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Review

Mitochondrial dismissal in mammals, from protein degradation to mitophagy☆,☆☆

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ABSTRACT

Mitochondria are double-membraned highly dynamic organelles; the shape, location and function of which are determined by a constant balance between opposing fusion and fission events. A fine modulation of mitochondrial structure is crucial for their correct functionality and for many physiological cell processes, the status of these organelles, being thus a key aspect in a cell's fate. Indeed, the homeostasis of mitochondria needs to be highly regulated for the above mentioned reasons, and since a) they are the major source of energy; b) they participate in various signaling pathways; albeit at the same time c) they are also the major source of reactive oxygen species (ROS, the main damaging detrimental players for all cell components). Elaborate mechanisms of mitochondrial quality control have evolved for maintaining a functional mitochondrial network and avoiding cell damage. The first mechanism is the removal of damaged mitochondrial proteins within the organelle via chaperones and protease; the second is the cytosolic ubiquitin–proteasome system (UPS), able to eliminate proteins embedded in the outer mitochondrial membrane; the third is the removal of the entire mitochondria through mitophagy, in the case of extensive organelle damage and dysfunction. In this review, we provide an overview of these mitochondria stability and quality control mechanisms, highlighting mitophagy, and emphasizing the central role of mitochondrial dynamics in this context. This article is part of a Special Issue entitled: Dynamic and ultrastructure of bioenergetic membranes and their components.

1. Introduction

Mitochondria are subcellular organelles crucial for the life of the cell. They are the main energy converters, and are essential components of various signaling pathways. They are sensors of metabolic homeostasis, and regulate the levels of intracellular signaling molecules, such as Ca^{2+}. Ca^{2+} is buffered in micro-domains at the ER proximity in order to modulate cytoplasmic signaling, and to support efficient oxidative phosphorylation and ATP production in the mitochondrial matrix [1]. On the other hand, mitochondria can be detrimental for the cell, being the major source of reactive oxygen species (ROS), which may oxidize and damage proteins, lipids and DNA [2], all of these becoming totally dysfunctional and dangerous for the cell life.

For all these reasons, mitochondria homeostasis needs to be highly regulated. Elaborate mechanisms of mitochondrial quality control have evolved to maintain a functional mitochondrial network and avoid cell damage. The crucial role of these defense pathways for cellular homeostasis and survival is supported by the fact that mitochondrial dysfunction is related to aging, cancer and a wide range of neurological pathologies [3–5]. The cell protects itself by removing or isolating what is damaged, from mitochondria-located proteins to the same organelles [6,7]. To this purpose, the cell accomplishes two main strategies. The first one is used to remove misfolded, denatured or oxidized proteins. This strategy is based both on the activity of mitochondrial proteases that remove proteins resident in the mitochondrial milieu [8,9], and on the activity of the cytosolic ubiquitin–proteasome system (UPS), which in turn recognizes and removes mistargeted or misfolded mitochondrial proteins before they reach the organelle, and mediates the degradation of proteins embedded in the outer mitochondrial membrane (OMM) [10,11].

The second strategy is based on the intrinsic characteristic of mitochondria being highly dynamic organelles, continually fusing and fragmenting in a strictly regulated manner [12,13], and in response to different physiological needs of the cell [14]. In this second defensive strategy, entire parts of the same organelles are removed. Thus,

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based on steric principles, damaged mitochondria are tilted toward a fragmented phenotype, so as to be more disposed to segregation and removal [15,16], while healthy or highly active mitochondria tend to fuse among themselves in order to favor the replacement of essential components, as well as maintaining the mitochondrial genome in the network [17–19]. The main mechanism used by the cell to remove damaged organelles and proteins is autophagy. The term autophagy encloses any processes regarding cytosolic component degradation via lysosomes. As we will see in more detail later, the process termed macroautophagy is generally responsible for the engulfment and removal of cytosolic components. However, a more specific autophagy pathway comes into play in conditions of severe mitochondrial dysfunction, this to selectively remove damaged mitochondria, hence the term 'mitophagy'.

Albeit beyond the scope of this review, we should also mention a third level of quality control, which occurs at a cellular level. It takes place when extensive mitochondrial damage promotes release of pro-apoptotic factors, so resulting in the cell's suicide through apoptosis [20].

In this review, we provide an overview of the mentioned mechanisms of mitochondria stability and quality control, focusing on the relationships existing between these processes (UPS and autophagy) and mitochondria dynamics, and on mitophagy, the latter being the principal process controlling mitochondria homeostasis. Considering the complexity of the subjects and the impressive amount of data present in the literature, we will give particular emphasis to recent findings in the field, focusing on mammals.

