Mitochondrial quality control pathways as determinants of metabolic health

*Ntsiki M. Held and Riekelt H. Houtkooper**

Mitochondrial function is key for maintaining cellular health, while mitochondrial failure is associated with various pathologies, including inherited metabolic disorders and age-related diseases. In order to maintain mitochondrial quality, several pathways of mitochondrial quality control have evolved. These systems monitor mitochondrial integrity through antioxidants, DNA repair systems, and chaperones and proteases involved in the mitochondrial unfolded protein response. Additional regulation of mitochondrial function involves dynamic exchange of components through mitochondrial fusion and fission. Sustained stress induces a selective autophagy – termed mitophagy – and ultimately leads to apoptosis. Together, these systems form a network that acts on the molecular, organellar, and cellular level. In this review, we highlight how these systems are regulated in an integrated context- and time-dependent network of mitochondrial quality control that is implicated in healthy aging.

Keywords:
- fission; fusion; mitochondrial dynamics; mitochondrial quality control; mitohormesis; mitophagy; ROS

*Corresponding author:
Riekelt H. Houtkooper
E-mail: r.h.houtkooper@amc.nl

Abbreviations:
ER, endoplasmic reticulum; **ETC,** electron transport chain; **IMM,** inner mitochondrial membrane; **mtDNA,** mitochondrial DNA; **nDNA,** nuclear DNA; **OMM,** outer mitochondrial membrane; **OXPHOS,** oxidative phosphorylation; **ROS,** reactive oxygen species; **UPR^mt,** mitochondrial unfolded protein response; **UPS,** ubiquitin-proteasome system.

Introduction

Mitochondria are double membrane-enclosed organelles that execute many metabolic functions, including ATP generation through oxidative phosphorylation (OXPHOS). Next to their role as energy suppliers, mitochondria are also involved in synthesis of biomolecules, maintenance of calcium homeostasis, production of reactive oxygen species (ROS), and apoptosis [1]. Mitochondria are unique organelles, in that they contain their own circular DNA (mtDNA) and transcription/translation machinery. They owe this characteristic to their endosymbiotic origin, having evolved from *Alphaproteobacteria* [2]. As a result, the human mtDNA encodes for only ~1% of mitochondrial proteins (mtDNA contains 13 protein-coding genes) while approximately 1,200 nuclear DNA (nDNA)-encoded mitochondrial proteins are synthesized in the cytosol and must be imported into the mitochondria [3]. A challenging consequence that arose from its endosymbiotic origin is the assembly of large multi-subunit OXPHOS complexes in the inner mitochondrial membrane (IMM), which require the import of nDNA-encoded proteins and coordinated expression and integration of mtDNA-encoded OXPHOS subunits [1]. Several systems of mitochondrial quality control have evolved at the organellar and cellular level to ensure the proper maintenance and, when necessary, repair of mitochondria [4, 5]. Mitochondrial quality control includes antioxidants to detoxify ROS, and chaperones, proteases, and the ubiquitin-proteasome system (UPS) to maintain mitochondrial proteostasis. The dynamic alteration of mitochondrial morphology through fusion and fission events allows exchange of mitochondrial content, and segregation of terminally damaged mitochondria to enable degradation by selective autophagy called mitophagy [6]. Ultimately, extensive mitochondrial damage can induce apoptosis via different pathways, for instance through the release of cytochrome *c*.

The significance of maintaining mitochondrial integrity is underscored by various diseases associated with mitochondrial dysfunction. These include inherited mitochondrial diseases caused by mutations in mtDNA or nDNA, resulting in mitochondrial defects that severely affect cells/tissues with high energy demands such as brain, muscle, liver, and kidney [7]. Deterioration of mitochondrial function and quality control is also implicated in aging and common age-related diseases such as metabolic diseases, neurodegenerative

diseases, and cancer [4, 5, 7, 8]. Accordingly, induction of mitochondrial stress response pathways prevents age-related decline and extends lifespan [9, 10]. In this review, we discuss the molecular pathways of mitochondrial quality control. We briefly discuss oxidative stress defense, but elaborate more on the recently discovered mitochondrial stress pathways such as the unfolded protein response, mitochondrial dynamics, and mitophagy. Furthermore, we discuss the dynamic regulation of and interplay between mitochondrial quality control pathways, as well as their promising role in maintaining cellular health and promoting longevity.

Bimodal regulation of reactive oxygen species (ROS) determines cell fate

The superoxide radical O_2^{-} is the primary ROS, and is produced when molecular oxygen is reduced by a single electron, which occurs at seven or more sites by IMM-associated proteins, in particular complexes I and III of the electron transport chain (ETC) [11]. As ROS can be damaging to various matrix biomolecules, there are several mechanisms available to keep ROS levels low. First, O_2^{-} is converted to H_2O_2 by the superoxide dismutases MnSOD in the matrix or CuZnSOD in the intermembrane space and cytosol. Mitochondrial H_2O_2 is then enzymatically scavenged by peroxiredoxins (Prxs) and glutathione peroxidases (GPxs) [12].

