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Mitochondrial Quality Control in the Myocardium: Cooperation between Protein Degradation and Mitophagy

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Abstract

Mitochondria are critical for cardiomyocyte survival and maintenance of normal cardiac function. However, changes in the extra- or intracellular environments during stress can cause excessive damage to mitochondria and lead to activation of cell death. In fact, there is evidence that mitochondrial dysfunction is an important contributor to both development of heart failure and the aging process. To counteract the adverse effects resulting from mitochondrial damage, cells have evolved mitochondrial quality control pathways that act at both the protein and organelle levels. Quality control of proteins in the outer mitochondrial membrane is monitored by the ubiquitin-protease system, whereas chaperons and proteases act in the various compartments of the mitochondria. When the damage is too excessive and the degradation machinery is overwhelmed, the entire mitochondrion is eliminated by an autophagosome. Together, these pathways ensure that myocytes maintain a functional network of mitochondria which provides ATP for contraction. Unfortunately, chronic stress and aging can negatively affect proteins that are involved in the mitochondrial quality control pathways which leads to accumulation of dysfunctional mitochondria and loss of myocytes. In this review, we provide an overview of the proteins and pathways that regulate mitochondrial quality control in the cell with an emphasis on pathways involved in maintaining protein and organelle homeostasis. We also delve into the effects of reduced mitochondrial quality control on aging and cardiovascular disease.

Keywords

Mitochondria; Autophagy; Proteases; Parkin; Aging; Mitophagy

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Disclosures

None

1. Introduction

Mitochondria are critical for myocyte function. The contracting cardiac myocyte requires a lot of energy in the form of ATP which is provided by mitochondria via oxidative phosphorylation. To ensure a continuous supply of ATP, the myocytes are densely packed with mitochondria. Unfortunately, reactive oxygen species (ROS) are byproducts of this process and are generated primarily by Complexes I and III of the electron transport chain during oxidative phosphorylation. The myocytes have developed a strong ROS-neutralizing defense which can deactivate the excess ROS that is produced. However, when the levels of ROS overwhelm the detoxifying systems, these species, such as hydrogen peroxide and superoxide anion, can damage cellular components as well as produce misfolded mitochondrial proteins [1]. Accumulation of damaged proteins can impair mitochondrial function and lead to activation of cell death. Consequently, the removal of damaged mitochondria is important for cell survival, particularly in high-energy post-mitotic tissues such as the heart. Therefore, it is not surprising that cells have developed mitochondrial quality control pathways that act at both the protein and organelle levels to counteract the adverse effects resulting from protein/organelle damage. These pathways ensure that the cells can maintain a functional network of mitochondria to prevent unnecessary cell death. There is evidence that mitochondrial dysfunction is an important contributor to both development of heart failure and the aging process, and studies indicate that this is due in part to reduced mitochondrial quality control.

Mitochondria are composed of four different compartments (the outer mitochondrial membrane, intermembrane space, inner mitochondrial membrane, and the mitochondrial matrix) and therefore contain a number of different proteins that are involved in mitochondrial protein quality control (Figure 1 and Table 1). Quality control (QC) is monitored by chaperons and proteases in the various compartments of this organelle. For instance, mitochondrial chaperons, such as HSP60, and HSP70, contribute to mitochondrial QC by mediating the refolding of misfolded proteins back to their native structures [2] and [3]. The methionine sulfide reductase system, consisting of MsrA and MsrB, reduces oxidized methionine back to methionine [4]. However, accumulation of unfolded or damaged mitochondrial proteins above a certain threshold overwhelms the mitochondrial refolding and repair capacity and thus requires activation of mitochondrial protein degradation machinery to remove damaged proteins [5]. Furthermore, when the degradation machinery is unable to remove damaged components, it leads to mitochondrial dysfunction and subsequent activation of autophagy [6]. This pathway is responsible for eliminating the entire organelle where the dysfunctional mitochondrion is sequestered inside an autophagosome and subsequently delivered to a lysosome for degradation.

Studies indicate that the accumulation of dysfunctional mitochondria in diseased or aged tissues might, in part, be due to reduced mitochondrial quality control. Chronic stress and aging affects proteins that are involved in the mitochondrial quality control pathways. In this review, we provide an overview of the proteins and pathways that are involved in mitochondrial QC with an emphasis on pathways involved in maintaining protein and organelle homeostasis in cells. We also delve into the effects of reduced mitochondrial quality control on aging and cardiovascular disease.

