Linking mitochondrial dynamics, cristae remodeling and supercomplex formation

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Abstract:

The dynamic and fluid nature of mitochondria allows for modifications in mitochondrial shape, connectivity and cristae architecture. The precise balance of mitochondrial dynamics is among the most critical features in the control of mitochondrial function. In the past few years mitochondrial shape has emerged as a key regulatory factor in the determination of the bioenergetic capacity of cells. This is mostly due to the recent discoveries linking changes in cristae organization with supercomplex assembly of the electron transport chain (ETC), also defined as the formation of respirosomes. Here we will review the most current advances demonstrating the impact of mitochondrial dynamics and cristae shape on oxidative metabolism, respiratory efficiency, and redox state. Furthermore, we will discuss the implications of disruptions in mitochondrial dynamics in diseases and during aging.

Mitochondrial Dynamics

Mitochondria are most commonly known for their role in energy production, however mitochondria are also important regulators of many cellular processes, including cell death, ROS generation, calcium homeostasis, and cell signaling (REF). Mitochondrial size, connectivity and ultrastructure is controlled through balanced and dynamic cycles of fission, fusion and cristae modifications, globally defined as mitochondrial dynamics. These changes in morphology are closely associated with mitochondrial function, such as

energy production through ATP synthesis, mitochondrial biogenesis, maintenance of mitochondrial DNA, and regulation of mitophagy and autophagy [1-3] . Since mitochondria play such a key role in several aspects of cell function, balancing mitochondrial dynamics is crucial for cell survival.

The dynamic nature of mitochondrial is controlled by a series of Dynamin-like GTPase proteins the mediate fission and fusion events. The fission of mitochondria begins with the recruitment of the cytosolic Dynamin-related protein 1 (DRP1), the key effector of mitochondrial fission, to mitochondria and association with the mitochondrial fission factor (*Mff*) [3] or it's receptor Fis1 (ref). Translocation of DRP1 to the outer mitochondrial membrane (OMM) is then followed by hydrolysis of GTP allows DRP1-mediated OMM constriction and subsequent fragmentation of mitochondria [4] . In addition, the point of constriction of mitochondria and the precise location to which Drp1 is recruited relies on ER contact sites [26-28] (elaborate here, REF).

Mitochondrial fusion is mediated by three dynamin related large GTPases, known as Mitofusin 1 (MFN1), Mitofusin 2 (MFN2) and Optic atrophy protein 1 (Opa1) [21, 22]. Given that mitochondria are double-membraned organelle, the fusion of mitochondria is a two-step process consisting of inner and outer membrane fusion. During outer membrane fusion, the outer transmembrane fusion proteins MFN1 and MFN2 form homo- and hetero-oligomeric complexes to tether the outer membranes of two mitochondria [21]. Both proteins have been shown to concentrate around the areas of close contact between two mitochondria in order to initiate OMM [5] . OMMs of two adjacent mitochondria become tethered, which is mediated through a heptad repeat

region (HR2) on MFN1. The OMMs are then fused by conformational changes in MFNs, achieved by GTP hydrolysis [6, 7] . MFNs 1 and 2 each have distinct roles in OMM fusion, thus both are required for functional elongation of mitochondria. Loss of either MFN isoform poses unique defects in mitochondrial fusion [5] . For instance, mutations in the GTPase domain in MFN2 induces fragmentation of mitochondria, while MFN1 GTPase mutants are unable to form large tubular networks of mitochondria [5] . Meanwhile, the intermembrane fusion protein OPA1 tethers the inner membranes to direct to direct inner membrane fusion[20]. An important note to be made here, is that in addition to OPA1 being an inner membrane fusion protein, it is also a master regulator of cristae structure and remodeling [22, 23].

The opposing processes of mitochondrial fission and fusion are crucial for many aspects of mitochondrial health and cellular adaptations (REF). However, this directionality must be governed by both a balance and plasticity of mitochondrial dynamics. In light of the importance of mitochondrial dynamics, cases of imbalance or like of adaptive plasticity have been shown to have detrimental effects. For example, mitochondrial fission has been observed to be important for mitochondrial quality control, known as mitopagy, as it acts to remove the damaged sections of mitochondria [8] . In response to oxidative stress, the adenosine monophosphate activated protein kinase (AMPK) pathway is activated, stimulating *Mff* to recruit DRP1 and initiate mitochondrial fission [8] . Although mitochondrial fission is required for maintenance of mitochondrial quality and dynamics, excessive mitochondrial fragmentation has been observed in

