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Review

Proteolytic control of mitochondrial function and morphogenesis ☆

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ABSTRACT

Mitochondrial proteostasis depends on a hierarchical system of tightly controlled quality surveillance mechanisms. Proteases within mitochondria take center stage in this network. They eliminate misfolded and damaged proteins and ensure the biogenesis and morphogenesis of mitochondria by processing or degrading short-lived regulatory proteins. Mitochondrial gene expression, the mitochondrial phospholipid metabolism and the fusion of mitochondrial membranes are under proteolytic control. Furthermore, in response to stress and mitochondrial dysfunction, proteolysis inhibits fusion and facilitates mitophagy and apoptosis. Defining these versatile activities of mitochondrial proteases will be pivotal for understanding the pathogenesis of various neurodegenerative disorders associated with defective mitochondria-associated proteolysis. This article is part of a Special Issue entitled: Mitochondrial dynamics and physiology.

1. Introduction

Mitochondria are essential organelles housing the respiratory chain complexes, which generate most of the cellular energy. They are the sites of many biosynthetic events, buffer cellular calcium and play an integral role in numerous cellular signaling pathways including programmed cell death [1–4]. Dysfunction of mitochondria is detrimental to cellular viability, has been linked to many diseases, and is associated with aging [5,6].

Recent proteomics and bioinformatics approaches defined a comprehensive inventory of mitochondrial proteins [7,8]. Numerous cellular pathways are emerging that maintain the mitochondrial proteome, ensuring a healthy and functional mitochondrial network. These pathways counteract the many challenges that mitochondria face and that may influence their well-being. The first challenge arises from the unique property of the organelle in that their proteome is encoded by two distinct genomes. While the vast majority of mitochondrial proteins are encoded by nuclear genes and synthesized in the cytosol, mitochondria also harbor their own genome, encoding for 13 polypeptides in mammalian cells (8 in yeast). All mitochondrial-encoded proteins constitute core components of the respiratory chain complexes. In order to achieve efficient respiratory chain complex assembly, it is imperative that there is coordinated expression and import of nuclear-encoded proteins to assemble with the mitochondrial-encoded proteins. Failure to coordinate this process may result in unpartnered proteins that are prone to misfolding or aggregation. The second challenge imposed upon mitochondria arises due to production of reactive oxygen species (ROS), which is an inevitable byproduct of the generation of ATP through oxidative phosphorylation. ROS can react with proteins, DNA and lipids resulting in accumulation of oxidatively damaged products. Hence, mitochondrial proteins, lipids and DNA are more prone to oxidative damage, which can lead to depolarization of the mitochondrial inner membrane. This may ultimately trigger the permeabilization of the outer membrane, the release of pro-apoptotic proteins from the intermembrane space into the cytosol and the initiation of the apoptotic cascade [9,10].

2. Mitochondrial quality control at a glance

In order to maintain a functional mitochondrial network, elaborate quality control mechanisms have evolved that remove damaged proteins or sequester and remove damaged organelles (Fig. 1) [11–15]. Impaired quality control of mitochondria is detrimental to cell health and has been linked to aging and various diseases, including prevalent neurological disorders like Parkinson's disease and spinocerebellar ataxia, highlighting the relevance of these defense pathways for cellular homeostasis and survival [16].

Mitochondrial proteases and the cytosolic ubiquitin–proteasome system (UPS) comprise the first line of cellular defense by facilitating the removal of damaged, oxidized or misfolded mitochondrial proteins (Figs. 1A and 2). Two membrane-bound AAA protease complexes conduct quality control surveillance across the mitochondrial

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inner membrane [17]. The *m*-AAA protease exposes its catalytic domain towards the matrix and the *i*-AAA protease towards the intermembrane space. Additional peptidases, such as the metallopeptidase OMA1, extend the capacity of this proteolytic system and contribute to the quality control of inner membrane proteins [18,19]. The Lon protease (Pim1 in yeast) eliminates denatured or oxidatively damaged proteins from the mitochondrial matrix [20–22]. Substrates of another matrix-localized peptidase, the ClpXP protease, remain to be identified. ClpXP from *Caenorhabditis elegans* is involved in the mitochondrial unfolded protein response (mtUPR), a compartment-specific stress response, regulating the levels of mitochondrial proteases and molecular chaperones to accommodate the unfolded protein load [23–25]. In addition to proteases localized within mitochondria, it is becoming clear that the cytosolic UPS also contributes to mitochondrial quality control (Fig. 2). Mitochondrial proteins that become mistargeted or misfolded *en route* to mitochondria are recognized and removed by the UPS [26,27]. Moreover, the UPS degrades proteins residing in the outer membrane of mitochondria in a process resembling ER-associated protein degradation (ERAD) and is therefore referred to as OMMAD (Fig. 2) (for outer mitochondrial membrane-associated degradation) [26,28].