2. Mitochondrial Protein Quality Control

2.1. Outer Mitochondrial Membrane (OMM)

The ubiquitin protease degradation system (UPS), known for its role in the breakdown of cytosolic proteins, also contributes to degradation of mitochondrial proteins. These proteins are post-translationally modified by ubiquitination, extracted from the membrane, and delivered to the 26S proteasome, where they are finally degraded [5] (Figure 1). A proteomics study analyzing ubiquitinated proteins in the mouse heart found that mitochondrial proteins were the majority of substrates for ubiquitination in the heart [7]. Remarkably, these proteins were from all four different mitochondrial compartments. In this case, these proteins are most likely retro-translocated from their compartment of origin to the outer mitochondrial membrane, similar to the degradation of ER proteins via the ERAD pathway [5]. There are several mitochondrial ubiquitin ligases that are localized on the cytosolic side of the OMM, such as March5, MULAN/MAPL, RNF185, and MULE/ARF-BP1 [8] and [9]. These proteins localize to the OMM, with the exception of MULE, which localizes to the cytoplasm and nucleus as well. These proteins are responsible for ubiquitinating proteins that need to be degraded, which marks them for removal by the proteasome. Although the UPS plays an essential role in protein quality control, there is also evidence that excessive activation of the UPS can be detrimental to cells. For instance, Lokireddy *et al.* found that the E3 ubiquitin ligase MULAN/MAPL is responsible for mitochondrial dysfunction and clearance observed in skeletal muscle wasting [10]. To date, none of the ubiquitin ligases discussed above has been investigated in the heart. Nonetheless, it is quite likely that aberrant expression would present with cardiac phenotypes given the importance of mitochondrial turnover in the heart.

In contrast, the E3 ubiquitin ligase Parkin is normally localized to the cytosol and translocates to dysfunctional mitochondria. Interestingly, Parkin has been reported to play a role both in UPS-mediated protein degradation and clearance of mitochondria via autophagy (“mitophagy”, discussed in detail below). Parkin ubiquitinates several OMM proteins, such as Hexokinase I, Mfn1/2, VDAC, and Miro, resulting in their degradation through the UPS [11], [12], [13] and [14]. The UPS-mediated degradation of Mfn1/2 and Miro appears to be related to Parkin’s role in mitophagy rather than individual protein quality control of these proteins. In addition, the AAA ATPase VCP/p97 extracts ubiquitinated proteins from multimeric complexes or structures for recycling or degradation by the proteasome [15]. VCP recruitment to damaged mitochondria is dependent on Parkin [16]. Ubiquitination of certain proteins by Parkin allows for p62 binding and subsequent removal of the entire organelle via autophagy [11]. Thus, these mitochondrial ubiquitin ligases are involved in regulating both remodeling of the mitochondrial proteome and mitophagy.

2.2. Intermembrane Space (IMS)

Protein quality control in the IMS is handled primarily by the protease HtrA2/Omi, the only soluble quality control protease found in this compartment [17]. The role of HtrA2 in the context of apoptosis has been well characterized [18]. This protein is released from mitochondria into the cytosol upon activation of apoptosis and is responsible for cleaving inhibitors of apoptosis [18]. Myocardial ischemia/reperfusion (I/R) injury results in the

translocation of HtrA2 from mitochondria to the cytosol where it promotes myocyte apoptosis [19]. Remarkably, HtrA2 levels have been positively correlated with age in mice, contributing to greater vulnerability of myocytes to I/R injury [20]. However, the functional role of HtrA2 in mitochondrial quality control is less well studied. HtrA2 deficiency results in mitochondrial malfunction, altered mitochondrial morphology, and ROS generation [21], which in turn damages mitochondrial DNA (mtDNA) [22]. Knockout mice of HtrA2 have smaller hearts, and die by 30 days of age due to neurodegenerative disorder [21]. The mutant *mnd2* mice, possessing a missense mutation in HtrA2, also die young by day 30–40, but are rescued by wild-type HtrA2 gene expression in the central nervous system [23]. Nevertheless, these rescued mice develop an accelerated aging phenotype in adulthood, have cardiac enlargement, and die by 12–17 months of age.

2.3. Inner Mitochondrial Membrane (IMM)

Quality control in the IMM is primarily dependent on two members of the ATPases Associated with diverse cellular activities (AAA) family: the m-AAA and i-AAA proteases. These protease complexes are embedded within the IMM, with their catalytic domains either exposed to the matrix (m-) or the intermembrane (i-) side. Chaperone-like domains in these two proteases recognize hydrophobic stretches of misfolded polypeptide chains of membrane-spanning proteins or unassembled subunits of respiratory complexes for degradation [24]. The AAA proteases actively extract transmembrane segments for complete protein degradation [24].

Deletion of a single gene encoding one of the two proteases in yeast impairs the mutants' respiratory capacity and results in aberrant mitochondrial morphology. In humans, mutations in one of the m-AAA protease subunits cause a form of hereditary spastic paraplegia [25]. Although these AAA proteases are present in cardiac mitochondria [26], little is known about their biological role or how these proteins are regulated under pathological conditions. The role of these AAA proteases in the heart requires further investigation.

