

Dispatch

## Mitochondria: Cristae-lization of Respiratory Chain Complexes

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For over 100 years mitochondria have been known for their distinctive morphology featuring elaborately folded cristae, and their role as ‘the powerhouse of the cell’. New research shows that these two characteristics are more dependent on each other than previously thought.

Mitochondria are double membrane organelles that generate the bulk of cellular ATP through oxidative phosphorylation. Mitochondria contain a series of discrete subcompartments that house subsets of the ~1000 different proteins that make up this organelle — the outer membrane, intermembrane space, inner membrane and the protein dense matrix. Additional complexity exists with the close apposition between the inner and outer membranes termed the inner boundary membrane, and the folds of the inner membrane making up the cristae. The groups of Scorrano and Enriquez [1] have now revealed a close connection between cristae shape and the activity and assembly of oxidative phosphorylation complexes.

While many textbook depictions show that mitochondrial cristae form regular folds or baffles, electron tomographic imaging has revealed that cristae actually form diverse shapes that are connected to the intermembrane space through small tubular structures termed crista junctions of approximately 30 nm in diameter [2]. Cristae are known to house the respiratory

chain complexes, along with cytochrome *c* and ATP synthase [3] (Figure 1A). Following his invention of blue native PAGE for the separation of membrane protein complexes, Herman Schägger [4] found that respiratory chain complexes assemble into higher ordered supercomplexes. The composition of these complexes varies and is dependent on the relative stoichiometries – predominantly these consist of complex I with a dimer of complex III and a monomer of complex IV, or complex I with the complex III dimer alone (Figure 1B). Complex II is not found in supercomplex form, while complex V can exist in a dimeric form.

While some argued that these complexes were merely post-lysis artefacts, Schägger's findings have stood the test of time and have been supported by a number of additional [discoveries](#), including roles of complex III and IV in stabilizing complex I; a role for the mitochondrial lipid cardiolipin in stabilizing supercomplexes; and the presence of key proteins that stabilize inter-complex contacts between complex III and IV. Schägger [4] postulated that supercomplexes enable efficient substrate channeling and enhance the catalytic activities by reducing diffusion times of mobile substrates coenzyme Q and cytochrome *c*. This has been supported in part by the recent findings that supercomplexes exist in dynamic forms that enable different assemblies to co-exist so that reducing equivalents can be fed into the respiratory chain at different sites [5]. Additional tomographic electron microscopic approaches have revealed that ATP synthase is found at the tips of the cristae and the complex I-containing supercomplexes assemble along the length of the tubules [3].

Using a number of elegant approaches to modulate cristae structure, Cogliati *et al.* [1] found that the shape of cristae is important in regulating the biogenesis of respiratory chain supercomplexes and for maintaining optimal mitochondrial respiration. The formation of cristae is supported by a protein termed optic atrophy 1 (Opa1). Opa1 is found in two forms: a long form that is inner membrane integrated, and shorter soluble forms that oligomerize

with the longer form, presumably supporting the structure of the narrow crista tubules. Opa1 is a member of the dynamin-related protein family of GTPases and is also involved in fusion of the mitochondrial inner membrane. During apoptosis, the mitochondrial outer membrane becomes permeabilized; this can lead to the entry of BH3-only family proteins such as tBID which can disrupt the Opa1 oligomers, resulting in destruction of the crista tubules and thereby liberating the major pool of cytochrome *c* from the cristae into the cytosol [6] (Figure 1C).

In their study, Cogliati *et al.* [1] identified a mutant of tBID that could permeabilize the outer membrane but not accelerate the release of cytochrome *c*. The authors found that this mutant did not result in the disruption of Opa1 complexes, and cristae were not remodeled. In this case the respiratory chain supercomplexes, in particular those containing complex I, were maintained in their active forms. Importantly, mutant tBID was unable to efficiently induce cell death upon its cellular expression. The authors also complemented their studies with elegant mouse models — a conditional Opa1 knockout that was shown to release cytochrome *c* readily and also have impaired respiratory complexes, and a transgenic mouse where cells containing slightly elevated levels of Opa1 had apparent tighter crista tubules, more active respiratory chain enzymes and cells grew faster than control cells in galactose, where there is a mitochondrial dependence for cellular ATP. Interestingly, loss of Opa1 also led to decreased assembly of new supercomplexes indicating that cristae act to organize this pathway of assembly.

A number of important questions still remain to be answered. In particular, Cogliati *et al.* [1] did not address the importance of the newly identified multi-subunit protein complex termed MINOS/MICOS/MitOS that connects the inner boundary membrane and the outer membrane at crista junction sites via interaction partners anchored in both membranes [7]. Disruption of key proteins within this complex can lead to the loss of crista junctions

generating an enclosed compartment, and in yeast, are nevertheless respiratory competent. Furthermore, it is interesting that the authors found that slight upregulation of Opa1 can result in the formation of tighter cristae and more respiratory competent mitochondria. How this is achieved is unknown. Interestingly, Opa1 is also involved in inner membrane fusion and its overexpression can induce mitochondrial fragmentation and impaired mtDNA biogenesis leading to respiratory chain defects.

The report by Cogliati *et al.* [1] extends studies between mitochondrial cristae and mitochondrial bioenergetic function previously made by a number of groups. Notably, Hackenbrock [8] showed changes in the appearance of mitochondrial cristae by modulating different metabolic states, while the group of Kuhlbrandt [9] showed that ATP synthase dimers assemble into long rows at the cristae tips where the membrane is most curved. This led to the proposal that such positioning could enable the formation of a proton sink for optimal ATP synthesis. More recently, Daum *et al.* [10] used a fungal model of ageing to show that mitochondrial cristae lose structure and vesiculate in senescent cultures. The loss of cristae shape correlates to a dissociation of complex V dimer rows into free dimers and then monomers and decreased overall fitness. The loss of cristae during aging was suggested to be a result of accumulating oxidative damage. It will be interesting to determine whether this oxidative damage leads to defects in the assembly of the other respiratory chain supercomplexes, Opa1 homologs and/or other structures that are involved in maintaining the shape of the organelle.

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Figure 1. Interconnectivity between mitochondrial respiration and cristae morphology.

(A) General composition of the mitochondrial respiratory chain. The increased curvature at cristae tips due to alignment of ATP synthase (V) dimers promotes an increased proton density used by the enzyme for efficient ATP production [9]. I, NADH dehydrogenase, complex I; II, succinate dehydrogenase, complex II; III, cytochrome bc<sub>1</sub> complex, complex III; IV, cytochrome c oxidase, complex IV; V, ATP synthase, complex V. (B) Respiratory chain supercomplexes and complexes involved in maintenance of cristae morphology. Double-edged arrows indicate potential interaction partners involved in cristae structure [7]. OM, outer membrane; IM, inner membrane; Cyt. *c*, Cytochrome *c*; Co. Q, Coenzyme Q. (C) Cristae remodeling in apoptosis. BH3-only BCL-2 proteins for example, tBID, BIM-S, BNIP3) trigger Bax/Bak dependent outer membrane permeabilization and disruption of Opa1 oligomers that in-turn results in cristae remodeling and the release of cytochrome *c*.