ROS levels are not only maintained at low level to prevent damage, they are also tightly controlled because H_2O_2 is involved in signaling pathways that maintain cellular function [9, 12]. H_2O_2 is membrane-permeable and has a relatively long half-life: therefore, it can diffuse to the cytosol to alter protein activity through reversible oxidation of sulfur-containing methionine and active site cysteine residues [13]. Furthermore, there has been growing evidence that a moderate increase in ROS production can activate cell signaling pathways that promote health and extend lifespan [14]. For instance, in *Caenorhabditis elegans* glucose restriction increased mitochondrial respiration and ROS generation, but at the same time improved ROS scavenging capacity, and ultimately extended worm lifespan. Of note, the outcome of ROS exposure depended on the ROS levels, because the administration of antioxidants prohibited the glucose restriction-mediated extension of lifespan [15]. High ROS levels induce oxidative stress, cellular damage, and eventually cell death. On the other hand, low ROS levels are essential for maintenance of cellular function, they improve resistance to oxidative stress and may eventually extend lifespan. This dual response to ROS exposure has been called mitohormesis [14, 15], though multiple stressors – such as hypoxia, misfolded proteins, and alterations in metabolic signaling pathways – can induce a similar hormetic response [9, 16, 17].

Mitochondrial proteostasis is managed at multiple levels in a subcompartment-specific manner

Given that only ∼1% of all mitochondrial proteins are mtDNA-encoded, the majority of the mitochondrial proteins have to be imported in a tightly regulated manner [1]. Many mitochondrial proteins synthesized in the cytosol possess a mitochondrial target signal that allows subcompartment-specific import via different routes. The most common route is the presequence pathway, which delivers proteins to the matrix or IMM through the translocase complexes of the outer membrane (TOM) and inner membrane (TIM) [18]. Import into the matrix is primarily driven by the mitochondrial membrane potential ($\Delta\Psi_m$), but also depends on the presequence translocase-associated motor (PAM), and the ATPase activity of mitochondrial heat shock protein 70 (mtHsp70) [3]. Proteins targeted to the IMM are arrested in the TIM23 complex due to a hydrophobic sorting signal that is typically located behind the presequence, hence resulting in the lateral release in the IMM, although the driving force of this translocation remains unestablished [18]. Upon import or membrane insertion, the sorting signals are usually proteolytically removed by the mitochondrial processing peptidase (MPP) and/or inner membrane peptidase (IMP) [19]. Matrix proteins are further stabilized by the mtHsp70 and Hsp60 chaperones, which facilitate folding and prevent protein aggregation; proteins that fail to fold properly are degraded by mitochondrial proteases (reviewed in [20]).

Mitochondrial proteolysis controls protein turnover and function

The mitochondrial proteolytic system consists of subcompartment-specific proteases and the ubiquitin-proteasome system (UPS) that together regulate mitochondrial protein turnover [21]. In the mitochondrial matrix, three major AAA proteases are involved in protein degradation, including two soluble proteases Lon and ClpP, and the membrane bound protease m-AAA [20]. Lon protease has a preference for oxidized or misfolded proteins [22]. ClpP, which is activated upon mitochondrial proteotoxic stress and is required for the activation of the mitochondrial unfolded protein response (UPR^{mt}) [23, 24], degrades misfolded proteins as well [22]. The m-AAA is an hetero-oligomeric protease that has a wide variety of substrates, and depending on its subunit composition is involved in degradation of misfolded/misassembled OXPHOS subunits [22], assembly of OXPHOS complexes through a chaperone-like activity [20], or processing peptidase activity regulating the function of the mitochondrial ribosomal protein MRPL32 and the mitochondrial fusion protein OPA1 [25, 26].

In the inter-membrane space (IMS), protein quality is controlled by the membrane-bound protease i-AAA and soluble protease HtrA2/Omi [20], which are both induced upon proteotoxic stress [27, 28]. The i-AAA always consists of the same subunit, i.e. YME1L1, but – similar to m-AAA – it is also involved in the maintenance of OXPHOS complexes and OPA1 processing [29]. The role of HtrA2/Omi as a quality control protease in mammals has not been extensively determined. Given that it has functional resemblances with the bacterial HtrA2/Omi orthologs that have been characterized as quality control proteases involved in the adaptive response to proteotoxic stress, it is suggested to have similar roles in mammals [27]. In addition, in mammalian cells

apoptotic stimuli can trigger the cytosolic release of HtrA2/Omi which induces apoptosis through proteolytic elimination of inhibitor of apoptosis proteins such as c-IAP1 and XIAP [30]. Under non-apoptotic conditions, however, HtrA2/Omi remains in the IMS and is also implicated in processing of proteins involved in mitochondrial fusion and mitophagy [31, 32].

The IMM houses two other proteases that have essential functions in mitochondrial quality control. First, metallopeptidase OMA1 has similar functions as the membrane-bound AAA proteases [4], and serves as a stress-regulated protease that determines mitochondrial morphology by OPA1 processing in mammals [33]. Second, the rhomboid protease PARL may be also involved in OPA1 processing [34], and constitutively cleaves the mitophagy protein PINK1 preventing mitophagy induction in healthy mitochondria [35].

