating three states for CIV [32]. CII represents all the delivery of electrons to the CoQ pool thought FADH<sub>2</sub>. DHODH: dihydroorotate dehydrogenase; G3PDH: glycerol-3-phosphate dehydrogenase; ETF-QO: electron transfer flavoprotein-ubiquinone oxidoreductase; SQR: sulfide CoQ reductase; Choline DH: choline dehydrogenase; Proline DH: proline dehydrogenase.



respiration and growth quality in non-fermentable substrates [34–36]. This could be interpreted as support for the solid model, and therefore as evidence against the fluid and plasticity models. However, these proteins are required for the assembly of the COX3 subunit into mature complex IV, and their ablation thus impairs complex IV assembly [34–36]. Mammalian orthologs have been identified for rcf1 (HIG1A and HIG2A). HIG1A interference does not influence respiratory complexes or SCs [36] and this protein has been ascribed a role in the regulation of mitochondrial  $\gamma$ -secretase function [37]. HIG2A interference induces a very moderate reduction in the respirasome levels but, as occurs in yeast, complex IV assembly is also impaired to the same extent [36]. These proteins should therefore be regarded more as complex IV assembly factors than as SC assembly factors. A true SC chaperone would allow assembly of SCs but not of the individual complexes. The first protein for which these properties have been identified, Cox7a2l, is required for stable interaction between complexes III and IV and has been renamed supercomplex assembly factor I (SCAFI) [32]. Mouse cells expressing mutated Cox7a2l do not build SCs containing complex III + IV or the respirasome, an effect rescued by expression of wild-type Cox7a2l. Screens of mouse strains showed that C57Bl/6J and Balb/cJ mice harbor a mutation in Cox7a2l that renders it unstable, and their mitochondria are consequently unable to build respiratory SCs containing complex IV. Cox7a2l/SCAFI thus defines three populations of complex IV: the fraction assembled with complexes I and III in the respirasome, which can receive electrons only from NADH; the fraction assembled with complex III alone, which receives electrons only from FAD-containing enzymes; and a non-interacting fraction that can receive electrons from any substrate (Fig. 2B). SCAFI modulates the interaction between complexes III and IV without affecting the stability of the individual complexes, thus satisfying the requirement for a *bona fide* SC assembly factor. The plasticity model is substantially validated by the identification of SCAFI as a modulator of CIV assembly into SCs and by the viability of animals whose mitochondria lack SCAFI, and therefore cannot form complex IV-containing SCs [32].

A recent study of mitochondria from *S. cerevisiae* questioned the functional relevance of mitochondrial SCs [38]. However, there are several problems with extrapolating these results to mammalian mitochondria. First, the mtETC of *S. cerevisiae* is very different from that of mammals because it lacks complex I, and the analysis therefore relates only to assemblies containing complexes III and IV. Second, the authors do not present a parallel analysis of the status of interaction between respiratory complexes. For their analysis they poisoned the mtETC with inhibitors of complex III or with carbon monoxide (a complex IV toxin), and performed experiments at low oxygen, even exposing cells to the absence of oxygen. Unfortunately, however, they did not explore the consequences of this rather strong manipulation on the structure and function of the mtETC. Furthermore, they used several genetically modified strains for which they did not experimentally confirm the assumed structure of the mtETC under their growth conditions. The conclusions of this study moreover contradict the results of other studies in *S. cerevisiae* that propose that the mtETC functions as a single unit [39]. Specifically, we showed that substrates for NADH (pyruvate + malate) and FADH<sub>2</sub> (succinate) have an additive effect on respiratory activity when added to mitochondria with SCs containing III + IV, but that this additive behavior is lacking or very reduced in the absence of complex IV plus III superassembly. This additive effect allows mitochondria to optimize the simultaneous use of different carbon substrates whose oxidization generates variable proportions of NADH and FADH<sub>2</sub> [32].

Several attempts have been tried to quantify the proportion of the respiratory SC that is assembled in the respirasomes under defined conditions, combining blue native electrophoresis and in gel histochemical enzymatic activity of the respiratory complexes [40,41]. However, the data available are not accurate enough to enable a proper quantification of these phenomena. In order to achieve quantification; proteomics approach such as iTRAQ [42] would be needed to estimate and determine

precisely SC distribution upon different conditions since mitochondrial SC organization is a dynamic process.

#### 4. SC dynamics and turnover

The demonstration that the structural organization of the mtETC is variable and complex and that differences in this organization are related to differences in function has important implications for our understanding of the regulation of this system. Dynamic superassembly of mtETC complexes allows the cell to adapt to different carbon sources and varying physiological conditions, and greater understanding of these processes promises insight into the implication of the OXPHOS system in human disease.