2.4. Mitochondrial Matrix

The greatest level of mitochondrial protein regulation and quality control occurs in the matrix of the mitochondrion. The matrix is very protein dense and contains the mitochondrial translation machinery as well as enzymes of the TCA cycle and other metabolic pathways. Here, protein folding is mainly dependent on the chaperones HSP70 and HSP60 [2] and [3]. The matrix also contains two AAA proteases, Lon and ClpXP. Lon removes oxidized proteins in the matrix, particularly iron-sulfur containing proteins such as aconitase, which are susceptible to oxidative damage [27]. The important role of Lon in protein quality control in mammalian cells was initially demonstrated by Bota et al. They found that knockdown of Lon expression using RNAi in human lung fibroblasts leads to abnormal mitochondrial function and morphology, as well as activation of apoptosis [28]. In contrast, overexpression of Lon in the fungus *podospora anserina* results in enhanced mitochondrial quality control and increased lifespan of the transgenic strains [29]. Human Lon has been found to bind mtDNA which serves to protect the DNA against oxidative stress-mediated damage [30].

Human ClpXP is an ATP-dependent protease which plays a role in the unfolded protein response and is composed of a double-stack of ClpP subunits flanked on each end by a ClpX subunit (Figure 1) [31] and [32]. In *c. elegans*, nascent polypeptides unbound by chaperones are degraded by ClpP [32]. These degradation products are then transported into the cytosol via HAF-1, a mitochondria-localized peptide transporter. These peptides activate the transcription factor ZC376.7, which induces transcription of mitochondrial chaperone proteins and ClpP. Studies of ClpXP in mammalian systems are highly lacking; however, a ClpP null mouse has been generated which addresses some of its function. These mice present with infertility, hearing loss, impaired growth and motor activity, as well as reduced survival [33]. In ClpP deficient hearts, levels of mitochondrial chaperones are increased, respiratory supercomplexes are decreased, and there is an approximate 4-fold increase in the accumulation of mtDNA. No specific cardiac abnormalities were reported for these mice. Unfortunately, most of our knowledge of proteases such as Lon and ClpXP is limited to yeast strains. Relatively little is known about the specificity and biological roles of these proteases in eukaryotic cells. Additional studies must be performed to fill in this knowledge gap.

Peptides from numerous matrix and inner membrane proteins are exported from mitochondria in an ATP-dependent manner [34]. Most likely, these peptides are generated by proteases such as Lon and ClpXP, and then transported to the cytosol where they are ubiquitinated and subsequently degraded by the proteasome. There is also evidence that the UPS participates in the normal turnover of intra-mitochondrial proteins and that these proteins do not have to be cleaved by proteases prior to export and degradation. For instance, intact subunits from Complex I, Complex IV, and the ATP synthase retrotranslocate to the OMM where they are ubiquitinated and subsequently degraded by the proteasome [35]. In addition, inhibition of the proteasome results in increased oxidation, diminished intramitochondrial protein translation, increased glycolysis, and overall mitochondrial dysfunction in neurons [36]. These findings propose an interesting system which enables proteasome degradation of proteins that are otherwise untouchable by the UPS.

3. Mitochondrial Derived Vesicles

When damage to mitochondria occurs on a larger scale, the refolding and/or degradation by chaperones and proteases are inadequate. Instead, portions of mitochondria may be pinched off and segregated for degradation (Figure 2). These small vesicles, termed mitochondrial derived vesicles (MDVs), bud off functionally respiring mitochondria at a steady under baseline conditions [37]. The formation of the MDVs is significantly increased during oxidative stress [37]. These vesicles transport oxidized proteins to the lysosomes for degradation, suggesting that this is another mechanism of mitochondrial quality control. Interestingly, Soubannier et al. reported that cardiac mitochondria also form MDVs and that cargo of these MDVs is highly selective and depends on the type of oxidative stress [38]. For instance, subunits of Complexes II, III, and IV are differentially incorporated within these MDVs under various treatments, whereas proteins of Complex I and ATP Synthase are excluded [38]. It is still unclear why certain proteins are excluded from these vesicles. It is possible that it is due to size difference or that certain proteins are only removed in response to a specific stress. In addition, these vesicles are formed independently of Drp1-mediated

mitochondrial fission and autophagy, suggesting that this is not merely a form of asymmetric fission [39]. Interestingly, it was recently demonstrated that the formation of these MDVs in response to oxidative stress requires the presence of PINK1 and Parkin [40]. While PINK1 and Parkin have well established roles in mitophagy (as reviewed below), it appears that this role in MDV formation is a separate, independent pathway, and is currently a subject under active investigation.

4. Mitochondrial Autophagy (Mitophagy)

Upon extensive or prolonged damage, often entire mitochondria must be eliminated. This occurs through a process called mitochondrial autophagy, or “mitophagy”, where the damaged organelle is engulfed by an autophagosome and subsequently delivered to the lysosome for degradation [6] (Figure 3). Autophagy is used for the elimination of both dysfunctional organelles and protein aggregates. It also functions as a recycling pathway during fasting or caloric restriction to generate amino acids to maintain ATP energy production [41]. Autophagy is an all-inclusive process, as it has been found to target not only mitochondria, but the ER, peroxisomes, and parts of the nucleus [42]. It was previously believed that autophagy sequestered cargo indiscriminately. However, accumulating evidence indicates that this is not the case. To date, two pathways that regulate mitophagy have been characterized.