a variety of diseases. For instance, a decrease in both mitochondrial length and density has been noted in hereditary spastic paraplegias (HSP), while treatment with the DRP1 inhibitor mdivi-1, or direct knockdown of DRP1 rescues dysfunctional mitochondrial dynamics and improves HSP specific defects, suggesting that impaired mitochondrial dynamics is a pathologic factor in HSPs [9] . Conversely, Chen et al. (2015) demonstrate that mice devoid of *Mff* have increased mitophagy and decreases in mitochondrial density [3] . Importantly, *Mff* deficient mice develop dilated cardiomyopathy, which is fatal around 13 weeks of age. As abnormal mitochondrial fission has been implicated in a variety of conditions, and thus targeting DRP1 and other mitochondrial fission-related proteins may be promising for therapeutic treatments of diseases and aging.

Mitochondrial fusion serves as an adaptive response to cellular stress. It has been observed that upon oxidative stress induced by the presence of oxidized gluathione, MFN2 mediates mitochondrial elongation and hyperfusion to maintain ATP production and thus may to serve to protect against mitophagy [10] . A link between MFN1 mediated mitochondrial fusion, glucose sensing and insulin release has also been identified in POMC neurons [11] . Loss of MFN1 in POMC neurons debilitates the flexibility of mitochondrial dynamics, thus interfering with proper glucose metabolism and altering control of insulin release [11] . Mitochondrial fusion is also an important process for substrate transfer between mitochondria and a variety of other organelles including the endoplasmic reticulum, lysosomes, and peroxisomes [12] . The discernable role of mitochondrial fusion in metabolism, mitochondrial

biogenesis, and mitophagy demonstrates the importance of mitochondrial dynamics in maintaining healthy cells. Thus, it is unsurprising that there are many diseases arising from defects in the mitochondrial fusion machinery. For example, loss of essential fusion proteins severely impacts the maintenance of tissues and leads to severe neurodegeneration and muscle atrophy [2, 13, 14] . Mutation of MFN2 causes Charcot-Marie-Tooth (CMT2) disease characterized by severe neurological defects including physical weakness and sensory loss [1, 15] . It has also been noted in severe preeclampsia there is a large downregulation of the mitochondrial fusion genes [16] .

The plasticity of mitochondrial dynamics largely allows for the proper regulation of post-mitotic cell processes, while an imbalance in mitochondrial dynamics to favour fission or fusion is threatening to cell and tissue homeostasis. Although fission and fusion are crucial operations individually, the ability of mitochondria to adjust their morphology to adapt to external stimuli is what is crucial for cell stability. Recently, Song et al. (2017) demonstrated that mitochondrial adynamism, meaning the absence of any functioning fission and fusion processes is less detrimental to cardiomyocytes than ablation of either fission or fusion [2] . Loss of the mitochondrial fission or fusion machinery causes acute defects including cardiac hypertrophy in MFN2 knockouts, and mitochondrial enlargement and accelerated mitophagy in DRP1 knockouts, whereas MFN1/MFN2/DRP1 triple knockout (adynamic) hearts survive much longer, but develop accelerated mitochondrial senescence leading to premature cardiomyopathy consistent

with aging [2] . This knowledge may provide insight into new therapeutic methods that target balancing mitochondrial dynamics, rather than selecting for fission and fusion in certain circumstances.

Cristae remodeling

In addition to mitochondrial outer membrane fusion, the inner mitochondrial membrane can undergo structural modifications to regulate cell processes. The inner mitochondrial membrane is made up of two sections, the inner boundary membrane and the cristae [17-19] . Cristae are defined as narrow invaginations formed from the inner mitochondrial membrane. The inner boundary membrane and the cristae are connected by the cristae junctions, which are formed and stabilized by the mitochondrial contact site and cristae organizing system (MICOS) complex [18, 20] . Cristae junctions are important barriers that allow the selective entrance and release of essential metabolites and proteins [21] . Cristae are vital to the function of mitochondria, as the machinery for the mitochondrial respiration, including the electron transport chain complexes are housed within the large surface area of the cristae [18, 21] . ATP-synthase, which is located at the apex of the cristae helps to define the curvature of the cristae due to its “ V”-like structure [18, 22] .