More recently, the major cytosolic proteolytic system – the UPS – was described to act in mitochondrial quality control as well [36]. The UPS is a highly selective proteolytic system: it marks proteins for proteasomal degradation through the covalent linkage of a chain of ubiquitin proteins [37]. A proteomic study in mouse heart revealed that numerous mitochondrial proteins are post-translationally modified by ubiquitin. Remarkably, these include not only outer mitochondrial membrane (OMM) proteins, but also IMS, IMM, and matrix proteins [38]. OMM proteins are more likely to be ubiquitinated and degraded by the UPS because they face the cytosol, but proteins destined for the mitochondrial matrix may also be targeted for degradation prior to import [39]. In addition, it has been suggested that the matrix protein OSCP, a subunit of OXPHOS complex V, can be retrotranslocated to the OMM, where it may be ubiquitinated [40]. This would imply that the UPS functions in a similar manner as at the endoplasmic reticulum (ER) [36, 40]; while the UPS has no access to the ER lumen, upon ER stress misfolded proteins are retrotranslocated across the ER membrane into the cytosol, polyubiquitinated, and degraded by the proteasome in a process called ER-associated degradation (ERAD) [41]. Certain key proteins that function in ERAD may have similar functions in mitochondria, hence it was postulated that mitochondria associated degradation (MAD) exists [36]. These proteins include the AAA ATPase p97 that is involved in the process of retrotranslocation and several E3 ubiquitin ligases including Parkin [42], Huwe1 [43], and MAPL/MULAN [44, 45], that associate with the OMM to mediate protein polyubiquitination. Furthermore, a complementary pathway to remove tail-anchored proteins that are mislocalized at the OMM has been recently described [46, 47]. Tail-anchored proteins are a distinct set of membrane proteins that contain a single transmembrane domain, which is inserted into the OMM [3]. Knockdown of AAA ATPase Msp1 (yeast) or ATAD1 (mammals) caused accumulation of these ectopic proteins at the OMM [46, 47], suggesting that these AAA ATPases are involved in extraction of mislocalized tail-anchored proteins from the OMM and targeting them for degradation by the proteasome [46, 47]. Altogether, although the full extent of UPS regulation of mitochondrial function has to be established, it may regulate multiple pathways of mitochondrial quality control by controlling protein turnover prior to mitochondrial import or upon retrotranslocalization.

The retrograde mitochondrial unfolded protein response relieves protein folding stress

Mitochondria may suffer from proteotoxic stress when the protein folding capacity is exceeded, for instance due to excessive ROS, mutations, or heat. Sustained proteotoxic stress induces the UPR^{mt}, a mitochondria-to-nucleus adaptive signaling involving attenuation of protein translation and induced expression of protein folding and proteolytic machineries [48, 49]. Early studies revealed that UPR^{mt} exists in mammalian cells following disruption of the stoichiometric balance between nDNA- and mtDNA-encoded proteins, or overexpression of a mutated form of the matrix protein ornithine transcarbamylase [23, 50]. However, the molecular mechanism is more extensively elucidated in C. elegans [24, 51–53]. Activation of the UPR^{mt} upon mitochondrial stress elicits expression of mitochondrial chaperones HSP-6 and HSP-60 in C. elegans (mtHsp70 and Hsp60 in mammals) [51]. This was used as a premise to screen for components of the UPR^{mt} signal transduction pathway using hsp-6 and hsp-60 reporter worms [24, 52, 53]. Canonical UPR^{mt} is initiated by the accumulation of unfolded proteins that activates the matrix protease CLPP-1 [24]. CLPP-1 derived peptides are exported to the IMS by the IMM transporter protein HAF-1 [24, 53], and subsequently diffuse into the cytosol. These peptides initiate a signaling cascade through the interaction with the transcription factor ATFS-1 that under unstressed conditions is imported into the mitochondrial matrix and degraded by Lon protease [53, 54]. The stress-mediated export of peptides leads to nuclear translocation of ATFS-1 in complex with transcriptional regulators UBL-5 and DVE-1, and induced expression of chaperones, proteases, and mitochondrial import proteins [24, 52, 54] (Fig. 1). In parallel to these retrograde signaling events, mitochondrial stress can also inhibit cytosolic translation via a ROS-dependent complementary signaling cascade involving GCN-2-mediated phosphorylation of eIF2α [55]. This decreased protein translation is also associated with attenuated mitochondrial protein import, through YME1L1-mediated degradation of TIM17A (part of TIM23 complex) [56]. Together these parallel events relieve the protein-folding load in mitochondria.