4.1. PINK1-Parkin Pathway

The PINK1-Parkin pathway is important in regulating clearance of dysfunctional mitochondria (Figure 3A). In a healthy cell, the serine-threonine kinase PINK1 is imported into mitochondria by the TOM complex and then actively degraded by MPP and PARL [43]. However, upon loss of mitochondrial membrane potential, PINK1 is no longer imported and it accumulates on the OMM [43]. Recently, it was reported that PINK1 can also accumulate on energetically healthy mitochondria in the event of misfolded protein aggregation in the matrix, possibly regulating the formation of MDVs [44]. When PINK1 accumulates on the OMM, it recruits the E3 ubiquitin ligase, Parkin [43] and [45]. Once recruited, Parkin initiates its ubiquitination of several OMM proteins such as Hexokinase I, VDAC1, Mfn1/2, and Miro [11], [12], [13] and [14]. Degradation of the fusion proteins Mfn1/2 maintains mitochondria in a fragmented state, a requirement for mitophagy to proceed. Miro is important in anchoring mitochondria to the cytoskeleton and degradation of this protein via the PINK1/Parkin pathway results in release and segregation of the damaged mitochondrion from the network of functional mitochondria [12]. Studies have reported that VDAC1 and Hexokinase I both play a critical role in Parkin-mediated mitophagy, but their exact role(s) in the mitophagy process is currently unclear.

To date, there is little consensus on how PINK1 recruits Parkin to mitochondria. It has been proposed that Parkin directly interacts with PINK1 at the OMM [46]. Alternatively, there's evidence that PINK1 and Parkin cooperate by sharing substrates. For instance, PINK1-mediated phosphorylation of Miro leads to its Parkin-mediated ubiquitination and degradation [12]. More recently, Chen and Dorn reported that Mfn2 interacts with Parkin in a PINK1-dependent manner and that phosphorylation of Mfn2 is a prerequisite for Parkin recruitment to mitochondria [47]. Further studies are needed to determine exactly how

PINK1 recruits Parkin to mitochondria. In addition, although only a few substrates of Parkin have been identified to date, it is likely that numerous substrates exist for Parkin and that these serve a redundancy to ensure efficient removal of damaged mitochondria.

The addition of ubiquitin to mitochondrial substrates serves as signal for autophagic degradation. The p62 protein binds both ubiquitinated substrates via its ubiquitin associated domain (UBA) and to LC3 on the autophagosomal membrane [48]. Hence, it physically links ubiquitin-tagged mitochondria to autophagosomes for engulfment by binding both LC3 and ubiquitin simultaneously. Parkin has also been found to interact with Ambra1, a promoter of phagophore nucleation, independent of its ubiquitin ligase function [49]. It is possible that Parkin also initiates autophagy in response to mitochondrial damage through this mechanism. This notion is further supported by the lack of autophagosomes in Parkin deficient myocytes after treatment with rotenone [50]. Thus, these studies suggest that the PINK1/Parkin quality control system regulates mitochondrial clearance at multiple levels: promoting fission by degrading fusion proteins, releasing and segregating dysfunctional mitochondria from the network, marking mitochondria for degradation via ubiquitination, and initiating formation of the autophagosome at the mitochondrion that needs to be degraded.

The importance of the PINK1/Parkin pathway in clearing dysfunctional mitochondria in the heart has been demonstrated by several studies. For instance, Parkin deficient mice have hampered recovery of cardiac contractility after sepsis activation [51], and impaired mitophagy after myocardial infarction, resulting in reduced survival and larger infarct sizes relative to wild type [50]. Parkin-mediated mitophagy also plays an important role in the preconditioning process [52]. Similarly, PINK1-deficient hearts are more susceptible to *ex vivo* I/R injury compared to wild type [53], and overexpression of PINK1 in HL-1 cardiac cells protects against simulated I/R-mediated cell death [53]. Billia et al. found that PINK1 deficiency leads to ventricular dysfunction and hypertrophy at 2 months of age [54]. These hearts have increased fibrosis, apoptosis, oxidative stress, and reduced mitochondrial respiration [53] and [54]. This is in contrast to Parkin^{-/-} mice which have normal mitochondrial and cardiac function at this age [50]. Collectively, this suggest that the PINK1/Parkin pathway plays an important role in clearing damaged mitochondria after stress and that PINK1 might have additional functions in myocytes.