The growth of yeast with glucose or lactose as the carbon source shows that the dynamics of SC formation varies with cell growth and physiological conditions [8]. In mammalian cell lines, SC assembly is increased by growth in the presence of galactose instead of glucose, thereby forcing the mtETC to work at maximum capacity (authors' unpublished observations). When interpreting results from cultured cells it is important to consider the state of confluence, since this strongly affects mitochondrial energy production and metabolism. Overconfluent enter a pseudo-hypoxic state accompanied by acidification of the media, which compromises oxygen consumption and reduces the maximal respiration rate [43–45]. Under these conditions, mitochondrial SCs undergo reorganization similar to that occurring in hypoxia (personal observations). Study of the control of cytochrome oxidase flux has revealed that the assembly status of SCs is altered in the uncoupled condition [46] and is also affected by both the voltage ( $\Delta\Psi(m)$ ) and the proton ( $\Delta pH(m)$ ) gradient [47]. In the latter study, the same observations were made for NADH dehydrogenase and bc(1) complexes, suggesting a dynamic equilibrium between SCs and individual complexes, and thus supporting the plasticity model [15]. It was recently reported that hypoxia and mitochondrial matrix pH also regulate SC assembly and activity in plants [48]: sustained hypoxia and low pH result in a drop in the activity of SC-assembled complex I in favor of individual complex I activity, a situation reversed upon re-oxygenation.

Many of the most dynamic and rapid cellular processes are mediated by posttranslational modifications. Activation of kinases and phosphatases and their downstream targets occurs over short time frames, enabling the cell to adapt to emerging challenges. Phosphorylation/dephosphorylation, acetylation/deacetylation, and redox alterations can affect SC components, potentially altering SC stability or function. OXPHOS activity is affected by several posttranslational modifications [49–52], but these studies did not address whether SCs were disrupted or stabilized. In the context of heart preconditioning, where mitochondria have been postulated to exert a protective role [53,54], preconditioning has been linked to complex V phosphorylation in ATPase subunit  $\beta$ . In order to model these phosphorylation events and their physiological relevance, the yeast ATPase subunit  $\beta$  has been subjected to series of mutations with nonphosphorylatable and phosphomimetic analogs corresponding to the mammalian sequence [55]. Some of the mutations have an effect on ATPase activity and the formation and stability of the free F<sub>1</sub> component of complex V. Interestingly, phosphorylation of T58 promoted a decrease in the formation and stability of complex V dimers, and thus ATPase activity. This is the first evidence that a posttranslational modification can alter SC levels. Although similar studies could be performed with phosphoproteins from other respiratory complexes, no such analysis has been reported yet.

The mitochondrial network is controlled by a balance between fusion and fission [56–60]. The reorganization of mitochondrial cristae during fusion and fission requires SCs to be relocated in order to maintain proper OXPHOS function. This requires their disassembly and reassembly. Several proteins are implicated in maintaining the cristae junctions tight, including the dynamin-related GTPase protein OPA1 [61,62] and the conserved adaptor Fc1/mitofilin [63]. In a recent

study we defined the relationship between SCs and mitochondrial ultrastructure [64]. Genetic ablation or overexpression of OPA1 revealed that SC assembly stability and function require an intact cristae structure. Induction of apoptosis promotes the rupture of cristae junctions, which releases cytochrome c to the cytosol, thereby destabilizing SCs. This constitutes the first experimental evidence of a direct link between mitochondrial inner membrane organization and SC arrangement, establishing a strong correlation of cristae ultrastructure and the incorporation of functional SCs.

The integrity of mitochondrial membranes and thus SC levels is also determined by the control of mitochondrial quality through the action of proteases or selective elimination by mitophagy. Dissipation of membrane potential by the uncoupler CCCP promotes mitochondrial fragmentation and mitophagy [65,66]. Work by Cogliati et al. [64] showing that cristae remodeling destabilizes SCs, together with our finding that CCCP decreases SC levels, suggests that mitophagy likely reduces SC formation and function; however, as yet no specific studies in this respect have been reported.

Last but not the least, the use by mitochondria of different fuels determines the metabolic switch. We recently showed that starvation, which triggers a preferential use of fatty acid instead of glucose as the OXPHOS fuel, reduces the levels of SC containing complex I. This response results in more free complex III accessible to electrons coming from FADH<sub>2</sub>, revealing a role of mitochondrial SCs in the adaptation to substrate availability [32].

## 5. Physiological relevance of SC function

A considerable body of evidence gathered in recent years points to an involvement of mitochondria in several disease processes and aging [67–78]. Given the fundamental and diverse roles of mitochondria, any malfunction is likely to lead to disease. An immediate effect of impaired mitochondrial function is increased ROS production, which itself affects mtETC activity and creates a vicious cycle that promotes a further decline of mitochondrial fitness. ROS have a strong influence on the maintenance and performance of SCs, and a growing number of studies show that ROS levels are correlated with a decrease in SC assembly and disease.