The initial work in mammalian cell culture demonstrated that disturbances of mitochondrial protein balance elicited UPR^{mt}, showing that mitochondrial stress triggered the upregulation of mitochondrial chaperones Hsp60, Hsp10, mtDnaJ, and the mitochondrial protease ClpP, while the levels of ER-specific chaperones remained the same [23, 50]. The sequence of events that drive induction of mammalian UPR^{mt} is not yet defined, although it requires ClpP activity and involves PKR-mediated eIF2α phosphorylation [57]. In mammals, phosphorylated eIF2α is also implicated in degradation of TIM17A by YME1L1 and subsequent attenuation of protein import [56]. Soon after commencement of mitochondrial proteotoxic stress, transcription factors CHOP and C/EBPß are upregulated via JNK2-mediated signaling, and in turn induce the expression of UPR^{mt} responsive genes [23, 58]. Of note, it has been suggested that PKR contributes to UPR^{mt} induction as well through the activation of c-Jun and/or JNK2 (Fig. 1) [57]. Downstream of c-Jun, however, there may be more unidentified transcription factors involved. Bioinformatic analysis suggested that the promoter region of UPR^{mt}
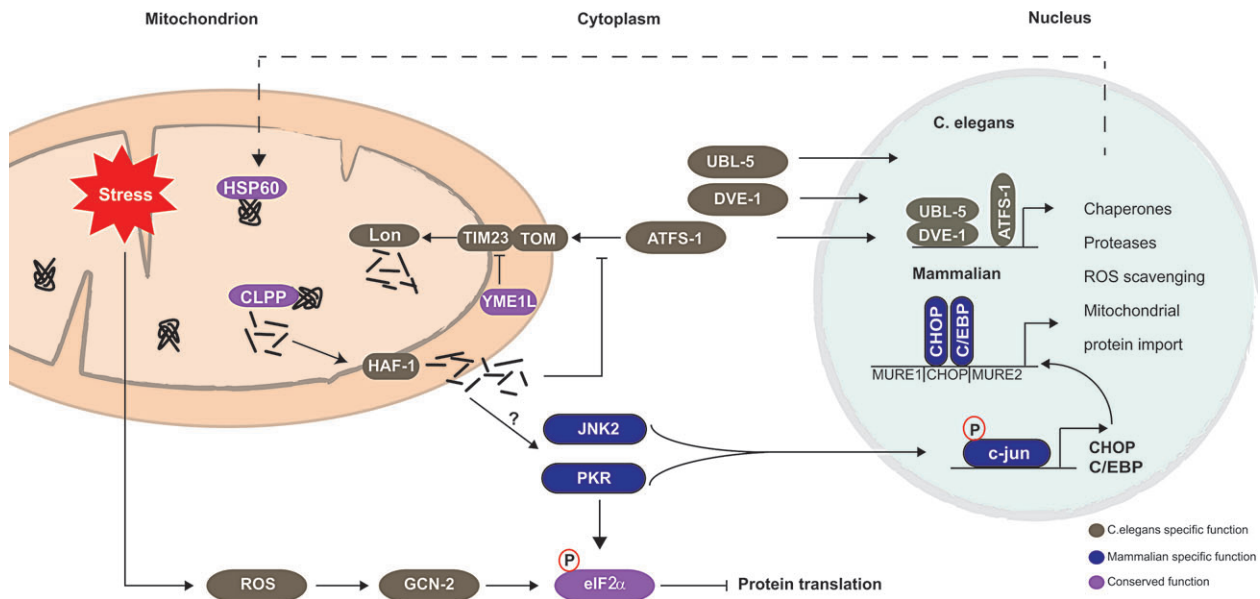
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Figure 1. UPR[mt] signal transduction pathway in mammals and worms. Upon mitochondrial proteotoxic stress, CLPP-1 activity is required to induce UPR[mt]. In *C. elegans*, peptides processed by CLPP-1 are exported into the cytosol by the transporter protein HAF-1 and attenuate mitochondrial protein import. Consequently, the transcription factor ATFS-1, which would normally be degraded in the matrix by Lon protease, accumulates in the cytosol. Upon accumulation, ATFS-1 translocates to the nucleus together with DVE-1 and UBL-5 to activate UPR[mt] responsive genes. Protein translation can be attenuated in a complementary pathway that depends on ROS signaling. In mammals, it is not completely clear how the signal is propagated to the nucleus. ClpP activity is also required for mammalian UPR[mt] induction, although the putative transporter protein (HAF-1 ortholog) is yet to be identified. Furthermore, it involves attenuation of mitochondrial import through YME1L1-mediated degradation of TIM23 component (TIM17A). Activation of JNK2 kinase and subsequent activation of transcription factor c-Jun induces expression of CHOP and C/EBPß leading to the induction of UPR[mt] responsive genes. PKR inhibits translation through the phosphorylating eIF2α, and is also involved in c-Jun activation. The UPR[mt] signaling components confirmed in worms are depicted in gray, the mammalian confirmed signaling components in blue, and proteins confirmed in both are shown in purple.

responsive genes contains two mitochondrial unfolded response elements (MURE1 and MURE2) flanking the CHOP-C/EBPß binding site [28]. The fact that only a small number of genes contains all three transcriptional elements – CHOP-C/EBPß, MURE1, and MURE2 – suggests that they provide specificity for selective induction of UPR[mt] genes [28]. Despite the new insights in UPR[mt] mechanisms, some major questions remain. For example, how do mitochondria prevent import of a signaling protein such as ATFS-1, yet allow import of chaperones, proteases, and other UPR[mt]-induced proteins? Further research should elucidate the temporal regulation of these signaling pathways in which chaperones are upregulated, while protein import and translation are attenuated.