4.2. Mitochondrial Autophagy Receptors

Studies have discovered that there are proteins and lipids present in the outer mitochondrial membrane that can directly function as mitophagy receptors, thus eliminating the need for ubiquitination and adaptor proteins (Figure 3B). For instance, mitochondrial cardiolipin [55] and FUNDC1 [56] can directly bind to LC3 on the autophagosome and fulfill this role. Similarly, Nix/BNIP3L and BNIP3 are pro-apoptotic BH3-only proteins that are located on the OMM in cells [57] and [58]. Although they are both known to induce mitochondrial dysfunction and cell death, they can also direct mitochondria to autophagosomes by directly binding to LC3 or gamma-aminobutyric acid receptor-associated protein (GABARAP) on the autophagosome [57], [58] and [59]. Interestingly, while abrogation of the interaction between BNIP3 and LC3 significantly reduces mitophagy, it is not fully eliminated [58],

confirming the presence of additional autophagy receptors on mitochondria. Nix as a mitophagy receptor seems to have two distinct roles: inducing autophagy in response to ROS, and promoting Parkin translocation [60]. Similarly, BNIP3-mediated autophagy is reduced in Parkin-deficient myocytes [61]. However, since both Nix and BNIP3 can directly bind to LC3 on the autophagosome, it is unclear why Parkin is needed for Nix/BNIP3-mediated mitophagy.

Nix and BNIP3 have important roles in the heart. Disruption of Nix results in the accumulation of dysfunctional mitochondria with age which correlates with development of cardiac dysfunction [62]. Interestingly, mice lacking both Nix and BNIP3 develop accelerated cardiac hypertrophy and mitochondria dysfunction compared to the single knockout mice, illustrating the overlapping functions of these two proteins. This suggests that Nix and BNIP3 are important for the maintenance of a healthy mitochondria pool under normal conditions. However, exactly when these proteins activate apoptosis instead of mitophagy is still unclear. Also, how mitochondrial stress activates receptor-mediated mitophagy remains poorly understood.

Thus, mitophagy appears to be regulated by two distinct pathways. Studies indicate that the Nix/BNIP3 pathway is important for mitochondrial health under baseline conditions, as demonstrated by the accumulation of dysfunctional mitochondria with age, ultimately resulting in heart failure in double knockout mice. Conversely, under stress conditions, the PINK1/Parkin pathway takes the front seat in clearing dysfunctional mitochondria. While these functions may seem distinct, there is likely substantial cross-talk between these two pathways.

It is important to note, however, that autophagy must be balanced. While minimum levels of autophagy are required for effective cellular homeostasis, excessive and chronic autophagy can result in cell death due to excessive clearance of essential proteins and organelles. Specifically in the heart, excess autophagy has been linked to myocyte death in various conditions including pressure overload [63] and myocardial ischemia/reperfusion [64].

5. Mitochondrial Fusion and Fission

Fission and fusion are important regulators of mitochondrial autophagy. These processes are tightly regulated and mediated by GTPases in the dynamin family [65]. Fusion of mitochondria involves two components to form a functional mitochondrion: fusion of the outer membranes, as well as fusion of the inner membranes. Outer membrane fusion is dependent on mitofusin 1 (Mfn1) and mitofusin2 (Mfn2) in mammals [66]. The importance of these fusion proteins is evident by mice with knockouts of these genes. Single global knockout mice of either of Mfn1 or Mfn2 die in midgestation, whereas double knockout mice die even earlier [66]. Also, cardiac specific deletion of Mfn1/Mfn2 is embryonic lethal and conditional deletion in the adult heart leads to mitochondrial dysfunction and a rapid progression to lethal dilated cardiomyopathy [67]. However, a later study by Papanicolaou et al. found that cardiac specific Mitofusin1/2 double-knockout (DKO) mice are normal at birth but quickly accumulate abnormal mitochondria in myocytes. These mice develop cardiomyopathy and do not survive past 16 days after birth [68]. Although it is unclear why

the phenotypes of the Mfn1/2 cardiac specific DKO mice differ in these two studies, both clearly demonstrate the importance of Mfn1/2 in maintaining normal mitochondrial morphology and function in myocytes.

Mitochondrial fusion prevents mitophagy and can therefore protect cells against excessive degradation of mitochondria [69]. Fusion can also help to rescue damaged mitochondria by diluting out the damaged proteins or allowing wild type mtDNA to compensate for defects [65]. However, there exists a threshold for this protective process in cells. Bhandari et al. discovered that abrogation of Parkin-mediated mitophagy in *Drosophila* leads to re-fusion of the dysfunctional mitochondria with healthy ones, resulting in extensive mitochondrial dysfunction and development of cardiomyopathy [70].

Fusion of the inner mitochondrial membranes is driven by the GTPase Opa1 [65]. It was recently found that pathological stress induces acetylation of Opa1 at lysine residues 926 and 931 in mouse hearts, impairing its function [71]. Sirt3, a protein that deacetylates Opa1, elevates its GTPase activity and protects cardiomyocytes from doxorubicin-mediated cell death. Genetic deletion of *Opa1* is embryonic lethal, though heterozygous mice display reduced mtDNA levels and develop both cardiac and mitochondrial dysfunction by 12 months of age [72].