2. Outlines on mitochondrial dynamics

As mentioned, mitochondria are highly dynamic organelles, their shape, location and function being defined by a constant balance and equilibrium between opposing fusion and fission events. These dynamic processes are crucial in many physiological and pathophysiological cellular conditions, the modulation of the same, being pivotal for determining cell life and death [14]. Thus, mitochondria morphology needs to be strictly regulated by a set of 'mitochondria-shaping' proteins, the number of which is constantly increasing. The machineries involved in mitochondrial fusion and fission are highly complex; we will only summarize them briefly here: For further extensive reading see other reviews (i.e. [12,21,22]). In mammals, mitofusin 1 (Mfn1) and 2 (Mfn2) together with OPA1 are required for the fusion of the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM), respectively. Mfns are integrated in the OMM and form homo- and hetero-oligomers, which promote tethering and fusion of the OMMs from two different mitochondria [23,24]. The pleiotropic protein OPA1 localizes in complexes at the IMM, facing the inter membrane space (IMS), and drives fusion on the IMM. OPA1 complexes are composed of post-translational proteolytically cleaved short and long forms, deriving from the several splicing variants existing [25]. Fusion, along with the anti-apoptotic role that OPA1 exerts on the mitochondrial cristae structures [26], requires both the short and long forms of OPA1 [27].

Mitochondrial fission depends on the GTPase cytosolic dynamin-related protein 1 (Drp1), which is located in the cytosol and needs to be activated and to translocate to mitochondria in order to constrict and cut the organelles [28]. Post-translational modifications, such as phosphorylation, SUMOylation and ubiquitylation modulate Drp1 activity so as to ensure adaptation to the various cellular needs [21,29,30]. Furthermore, the OMM protein hFis1 has been proposed as a Drp1 receptor, being so necessary for mitochondrial fission. hFis1 mechanism of action is still highly controversial [31,32]; on the other hand, new possible Drp1 receptors have been recently identified: Mff (the OMM-anchored mitochondrial fission factor, [32]), and human MIEF1/MiD51 (the OMM-bound mitochondrial elongation factor1/mitochondrial dynamics 51) with its variant MiD49 [33,34]. The latter were reported to directly and specifically recruit Drp1 in a Fis1-independent manner, but with opposite effects on the mitochondrial morphology, depending on the protein levels [33–35].

Changes in mitochondria morphology influence crucial physiological functions in the cells, such as Ca^{2+} signaling, generation of reactive oxygen species, neuronal plasticity and lymphocyte migration [14]. Moreover, the ultrastructure and shape of the organelles have also been linked to pathophysiological aspects, from muscle atrophy to lifespan determination, and to apoptosis [14], and are affected in several human genetic diseases [22] (see Table 1).

The remarkable structural dynamism of mitochondria has a particular bearing upon the mitochondrial quality control system. The strict interconnection between mitochondria dynamics and mitochondrial quality control, detectable at different levels, will be discussed in the following paragraphs.

3. Mitochondrial proteostasis: the degradation of mitochondrial proteins

3.1. Mitochondrial proteases and removal of proteins within the organelle

The mechanisms developed by mitochondria to maintain their homeostasis are numerous (see Fig. 1). The first line of defense is located in the organelles and acts at a molecular level in conditions of *mild* mitochondrial damage. It consists of several proteases and chaperones operating within the mitochondrion [36].

The majority of the mitochondrial proteins are synthesized in the cytosol and subsequently imported into the organelle [37]. The unwanted but probable interaction and aggregation among proteins entering the organelle in a relatively unfolded state are prevented by a group of chaperones, members of the heat shock family [38].

A highly conserved group of proteases is responsible for the removal of unfolded or damaged proteins within the mitochondria. For example, in concert with chaperones, the AAA^{+} (*A*TPase *a*ssociated with a wide variety of cellular *a*ctivities) soluble hLon protease family removes denatured or oxidatively mildly damaged proteins [39,40]. A second soluble protease group belongs to the bacterial Clp protein family (*c*aseinolytic *p*rotease [41]), mitochondrially represented by mtClpXP (chaperone-protease complex), the activity of which is still undefined. The inner mitochondrial membrane (IMM) is highly enriched in proteins, which are more susceptible to oxidative damage due to their proximity

Table 1
List of the main physiological and/or patho-physiological processes, in which mitochondria dynamics play a crucial role. The main organelle phenotype is shown, together with a selected number of correlated references.

Patho-/physiological process	Mitochondrial phenotype	References
Ca^{2+} signaling	Variable depending on the pathophysiological condition	Szabadkai et al. [155]
Generation of reactive oxygen species (ROS)	Fragmentation	Yu et al. [156]
Maintenance of dendritic spines and neuronal plasticity	Fragmentation	Li et al. [157]
Lymphocyte migration	Fragmentation and relocalization	Campello et al. [158]
Metastatic cell migration	Fragmentation and relocalization	Zhao et al. [159]
Muscle atrophy	Fragmentation	Romanello et al. [160]
Lifespan	Fragmentation (in aging)	Scheckhuber et al. [161]
Apoptosis	Fragmentation and cristae-remodeling	Scorrano [162]
Neurodegenerative diseases (AD, PD, HD, ADOA…)	Mostly fragmentation, in some cases: cristae disruption, impaired trafficking, reduction in number	Corrado et al. [22]; Cho et al. [5]
Cancer	Fragmentation	Grandemange et al. [163]; Qian et al. [164]; Corrado et al. [22]
Cardiovascular disease	Mainly fragmentation	Ong et al. [165]; Piquereau et al. [166]