Induction of the mitochondrial unfolded protein response improves organismal health and extends lifespan

Mitochondrial dysfunction is associated with aging and common age-related diseases [5, 59]. Paradoxically, electron transport chain (ETC) dysfunction and concomitant activation of the UPR[mt] has been found to increase lifespan in worms and flies [60–62]. In *C. elegans* impaired complex IV activity due to knockdown of *cco-1* during larval development induced the UPR[mt], which was maintained in adulthood and required for longevity [61]. Likewise, depletion of the mitochondrial ribosomal protein *mrps-5* disturbed mito-nuclear protein balance enough to induce the UPR[mt] and extend worm lifespan, a mechanism that was conserved in mammalian models [63]. Intriguingly, in *C. elegans* and *D. melanogaster* local perturbation of ETC function in brain, intestine, or muscle cells during larval development induces a systemic hormetic response leading to lifespan extension [61, 62]. These cell-non-autonomous effects are likely due to an unidentified mitochondrial signaling molecule or "mitokine" that perceives local stress and initiates distal stress response [61]. Mitochondrial derived peptide humanin and metabolic hormone FGF21 have been proposed to act as mitokines given that they are both implicated in adaptive responses to metabolic stress [7, 64, 65], but the causal link between these signaling molecules and UPR[mt] requires further investigation. In addition to spatial regulation, the timing of UPR[mt] induction is key, as illustrated by the fact that ETC disturbance during adulthood does not promote longevity [60, 61]. This suggests that early induction of the UPR[mt] activates adaptive responses that may be remembered through epigenetic alterations and contribute to an increased lifespan. The causal relation between the two mitohormetic pathways – UPR[mt] and antioxidants – is not fully elucidated. In several studies, UPR[mt] and lifespan extension are not attenuated by supplementation of antioxidants [61, 63], although others found that overexpression of ROS scavengers abolished UPR[mt]-dependent lifespan extension [62]. Interestingly,

lifespan extension caused by the induction of mitochondrial biogenesis involves both mitohormetic pathways, i.e. parallel activation of both UPR[mt] and antioxidants [16].

The mitochondrial network is maintained through dynamic fusion and fission

Although mitochondria are often depicted as individual rod-shaped organelles, they actually exist in interconnected networks that are highly dynamic. As a consequence, the number, shape, and localization of mitochondria are constantly changing. This dynamic character is a result of the continuous alternation between fusion and fission events. Fusion results in a more interconnected mitochondrial network [66], and allows exchange of mitochondrial content to maintain the overall integrity of the mitochondrial genome and proteome [67, 68]. On the contrary, fission events produce smaller mitochondria that can operate individually elsewhere in the cell or are degraded by mitophagy [69]. Since these two processes have opposing effects on the mitochondrial network, the balance between them is highly regulated. The preference for one process over the other allows mitochondria to adapt to changes in cellular energy demand or alterations in the mitochondrial environment [70–72].

Mitochondrial fusion requires coordinated fusion of outer and inner mitochondrial membranes

Mitochondrial fusion in mammals requires three membrane bound GTPases, the mitofusins Mfn1 and Mfn2 (Fzo1 in yeast) for OMM fusion, and optic atrophy 1 (OPA1; Mgm1 in yeast) for IMM fusion [29, 73]. Deletion of these GTPases, especially of Mfn1/2, hampers fusion events while fission events continue, and results in a network of small fragmented mitochondria [73–75]. In addition to being essential for IMM fusion, OPA1 is also involved in maintaining cristae integrity required for mitochondrial sequestration of cytochrome *c*, thereby protecting cells from apoptotic cell death [75]. OPA1 activity in IMM fusion and cristae maintenance depends on its post-transcriptional and post-translational processing [29, 76]. For IMM fusion, a balanced mixture of short and long OPA1 isoforms is required, which is constitutively regulated by the i-AAA protease YME1L1 [29]. In case of mitochondrial stress, however, the IMM-associated protease OMA1 is induced resulting in a complete conversion to short OPA1 isoforms [33], which hampers fusion and favors fission events to occur [33, 77, 78]. Stress-mediated inhibition of fusion through complete loss of long OPA1 isoforms not only prevents damaged mitochondria fusing with healthy mitochondria, but is also involved in cristae remodeling and induction of cytochrome *c*-mediated apoptosis [76–78]. The maintenance of narrow cristae junctions depends on the balance between the long membrane-bound OPA1 and the short soluble OPA1 in the IMS, which together form an OPA1 oligomer [76]. Complete destabilization of this oligomer widens the cristae, causes cytochrome *c* release and

concomitant induction of apoptosis [76]; while the preservation of the long OPA1 isoform alone is sufficient to prevent apoptosis [77, 78].

The regulatory mechanism of the multiple proteins involved in OMM fusion is still poorly understood [66]. Mitofusin ubiquitination is emerging as an important node of mitochondrial fusion regulation. In mammalian cells, mitofusin ubiquitination has been mainly associated with proteasomal degradation and inhibition of fusion during mitochondrial stress [79]. For instance, the E3 ligase Parkin mediates degradation of Mfn1 and Mfn2 upon mitochondrial depolarization in a PINK1-dependent manner [42]. Such inhibition of fusion upon mitochondrial stress is an essential step prior to induction of mitophagy (Fig. 2), which will be discussed in more detail later. In contrast, recent reports indicate that non-degradative ubiquitination of mitofusins may promote fusion in mammals [80, 81], similar to what has been described in yeast [82], although the E3 ligase responsible for this non-degradative ubiquitination is still unknown.