Mitochondrial fission is mediated by cytosolic Drp1, which forms a ring around the mitochondrion's exterior and pinches it into two daughters [73]. Fission plays an important role in the quality control mechanism for mitochondria. Dysfunctional proteins can be asymmetrically segregated through unknown mechanisms to one region of the mitochondrion. Fission of the mitochondrion between these two zones produces two daughters, of which the one with higher membrane potential has a great probability of undergoing fusion [74]. Studies in mice have demonstrated that inhibition of fission with a dominant negative Drp1 mutant (Drp1 K38A) or by Fis1 RNAi results in decreased mitophagy, accumulation of oxidized mitochondrial proteins, reduced respiration, and hyperfusion of the mitochondrial network [74]. Inhibition of Drp1 with Mdivi-1 or knockdown using siRNA in myocytes shows a protective effect by preserving mitochondrial morphology, reducing cytosolic calcium, and preventing cell death after simulated ischemia/reperfusion [75]. *In vivo*, Drp1 inhibition in rat hearts improves cardiac function and reduces infarct size post I/R [76].

6. Exocytosis/Mitoptosis

A few studies have reported that fragmented mitochondria can also be eliminated from cells by being directly released into the extracellular space. Lyamazaev et al. reported that inhibition of mitochondrial respiration combined with uncoupling of oxidative phosphorylation results in clustering of fragmented mitochondria in the perinuclear region followed by their elimination in surviving HeLa cells. These mitochondrial fragments were sequestered by a membrane, (termed a "mitoptotic" body). The vesicular contents degrade into smaller vesicles that were then extruded from the cell body [77]. This form of mitochondria elimination is referred to as mitochondrial apoptosis or "mitoptosis", and is independent of autophagy.

In addition, Nakajime et al. found that fragmented mitochondria were sequestered in vacuoles that originated from the plasma membrane in cultured c-flip^{-/-} mouse embryonic fibroblasts undergoing apoptosis [78]. These vesicles fused with the plasma membrane, resulting in the release of naked mitochondria into the extracellular space. Interestingly, this mechanism of mitochondrial elimination was specific to TNF α -mediated cell death. Treatment of cells with cisplatin, which induces apoptosis by inhibiting DNA synthesis, did not induce extrusion of mitochondria. This suggests that fragmented mitochondria can be released from apoptotic cells under certain stress conditions and might be responsible for eliciting an immune response. This group also demonstrated that mitochondria are extruded by hepatocytes in C57BL/6 mice injected with an anti-Fas antibody, suggesting that this phenomenon is not limited to cultured cell lines. TNF α mediates cell death primarily via the death receptor pathway though a limitation of this study is that they did not test whether direct activation of the intrinsic or mitochondrial pathway of cell death results in extrusion of mitochondria from cells.

More recently, this phenomenon has also been described in ciliated protozoan [79]. Mitochondria are expelled in response to heat shock or by clustering of immobilization antigens and elevated intracellular calcium levels. Protozoan mitoptosis promotes cell survival and is hypothesized to be an early evolutionary adaptation for clearing damaged mitochondria. Clearly, additional studies are required to determine if this process of mitochondrial elimination also occurs in the myocardium, and what markers are responsible for delegation of mitochondria to autophagy or direct extrusion pathways. It will also be interesting to determine what effects naked versus encapsulated mitochondria have on immune and other tissues responses *in vivo*.

7. Compromised Mitochondrial Quality Control in Aging and Disease

The mechanisms underlying the development of heart failure are very complex and not fully understood. However, recent studies have provided evidence that mitochondrial dysfunction is an important contributor to both development of heart failure and the aging process. For instance, mutations in genes that disrupt mitochondrial function are associated with cardiac dysfunction in both mice [80] and humans [81]. Also, mtDNA accumulates mutations with age which leads to impaired mitochondrial respiration, increased ROS production, and development of age-related cardiomyopathy [82].

Multiple lines of evidence indicate that the accumulation of dysfunctional mitochondria in diseased or aged tissues might, in part, be due to reduced mitochondrial quality control. Several studies have reported that chronic stress and/or aging affects proteins that are involved in the mitochondrial quality control pathways (at all levels). Impairment of mitochondrial protein quality control processes that leads to mitochondrial dysfunction and reduced mitophagy will result in accumulation of dysfunctional mitochondria in the cell. First, the UPS system is important in the quality control of mitochondrial proteins, particularly those on the OMM, and studies have found that inadequate proteasomal degradation exists in animal models of heart disease and in a large subset of human failing hearts (reviewed in [83]). Dysregulation of ubiquitination or even excess proteasomal activity can similarly result in detrimental effects. Ubiquitinated proteins as well as ubiquitin

itself are elevated in ischemic and dilated cardiomyopathy [84]. Excessive activity of the proteasome is observed in and thought to contribute to several conditions including cardiac atrophy [85], pressure overload-induced hypertrophic cardiomyopathy [86], and doxorubicin-induced cardiotoxicity [87].