Structural modifications to cristae, including changes in length, width, and tightness or openness of the cristae and cristae junctions, can occur with changes in energy substrate availability, or other stresses in the cell that require mitochondrial adaptation [21] . Notably, it has been observed that mitochondrial cristae tighten upon starvation. This allows for the assembly of

a functional ATP synthase monomer, thus promoting energy production via ATP-synthase linked respiration [19] . These adjustments to cristae structure, known as cristae remodelling can provide an adaptive and protective response to changes in cellular demands, and can also act to induce apoptosis when cellular stresses become detrimental [21] .

A vital protein in regulating cristae remodeling is Opa1. Opa1 independently functions as an inner membrane fusion protein and a modifier of cristae shape [23] . Opa1 situates at the cristae junctions in order to control cristae widening or constriction [21] . In a physiological setting, Opa1 acts to maintain closed cristae junctions in order to control release of pro-apoptotic proteins, including cytochrome c. Importantly, Opa1 is required to stabilize cristae structure for proper mitochondrial respiration efficiency and reactive oxygen species production [24] . Cells lacking Opa1 not only show highly fragmented mitochondria, but also reveal excessively widened and dysfunctional cristae, increasing cell susceptibility to apoptosis [23, 25, 26] . Conversely, controlled upregulation of Opa1 in mice is highly protective against muscular atrophy, ischemic damage in the heart and brain, and hepatocyte apoptosis by preventing mitochondrial dysfunction and pro-apoptotic signals induced by release of cytochrome c [24] . Recently, a role for ATPase inhibitory factor 1 (IF1) in prevention of apoptosis via Opa1-mediated cristae stabilization has been discovered. Increased IF1 activity has been shown to restrict the activity of OMA1, a stress-activated metalloprotease responsible for degradation of the long isoform of Opa1 (L-Opa1), thus preserving the function of Opa1, indicating a pro-survival function of IF1 [27] . This implicates

Opa1-maintained cristae structure as a central regulator of cellular health and life sustainability.

Cristae remodelling is also implemented when stress levels are detrimental to the cell. Upon necessary stress stimuli, mitochondrial fragmentation and cristae destabilization can facilitate the release of cytochrome c, initiating a pro-apoptotic cascade [21, 28] . One potential mechanism allowing for cristae destabilization and cytochrome c release has been proposed by Jiang et al (2014). This mechanism involves stress-induced activation of OMA1. OMA1 executes the processing of L-Opa1 to short isoforms of Opa1, disengaging cristae junctions to induce cytochrome c release [26] . Thus, Opa1 plays a key adaptive role in a tightly controlled mechanism regulating the release of pro-apoptotic factors in response to cellular stresses.

The interplay of mitochondrial dynamics, Cristae remodeling and Supercomplex assembly

Mitochondrial dynamics and cristae remodeling are important aspects of in determining cell fate, in terms of cell death. However, there has always been an aspect of enhanced cell survival and mitochondrial function that had been associated with mitochondrial fusion, especially when this is a response to cell stress stimuli. The equilibrium of fission and fusion events has been consistently associated with regulation of metabolism [10-12]. Though, it was unclear as to how mitochondrial shape can affect bioenergetic output in terms of ATP generation. Recently, however, a direct role for OPA1 in the regulation of mitochondrial respiratory efficiency through it’s ability to modify cristae shape and ETC supercomplexes [17].

Recent studies in the field now reveal that mitochondrial dynamics are directly involved in the energetic output of mitochondria [13-17].

In these cases,

Recently, however, a direct role for OPA1 in the regulation of mitochondrial respiratory efficiency has been established [17]. OPA1-dependent modulation of cristae shape regulates the assembly of the ETC into supercomplexes for maintaining optimal mitochondrial respiration and cell growth [17]. Disruption of cristae shape by acute loss of OPA1 was sufficient to disrupt supercomplex assembly and respiratory capacity prior to any impairment of mtDNA copy number. Meanwhile, a slight elevation in the levels of OPA1 led to tighter cristae, increased activity of respiratory enzymes and enhanced the respiratory efficiency of mitochondria [17]. This provides the first evidence that mitochondrial dynamics, cristae structure and respiratory function are interconnected.