Mitochondria are highly dynamic organelles, constantly undergoing fission and fusion events. This property of mitochondria offers a second line of defense against mitochondrial dysfunction (Fig. 1B) [29,30]. Mitochondrial fusion allows content mixing helping organelles deficient in certain components to replenish their stores (Fig. 1Bii) [31–33]. Consistently, mtDNA mutations accumulate upon inhibition of fusion, finally triggering the loss of the mitochondrial genome [32]. Moreover, the mitochondrial network becomes hyper-fused under certain stress conditions, for instance upon starvation, following inhibition of autophagy or protein synthesis and under oxidative stress conditions (Fig. 1Bii) [34–36]. This protects mitochondria against autophagy and increases cellular ATP production [35,36]. However, severe mitochondrial damage can trigger loss of the membrane potential across the mitochondrial inner membrane, which impairs mitochondrial fusion. Ongoing fission events then result in fragmentation of the mitochondrial network, a prerequisite for the mitochondria-specific form of autophagy [37], termed mitophagy [38], which represents another important form of mitochondrial quality control (Fig. 1Bi). Therefore, the fragmentation of mitochondria facilitates the segregation of damaged organelles from the healthy network and their removal by mitophagy. Moreover, fragmentation of the mitochondrial network precedes programmed cell death [28,39,40]. Although the precise reason that mitochondria fragment during apoptosis remains undefined, perhaps this facilitates the release of pro-apoptotic components ensuring turnover of cells unable to cope with high levels of mitochondrial dysfunction.

Recently, mitochondrial-derived vesicles (MDVs) were reported to facilitate mitochondrial quality control by delivery of selective mitochondrial cargo to lysosomes (Fig. 1Biii) [41–43]. These MDVs are generated as an early response to oxidative stress [41]. Their formation and delivery to lysosomes does not require mitochondrial depolarization or Drp1-mediated mitochondrial fission and is independent of the general autophagy machinery [41]. These findings suggest a quality control route parallel to mitophagy for the selective degradation of mitochondrial proteins in lysosomes.

While the complexity of mitochondrial quality control mechanisms is unfolding, it is becoming increasingly clear that mitochondrial proteases take center stage in these pathways. In addition to their conventional role in degrading damaged or misfolded mitochondrial proteins, many proteases have additional functions as processing peptidases and control the stability of proteins that regulate crucial steps during mitochondrial biogenesis, morphogenesis and turnover (Fig. 3). In this review, we describe how proteolysis can influence various mitochondrial functions and can impinge on mitochondrial quality control.

3. Proteolytic control of mitochondrial biogenesis

3.1. Lon protease regulates mitochondrial transcription

Lon is an ATP-dependent serine protease found in the mitochondrial matrix that mediates the degradation of misfolded and oxidatively damaged proteins [20–22]. It has also been recently illustrated that Lon plays a pivotal role in the regulation of mitochondrial gene expression [44,45]. In yeast, Lon/Pim1 is required for the expression of intron-containing genes encoded by mitochondrial DNA (mtDNA), including cytochrome *c* oxidase subunit 1 and cytochrome *b* [46]. In *Drosophila* cells, depletion of Lon increases the protein levels of mitochondrial transcription factor A (TFAM) and mtDNA copy number, resulting in enhanced transcription of mtDNA-encoded genes [44,47]. When the

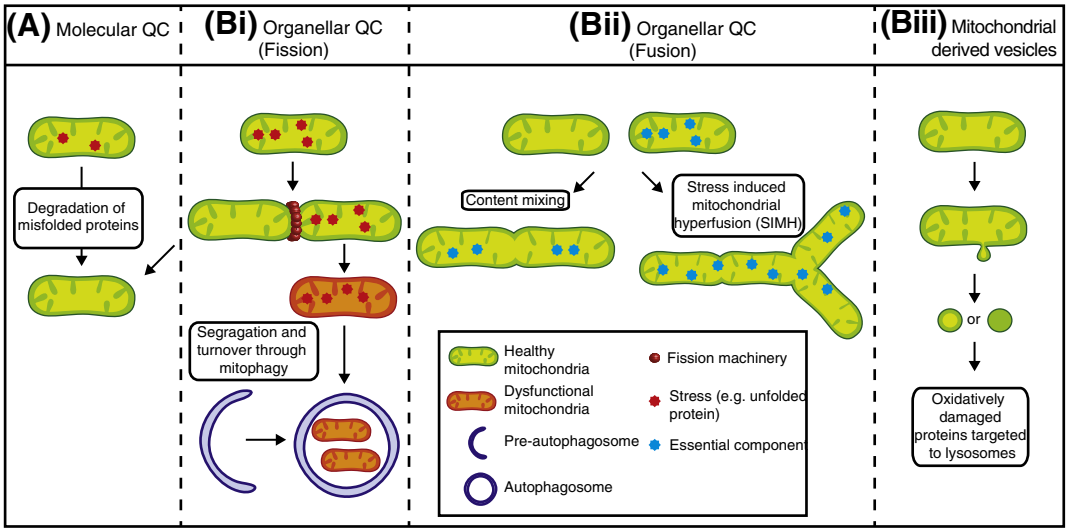

Fig. 1. Mechanisms of mitochondrial quality control. (A) Mitochondrial proteases and chaperones localized in different subcompartments of mitochondria degrade misfolded and damaged proteins. (B) Fusion and fission of mitochondria contribute to mitochondrial quality surveillance. (Bi) Mitochondrial dysfunction inhibits fusion and ongoing fission events segregate non-functional mitochondria from the healthy mitochondrial network. During mitophagy, autophagosomal membranes encapsulate dysfunctional mitochondria and fuse subsequently with lysosomes. (Bii) Mitochondrial fusion serves as a pro-survival mechanism. Content mixing allows complementing functional deficiencies within the mitochondria network. Stress conditions induce fusion (stress-induced mitochondrial hyperfusion; SIMH), resulting in elongation of mitochondrial tubules and protection against mitophagy. (Biii) Various populations of mitochondrial-derived vesicles (MDVs) containing selective cargo are budded from mitochondria during oxidative stress and targeted to lysosomes for degradation. QC, quality control.