Fission is regulated through modulation of Dynamin-related protein 1 function

Mitochondrial fission requires coordinated scission of both the OMM and IMM, which is mediated by just one cytosolic GTPase, Dynamin-related protein 1 (Drp1; Dnm1 in yeast) [83]. As a cytosolic protein, Drp1 has to translocate to mitochondria, bind to its receptor proteins at the OMM, and assemble into an oligomeric structure that encircles the mitochondrion. Upon GTP hydrolysis, the Drp1 oligomer mediates scission of both mitochondrial membranes, dividing it into two mitochondria [69]. The recruitment of Drp1 to the OMM is an intricate process that depends on Drp1 post-translational modifications and interacting receptor proteins [84]. These include phosphorylation, O-GlcNAcylation, SUMOylation, ubiquitination, and *S*-nitrosylation (reviewed in [69, 84]). Among these modifications, phosphorylation has been most extensively studied. Phosphorylation of Drp1 occurs on multiple serine residues by various kinases that depending on the stress stimulus can either promote or inhibit fission through Drp1 recruitment and activation [84]. Furthermore, Drp1 recruitment and oligomerization is also regulated by OMM receptor proteins. Studies in yeast demonstrated that Dnm1 (yeast ortholog of Drp1) requires membrane receptor Fis1 and the cytosolic adaptor proteins Mdv1/Caf4 for its recruitment to the OMM and subsequent oligomerization [69, 84]. Mammals possess Fis1 [85], yet lack Mdv1/Caf4 orthologous adaptor proteins. The OMM proteins Mff [86], MiD49, and MiD51 [87] have been recently described as putative alternative Drp1 receptors that possess the ability to independently recruit Drp1 [88]. Nevertheless, the functional consequence of post-translational modifications on Drp1 recruitment/activity is highly species-, cell-type- and stimulus-specific, and many of the mechanistic aspects remain elusive. Future research should clarify the orchestration of Drp1 recruitment and activation by receptor proteins and post-translational modifications.
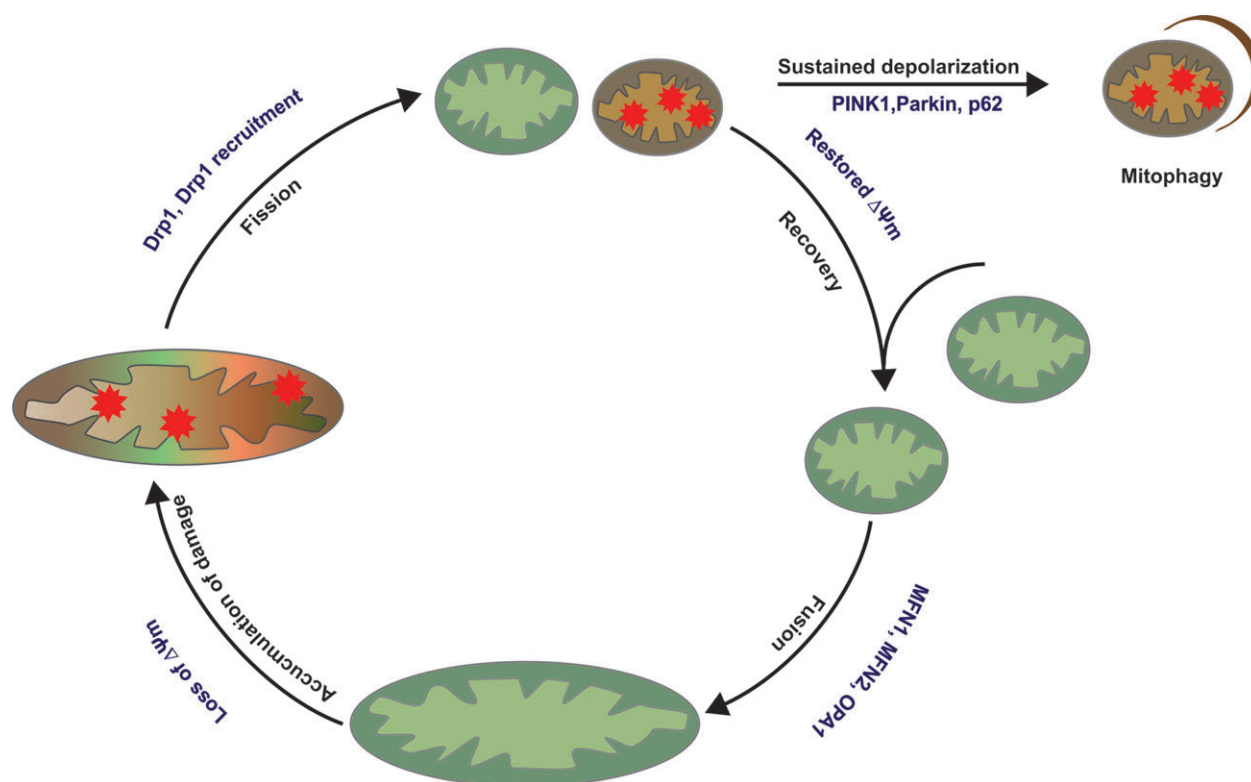
Figure 2. Mitochondrial life cycle. Mitochondria are continuously alternating between fusion and fission events. Fission allows segregation of damaged mitochondria that may be transiently depolarized. After separation, mitochondria are degraded by mitophagy if the depolarization sustains or if the mitochondrion is terminally damaged. The other daughter mitochondrion can continue to cycle between fusion and fission events.