Other studies have reported that reduced activity of mitochondrial proteases can contribute to heart disease and aging. For instance, mice that carry a mutation in HtrA2 that inactivates its protease activity in non-neuronal tissues exhibit a phenotype of premature aging with weight loss, osteoporosis, curvature of the spine, muscle atrophy, and cardiac hypertrophy [88]. Moreover, studies have found that aging is associated with reduced Lon protease activity in various tissues. For instance, Lon levels and activity in skeletal muscle mitochondria from old mice is significantly decreased compared to young mice, which correlates with increased levels of oxidized mitochondrial proteins [89]. This study also found that increased chronic oxidative stress in MnSOD heterozygous mice exacerbates the effects of aging on reduced Lon activity. Interestingly, Deval et al. found that Lon protein levels increases with age in rat hearts while overall Lon activity remains unchanged, suggesting that there is an accumulation of inactive Lon [90]. Recently, Hoshino et al. reported that Lon is subject to oxidative modifications which attenuate its protease activity in failing mouse hearts [91]. Thus, these studies demonstrate that chronic stress and aging negatively affect proteins that are involved in mitochondrial quality control.

In addition, multiple studies indicate that autophagy diminishes with aging and it has been proposed that the reduced autophagic response contributes directly to the aging phenotype [92]. For instance, tissue-specific knockouts of essential autophagy genes results in the appearance of many age-associated problems, such as the accumulation of inclusion bodies containing ubiquitinated proteins, accumulation of lipofusins containing lysosomes, disorganized mitochondria, and increased oxidative stress [93], [94], [95] and [96]. In contrast, increased autophagy delays aging and extends longevity. For instance, caloric restriction is a potent physiological inducer of autophagy and is well known to extend life span in animals. It reduces the incidence of diabetes, cardiovascular disease, cancer, and brain atrophy [97] and [98]. Interestingly, selective activation of mitophagy by Parkin delays the aging process. Cardiac specific Parkin transgenic mice are resistant to aging and aged mice exhibit fewer dysfunctional mitochondria compared to age-matched wild type controls [99]. Similarly, Parkin overexpression extends life span and improves mitochondrial function in *Drosophila melanogaster* [100]. In contrast, Parkin deficiency results in the accrual of abnormal mitochondria in aged myocytes [101]. This suggests that specifically activating Parkin-mediated mitophagy might be cardioprotective. Balance between mitochondrial biogenesis and mitophagy is critical as excessive mitophagy can overtax the remaining mitochondria, induce damage, and even initiate apoptosis.

8. Conclusion and Future Directions

Mitochondrial health is pivotal to cellular function and it is clear that accumulation of dysfunctional mitochondria in myocytes is associated with age-related pathologies and development of heart failure. Although cells have evolved intricate systems to remove damaged mitochondrial proteins and organelles under both baseline and stress conditions,

the effectiveness of the mitochondrial quality control pathways are reduced with age and under conditions of chronic stress. While substantial progress has been made in this field, studies have only begun to unravel the diverse contribution of mitochondrial quality control mechanisms in the complex interplay between mitochondria and other compartments in the cell. To date, studies have provided us with only a glimpse into the components of the mitochondrial quality control systems. Therefore, it is important for future research to be aimed at understanding the mechanism underlying mitochondrial QC dysfunction in heart disease and aging, and to identify the molecular pathways that regulate degradation of damaged mitochondrial proteins and dysfunctional mitochondria in myocytes. It is also critical to understand how the UPS, chaperons, proteases and autophagosomes coordinate under both normal and stress conditions to maintain mitochondrial homeostasis in cells. Advances in these areas will expand our knowledge of mitochondrial quality control in the myocardium and help identify potential new proteins that can be targeted to effectively treat or prevent heart disease.

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Highlights

- Mitochondrial quality control occurs at both the protein and whole organelle levels
- Protein quality control occurs in each mitochondrial compartment
- Mitochondrial derived vesicles degrade portions of mitochondria
- Entire damaged mitochondria are degraded through mitochondrial autophagy
- Reduced mitochondrial quality control contribute to aging and disease

Figure 1.

Protein quality control in the mitochondrion. Outer mitochondrial membrane (OMM) E3 ubiquitin ligases such as March5 and MAPL tag proteins for degradation by the 26S proteasome, which is also responsible for the breakdown of the majority of ubiquitinated cytosolic proteins. Within the intermembrane space (IMS), HtrA2 is the chief protease in charge of protein degradation. Two ATPases Associated with diverse cellular Activity proteases, the matrix (m-) and the intermembrane (i-) AAA, identify misfolded polypeptides on their respective side of the IMM for degradation. Lon and ClpXP are the two most important QC proteases in the mitochondrial matrix. Lon is primarily responsible for the removal of oxidized proteins. ClpXP, composed of two ClpP subunits flanked by ClpX, plays a role in the unfolded protein response, degrading proteins unbound by chaperones (pink and green).