The vicious cycle of ROS and mitochondrial dysfunction has been extensively studied in models of aging [75,79]. Mitochondrial ROS are emerging as signaling molecules that can have beneficial or detrimental effects, depending on their concentration. In the physiological range, ROS participate in homeostasis as modulators of growth factor signaling [80], activators of uncoupling proteins [81] and regulators of mitochondrial biogenesis [82]. In contrast, above the physiological threshold ROS are pathological causing lipid peroxidation, protein oxidation and mitochondrial DNA damage. Aging-dependent decay of mitochondrial function is directly linked to increased ROS production, but little is known about the molecular mechanisms involved. Recent hypotheses propose that the decline in mitochondrial function with age is related to the decrease in the levels of SCs in heart [83] and brain rat cortex [84]. Contrasting with this explanation, in rat skeletal muscle the highest molecular weight SCs accumulate in older animals, perhaps as a consequence of a molecular mechanism that enhances the catalytic activity of the respirasome by better channeling of fuels and by preventing ROS generation [85].

Since mitochondria produce ATP through the coupling of the mtETC to ATP synthase, tissues with a high-energy demand, such as the highly contractile heart, are deeply dependent on mitochondrial function. Mitochondria are involved in the progression of hypertrophy and heart failure (HF) [86,87], but although it is clear that mitochondrial function is diminished in failing hearts, the restructuring of mitochondrial SCs has not been studied in depth. In a canine model of HF, Rosca et al. found a decrease in state 3 mitochondrial respiration in both subsarcolemmal and interfibrillar mitochondria, accompanied by a reduction in complex-I-containing SCs [88] that was not due to a

modification in the lipid content of the inner mitochondrial membrane [89]. These authors conclude that the destabilization of the SCs is instead due to changes in the phosphorylation of specific complex IV subunits that alter protein–protein interactions or the stability of SCs containing complex IV [89]. Moreover, in Angiotensin II (AngII) models of cardiac insult it has been reported the ROS mediate crosstalk between the cytoskeleton network and mitochondrial function and integrity [90–92]. Although this finding appears to run counter to the detrimental action of ROS on mitochondrial integrity [93], so far no-one has studied HF and SC destabilization in the context of cardiac insult and HF.

In cancer, cell metabolism switches towards glycolysis (the Warburg effect) accompanied by a depression in OXPHOS. The mechanisms underlying this metabolic switch are not well understood. Mitochondrial function is impaired by K-ras expression and activation in a sequence of events. First, mitochondrial membrane potential and oxygen consumption decrease and ROS production is enhanced [94]. As a consequence, complex I content and activity decay and ROS defense mechanisms are depressed [94,95]. This mitochondrial response translates into a switch towards glycolysis, lactate production and apoptosis that leads to tumor formation and proliferation [94]. The possible influence of SC organization on the depression of the OXPHOS system, increased ROS production and tumorigenesis was not proposed until recently [96]. These authors suggest that ROS produced in tumorigenesis would alter SC formation, leading primarily to disruption of complex I assembly and activity and thus promoting a second peak of ROS generation, which would amplify the mitochondrial defect.

## 6. Unsolved questions and concluding remarks

To date, the knowledge on how mitochondrial SCs are organized and its relevance in physiology is increasing with the help of the new methodological approaches as well as genetic models. However there are still several open questions regarding the structural organization of the SCs. One major unresolved question is the degree of interaction between the different CoQ pools, and by the same token, if there is a unique CoQ pool for FADH<sub>2</sub> derived electrons or dedicated pools for different FADH<sub>2</sub> donors. The role of the different SCs containing I + III and how complex IV integration into the different SCs is regulated are still unknown. It is necessary to develop methodologies to accurately estimate the amount of SCs *in vivo*, since the data derive from Blue Native are conditioned by the use of detergents to solubilize the membrane. Equally, the true stoichiometry between complexes when associated in SC requires a more robust proteomic analysis.

Mitochondrial physiology and biogenesis are deeply involved in the initiation and progression of many pathological situations and aging. Production of ROS, altered quality control balance, energy deficiency and decrease in mitochondrial respirasome formation are some of the features controlled by mitochondria that are impaired with aging and disease. Cell fate is determined by the internal crosstalk among signaling pathways that determines decisions about which fuel source to use and even whether to live or die. Mitochondria are key organelles in modulating and switching metabolism to optimize the performance of the cell. Reorganization of SCs in the inner mitochondrial membrane in response to different stimuli, carbon sources or stress conditions is revealing an important and novel adaptive mechanism controlled by mitochondria. These findings suggest exciting and challenging approaches to controlling disease by adjusting SC levels to meet cells' physiological requirements at a given moment.

## Acknowledgements

We thank Simon Bartlett (CNIC) for English editing and Concepción Jiménez for management. This study was supported by grants from the Ministerio de Ciencia e Innovación (SAF2012-32776 & CSD2007-00020); the Comunidad de Madrid (CAM/API1009); and the Marie

Curie Career Integration Grant (UEO/MCA1108). RA-P is an investigator of the Ramon y Cajal research program from the Ministerio de Economía y Competitividad. The CNIC is supported by the Ministerio de Economía y Competitividad and the Pro-CNIC Foundation. All authors declare that they have no competing interests.

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