The balance between fusion and fission controls mitochondrial morphology and function

An equally significant aspect of mitochondrial dynamics regulation is how the alternating fusion and fission events are coordinated. This is substantiated by several diseases related to imbalances in mitochondrial fusion and fission (reviewed in [89]). In brief, mutations in the key proteins that regulate fusion and fission are associated with neurological disorders in which mutations in Mfn2 and Opa1 cause Charcot-Marie-Tooth neuropathy type 2A and autosomal dominant optic atrophy, respectively [90–92], and mutations in Drp1 cause a combined mitochondrial and peroxisomal fission defect that results in abnormal brain development and even neonatal lethality [93].

Over the past decade, great advances have been made on the molecular mechanism of fusion and fission. The contribution of individual proteins to fusion or fission regulation has mainly been studied in gain- and loss-of-function experiments. Despite of our current knowledge on these molecular aspects, we still know relatively little about how the fusion/fission events are orchestrated in normal physiology. Live cell imaging revealed that these events quickly alternate [94], and many factors such as mitochondrial membrane potential, mitochondrial motility, and length influence the fusion-fission cycle and cellular physiology [70, 95] (Fig. 2). Recently, it was shown that mitochondrial dynamics critically regulates physiology of brown adipocytes through its role in thermogenesis [96]. Adrenergic stimulation and cold exposure shift the balance toward fission through inhibition of fusion by OPA1 processing. This results in a fragmented mitochondrial network, which was required for mitochondrial depolarization and accompanying heat production [96]. Taken together, various proteins and/or stimuli influence the mitochondrial fusion and fission cycle, which is likely to occur in a cell- and context-dependent manner.

Mitophagy: The removal of non-functional mitochondria

Terminally damaged mitochondria can be degraded by a process called mitophagy, a selective autophagic route. In autophagy (macroautophagy), cytoplasmic components are sequestered in a double membrane vesicle (autophagosome) in a non-selective manner. The autophagosome then fuses with a lysosome, causing its contents to be degraded. Autophagy is an important cellular quality control process that permits the cell to remove and recycle its cell content upon starvation [97]. In contrast, mitophagy occurs under nutrient-rich conditions by selectively eliminating dysfunctional mitochondria [98]. As a system of mitochondrial quality control, mitophagy contributes to the maintenance of a

healthy mitochondrial network by preventing healthy mitochondria fusing with damaged ones [99] (Fig. 2). Given that damaged mitochondria can trigger apoptosis by releasing Ca^{2+} and cytochrome c [100], it prevents cellular harm and is crucial for cell survival.

Mitophagy is induced upon loss of $\Delta\Psi_m$, and involves the kinase PINK1 and the E3 ligase Parkin [101, 102]. PINK1 initiates mitophagy by flagging damaged mitochondria and recruiting Parkin. In addition to mitophagy, PINK1 may be involved in other mitochondrial processes such as ATP production through stimulation of complex I reductive activity [103]; in case of PINK1 deficiency, ATP production can be maintained by supplementation of electron carriers vitamin K2 or ubiquinone [104]. With respect to its role in mitophagy, PINK1 is regulated through localization-dependent degradation. Under normal conditions, it is imported into the IMM, cleaved by the IMM protease PARL, and subsequently degraded [35, 102]. Dissipation of $\Delta\Psi_m$ hampers PINK1 import, causing it to accumulate at the OMM [102], where it binds to the TOM complex [105]. Once on the OMM, PINK1 recruits Parkin and activates its ligase activity to enable OMM protein polyubiquitination [101]. It was recently shown that PINK1-mediated recruitment and activation of Parkin occurs through phosphorylation of Parkin [106, 107], but is especially accelerated when combined with phosphorylation of ubiquitin [108–110]. Parkin ubiquitinates various proteins on the OMM and in the cytosol and thereby facilitates recruitment of the autophagy machinery to ultimately degrade damaged mitochondria [102, 111]. These Parkin targets not only include Mfn1 and Mfn2 [42, 112], but also members of the TOM complex, apoptotic proteins, proteins that mediate mitochondrial transport, proteasomal subunits, and members from the autophagy machinery [111]. How these Parkin-mediated ubiquitination events induce mitophagy is not completely understood. It is possible that OMM protein ubiquitination induces mitophagy in several ways: (i) inducing prerequisite proteasomal degradation of proteins involved in mitochondrial fusion and transport [42, 113]; (ii) promoting recruitment of ubiquitin binding proteins such as p62 and HDAC6 that facilitate autophagosome formation [114, 115]; and/or (iii) the presence of ubiquitinated proteins on the OMM alone might stimulate recruitment of the autophagy machinery [105, 116, 117].