Figure 2.

Mitochondrial Derived Vesicles. Small vesicles containing mitochondrial proteins and lipids bud off from mitochondria under baseline conditions and under oxidative stress. Formation of these vesicles requires the presence of PINK1 and Parkin. After budding off from the mitochondria, these vesicles fuse with lysosomes for degradation.

Figure 3.

Mitochondrial Autophagy (mitophagy). **(A)** Upon loss of mitochondrial membrane potential, PINK1 accumulates on the OMM surface. PINK1 recruits Parkin, which ubiquitinates OMM proteins, thus inducing engulfment of the mitochondrion by the autophagosome through p62 and LC3. **(B)** Nix and BNIP3 function as autophagy receptors on mitochondria by binding to LC3 on the autophagosome. Both pathways result in the autophagic sequestration of the mitochondrion, fusion with a lysosome, and degradation of the organelle.

Table 1**Key Proteins and Protein Complexes Involved in Mitochondrial Quality Control**

Protein name	Unabbreviated name	Function
26S Proteasome		protease complex that degrades ubiquitinated proteins
Ambra1	Activating molecule in beclin1-regulated autophagy	promoter of phagophore nucleation
BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3	OMM pro-apoptotic BH3-only protein and mitophagy receptor
ClpXP		mitochondrial matrix QC protease, plays a role in the unfolded protein response
Drp1	Dynamin related protein 1	cytosolic protein involved in mitochondrial fission
Fis1	Fission 1	mitochondrial protein involved in fission, plays a role in recruiting Drp1 to the mitochondrial surface
Fundc1	FUN14 domain containing 1	an integral mitochondrial outer membrane protein that interacts with LC3
HAF-1		matrix peptide exporter required for mitochondrial unfolded protein response in <i>c. elegans</i>
HSP60	Heat shock protein 60	chaperone protein involved in mitochondrial protein import and folding
HSP70	Heat shock protein 70	chaperone protein involved in mitochondrial protein import and folding
HtrA2/Omi	HtrA serine peptidase 2	soluble mitochondrial IMS protease
i-AAA	Intermembrane-ATPases associated with diverse cellular activities	mitochondrial protease embedded in the IMM, with catalytic domain exposed to the intermembrane side
Lon		mitochondrial matrix protease that removes oxidized proteins, particularly iron-sulfur containing proteins such as aconitase
m-AAA	Matrix-ATPases associated with diverse cellular activities	mitochondrial protease embedded in the IMM, with catalytic domain exposed to the matrix side
March5	Membrane-associated ring finger 5	E3 ubiquitin ligase that tags proteins for degradation by the 26S proteasome
Mfn1/2	Mitofusin 1/2	Required for OMM fusion; inhibits mitophagy
Miro	Mitochondrial Rho	OMM protein that anchors mitochondria to the cytoskeleton
MPP	Mitochondrial processing peptidase/protease	soluble matrix endoprotease that cleaves off pre-sequence tags from matrix-bound proteins
MsrA/B	Methionine sulfoxide reductase A/B	Both isoforms reduce methionine sulfoxide to methionine
MULAN/MAPL	Mitochondrial ubiquitin ligase activator of NF- κ B/mitochondrial-anchored protein ligase	E3 ubiquitin ligase that promotes degradation of proteins through the 26S proteasome, mitochondrial fragmentation and mitophagy
MULE/ARF-BP1	Mcl-1 ubiquitin ligase E3/ARF-binding protein 1	BH3-only E3 ubiquitin ligase with a role in mitophagy and the degradation of the anti-apoptotic protein MCL-1
Nix/BNIP3L	Nix/BCL2/adenovirus E1B 19kDa interacting protein 3-like	OMM pro-apoptotic BH3-only protein and mitophagy receptor
Opa1	Optic atrophy 1	GTPase involved in the fusion of the IMM
p62/SQSTM1	Sequestosome 1	Adaptor protein; binds ubiquitinated proteins and autophagy proteins
Parkin		E3 ubiquitin ligase with roles in UPS-mediated protein degradation and mitophagy

Protein name	Unabbreviated name	Function
PARL	Presenilin associated rhomboid-like	mitochondrial membrane protein that is involved in the proteolytic processing of PINK1
PINK1	PTEN-inducible kinase 1	serine-threonine kinase which accumulates on depolarized mitochondria, initiating mitophagy
RNF185	Ring finger protein 185	E3 ubiquitin ligase on the OMM that regulates autophagy through BNIP1
TOM complex	Translocase of the outer membrane	protein complex in the OMM involved in the transport of protein into mitochondria
VCP/p97	Valosin containing protein/protein p97	involved in vesicle transport and fusion, and 26S proteasome function