Dynamic changes in mitochondrial architecture have a major impact on the ability of cells to

survive stress and modify their energy production. Our recent work demonstrates that mitochondria can respond to different environmental cues to modify their energetic output and promote cell survival [16, 34]. In essence, the state of the mitochondrial network and cristae organization can be reconfigured to reflect the metabolic demand of a cell under stress conditions [98-100]. OPA1 can respond rapidly to changes in nutrient levels in cycling cells to regulate cristae structure [16]. By forming oligomeric interactions, OPA1 can regulate cristae tightness to enhance the stability of

ETC complexes, drive the formation of supercomplexes and increase assembly of the ATP synthase to maintain mitochondrial respiration during cellular starvation (Figure 2). Importantly, in the absence of OPA1 cells were no longer resistant to starvation-induced cell death. Our recent work shows that mitochondrial elongation and cristae remodeling in neurons, by physiological cues during ischemic conditions, can go so far as to maintain efficient mitochondrial ATP production even amid conditions that cannot support this process, such as severe hypoxia [34].

The remodeling of mitochondria under such conditions can instigate a systemic reconfiguration of mitochondrial efficiency to extract more ATP per oxygen molecule, by driving the supercomplex assembly of ETC complexes and ramping up the respiratory reserve capacity. In doing so, mitochondrial structure can dictate the bioenergetic status of neurons and allows sustained ATP levels without the need for glycolysis. This study provides an explanation into the protective effect of mitochondrial fusion that goes beyond the inhibition of apoptotic signaling. Furthermore, it places the changes in mitochondrial architecture as a regulatory mechanism for the bioenergetic adaptation to metabolic demand that can quickly endorse the fate of cells.

Mitochondrial dynamics, Cristae remodeling and ROS production in stem cells

It is evident that mitochondrial dynamics is important for mitochondrial metabolism, mitochondrial biogenesis, and maintaining cell and mitochondrial quality through apoptosis and mitophagy. Throughout this

review the role of mitochondrial dynamics, cristae remodeling and supercomplex formation in somatic cell homeostasis has been discussed. Although these mitochondrial processes are also essential for stem cell vitality, they are additionally required for stem cell migration and differentiation.

In adult stem cells, mitochondria appear to be more fragmented than their progeny. Increased mitochondrial fragmentation and autophagy likely aid in the removal of older and damaged mitochondria, maintaining mitochondrial quality [29] . Indeed, increased mitochondrial fragmentation is observed in numerous types of stem cells when compared to differentiated cells [30] .

It is well established that regulated mitochondrial fission and fusion are required for proper stem cell function. Notably, mitochondrial fission has been implicated as an important process for stem cell migration, a necessary function of stem cells for tissue repair. DRP1-mediated mitochondrial fragmentation is a necessary action upon stem cell activation to ensure the equal distribution of mitochondria during mitotic division [31] . Recently, it has been demonstrated that succinate, a tricarboxylic acid (TCA) cycle metabolite, can induce phosphorylation of DRP1 through p38 MAPK, generating fragmented mitochondria. In a physiological state, succinate promotes mitochondrial fission and increases mitochondrial respiration, thus enhancing ATP production, and mitochondrial reactive oxygen species (ROS) output [31] . Interestingly, in a wound-healing model, increased ROS promoted mesenchymal stem cell migration by increasing F-actin production [31] . This model indicates a compelling role for mitochondrial fragmentation in stem

cell function, including migration and proliferation during necessary tissue repair.

Furthermore, mitochondrial dynamics and cristae structure are important regulators of stem cell fate decisions by governing necessary changes in metabolism and mitochondrial reactive oxygen species production. One example of this is the control of T cell fate through changes in mitochondrial structure and tightly organized cristae [32] . Memory T cells lacking Opa1 exhibit a broader cristae organization and a less efficient electron transport chain (ETC), potentially due to increased separation between ETC complexes (Buck et al., 2016). Inefficient electron flow through ETC complexes can lead to increased mitochondrial ROS, which has been implicated as a signaling molecule that drives stem cell commitment and differentiation [32-35] . Thus, Opa1-deficient T cells exhibiting widened cristae produce excess ROS, debilitating T cells to maintain their quiescence [32] .

Moreover, the role of mitochondrial dynamics as an upstream regulator of stem cell fate decisions has been thoroughly characterized in neural stem cells. Promoting mitochondrial fragmentation through acute loss of MFN1/2 or Opa1 drives neural stem cell commitment at the expense of self-renewal, leading to premature depletion of the stem cell pool [33] . Notably, induced mitochondrial fragmentation, through a ROS-mediated signaling mechanism can activate an NRF2-dependent nuclear transcription program that alters the expression of self-renewal and commitment genes, thus driving differentiation [33] . Therefore, strong evidence implicates mitochondrial dynamics and regulation of cristae structure in the maintenance of stem cell

identity, and that this is through control of metabolism and reactive oxygen species production.

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