The physiological relevance of PINK1/Parkin-mediated mitophagy

In addition to questions pertaining to the molecular regulation of mitophagy, it will be interesting to assess how PINK1 and Parkin regulate mitophagy in more physiological conditions, as experimental systems often rely on potent uncoupling agents such as CCCP. Given that both *PINK* and *PARK2* (encoding Parkin) genes have been found mutated in early-onset hereditary Parkinson's disease [118], the physiological relevance of PINK1/Parkin-mediated mitophagy is of particular interest in neurons. Regardless of that, the list of substrates and pathways that involve PINK1 and Parkin activity is expanding [119], and conceivably the outcome of PINK1/Parkin deficiency relies on compensatory pathways

that may be regulated differentially depending on the species, cell type, and mode of activation. In this context, it is interesting to note the marked mechanistic similarities between UPR^{mt} and mitophagy. These stress responses are both activated upon dissipation of $\Delta\Psi_m$ and extramitochondrial accumulation of signaling proteins, i.e. ATFS-1 and PINK1, respectively. In unstressed conditions, these proteins are constitutively imported and degraded in the mitochondrion, but stress-induced loss of $\Delta\Psi_m$ impairs mitochondrial protein import, alleviating the protein folding load and facilitating ATFS-1 and PINK1-dependent induction of the UPR^{mt} and mitophagy [48, 99]. One may wonder how mitochondria discriminate between inducing UPR^{mt} and mitophagy, if both signaling proteins are accumulating extramitochondrially following $\Delta\Psi_m$ dissipation. While both may indeed be induced upon mitochondrial depolarization, the kinetics of the two responses could be different, for instance requiring a prolonged activation state or changes in mitochondrial morphology [71, 94] (Fig. 2). Additionally, UPR^{mt} and mitophagy may require secondary signals or processes to fully engage their protective effects. Along these lines, it was recently shown that PINK1/Parkin are also involved in an emerging quality control system involving the release of mitochondria-derived vesicles (MDVs), that bud off from mitochondria and deliver damaged content to lysosomes for degradation [120]. Further investigation on PINK1/Parkin function in different systems of mitochondrial quality control may shed light on the induction thresholds of different stress responses that seem to converge in a context- and timing-dependent manner, allowing consecutive induction as well as cross-regulation of mitochondrial quality control pathways.
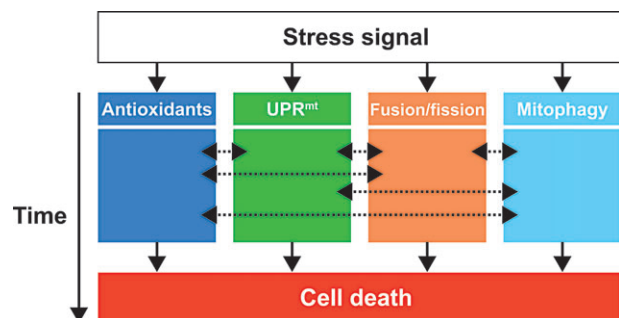

Figure 3. Mitochondrial quality control pathway interaction. Depending on the type of stress stimuli a corresponding mitochondrial stress response is induced. For instance, in case of oxidative stress antioxidants and DNA repair enzymes are activated. Simultaneously, mitochondrial chaperones and proteases may be upregulated via the mitochondrial unfolded response (UPR^{mt}). Damaged components can be diluted in the mitochondrial network through fusion, whereas severely damaged mitochondria are separated from the network by fission and subsequently degraded by mitophagy. In contrast to the classical hierarchical view of sequential mitochondrial quality control activation, we postulate that this activation is highly context- and time-dependent. The dashed arrows indicate that the period and level of stress activation lead to crosstalk between the different stress responses, which depend on the type of stress stimulus and its duration. Prolonged stress or severe damage not only elicits mitochondrial repair responses, but ultimately leads to apoptosis.

Review essays

Conclusions and prospects

Mitochondrial quality control pathways play a central role in mitochondrial health, which has major potential to improve health and lifespan. In the past decade, tremendous progress has been made in this field with the identification of various quality control pathways. The different systems of mitochondrial quality control are often described as a highly regulated and hierarchical network. In this classical view, each system has a maximum capacity, and crosstalk between them permits induction of the next system when the previous one is overwhelmed. In recent years, however, it has become evident that mitochondrial quality control pathways are not hierarchical, and that the sequence of events is highly dependent on various factors such as stress stimuli, stress duration, activation of auxiliary proteins, and degree of mitochondrial damage (Fig. 3). Future studies should aim to improve our understanding of UPR^{mt} and mitochondrial dynamics in mammals, as these processes have mostly been studied in worms and yeast. Also, how the network of quality control pathways is cross-regulated should receive more attention, with particular focus on post-translational modifications such as ubiquitination that seems to regulate all the systems of mitochondrial quality control. Moreover, getting a better grip on how mitohormesis is regulated may establish the mitochondrion and its quality control system as target in future therapeutic interventions. These may range from dietary, exercise-related, and pharmaceutical approaches that relieve mitochondrial dysfunction in disease, or create mitochondrial stress to induce a (mito-) hormetic response and eventually promote longevity. A better understanding of how these systems are coordinated holds the promise of potential future applications.

The authors have declared no conflicts of interest.

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