BBA - Molecular Basis of Disease 1865 (2019) 782–796

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Mitochondrial quality control in the cardiac system: An integrative view☆

Virgilio J.J. Cadete[a,b], Goutham Vasam[c], Keir J. Menzies[c,d], Yan Burelle[a,c,*]

[a] Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada
[b] Sinclair Centre for Regenerative Medicine, Ottawa Hospital Research Institute, Ottawa, ON, Canada
[c] Interdisciplinary School of Health Sciences, Faculty of Health Sciences, University of Ottawa, Ottawa, ON, Canada
[d] Department of Biochemistry, Microbiology and Immunology, University of Ottawa Brain and Mind Research Institute and Centre for Neuromuscular Disease, University of Ottawa, Ottawa, ON, Canada

ARTICLE INFO

Keywords:
Cardiac physiology and pathophysiology
Mitochondria
Mitochondrial quality control
Mitophagy
Mitochondrial-derived vesicles
Mitochondrial proteases

ABSTRACT

Recent studies have led to the discovery of multiple mitochondrial quality control (mQC) processes that operate at various scales, ranging from the degradation of proteins by mitochondrial proteases to the degradation of selected cargos or entire organelles in lysosomes. While the mechanisms governing these mQC processes are progressively being delineated, their role and importance remain unclear. Converging evidence however point to a complex system whereby multiple and partly overlapping processes are recruited to orchestrate a cell type specific mQC response that is adapted to the physiological state and level of stress encountered. Knowledge gained from basic model systems of mQC therefore need to be integrated within organ-specific (patho)physiological frameworks. Building on this notion, this article focuses on mQC in the heart, where developmental metabolic reprogramming, sustained contraction, and multiple pathophysiological conditions pose broadly different constraints. We provide an overview of current knowledge of mQC processes, and discuss their implication in cardiac mQC under normal and diseased conditions.

1. Introduction

Mitochondria are responsible for the production of ATP through oxidative phosphorylation (OXPHOS) and also influence several other facets of cell biology, including intra- and inter-cellular metabolic fluxes, redox and Ca^{2+} homeostasis, production of physiological levels of reactive oxygen species (ROS) for cell signaling, and modulation of nuclear gene expression through genetic and epigenetic mechanisms [1,2]. Not surprisingly, dysfunction of these organelles is linked to the development of numerous diseases through a variety of mechanisms that include energetic failure, dysregulation of Ca^{2+} flux, oxidative damage, genetic reprogramming, and triggering of cell death [2–4]. Moreover, stressed mitochondria can also release pro-inflammatory bacterial-like alarmins and self-antigens that can lead to potentially adverse immune responses [5–7].

Considering the central roles played by these organelles in health and disease, cells have evolved sophisticated mechanisms to prevent the accumulation of abnormalities within the mitochondrial population. These mechanisms involve the continuous replacement of mitochondrial biomass through biogenesis, which is a consequence of the coordinated induction of key transcription factors and co-activators that collectively regulate the expression of nuclear- and mitochondria-encoded genes [8–13]. In parallel, a variety of mitochondrial quality control processes (mQC) insure the repair or removal of various constituents [8,10,12,14,15], thus completing the mitochondrial life cycle.

Over the recent years, studies have led to the discovery of multiple mQC processes that operate at various scales, ranging from the degradation of proteins mediated by intra-mitochondrial proteases to the delivery of selected cargos or entire organelles to lysosomes for degradation. While the basic molecular mechanisms governing these mQC processes are progressively being delineated, their role, level of interdependence, and physiopathological importance still remain unclear. There is increasing consensus that *in vivo* maintenance of mitochondrial health results from the coordinated action of multiple complimentary mechanisms that likely vary according to the cell type, the physiological state, and the type and impact of pathological stress. For this reason, knowledge gained from basic model systems of mQC need to be integrated within organ-specific (patho)physiological frameworks.

☆ This article is part of a Special Issue entitled: The power of metabolism: Linking energy supply and demand to contractile function edited by Torsten Doenst, Michael Schwarzer and Christine Des Rosiers.

* Corresponding author at: Interdisciplinary School of Health Sciences, Faculty of Health Sciences, University of Ottawa, RGN building, 451 Smyth Road, Ottawa, ON K1N 8M5, Canada.
E-mail address: yburell2@uottawa.ca (Y. Burelle).

https://doi.org/10.1016/j.bbadis.2018.11.018
Received 19 April 2018; Received in revised form 29 October 2018; Accepted 15 November 2018
Available online 22 November 2018
0925-4439/

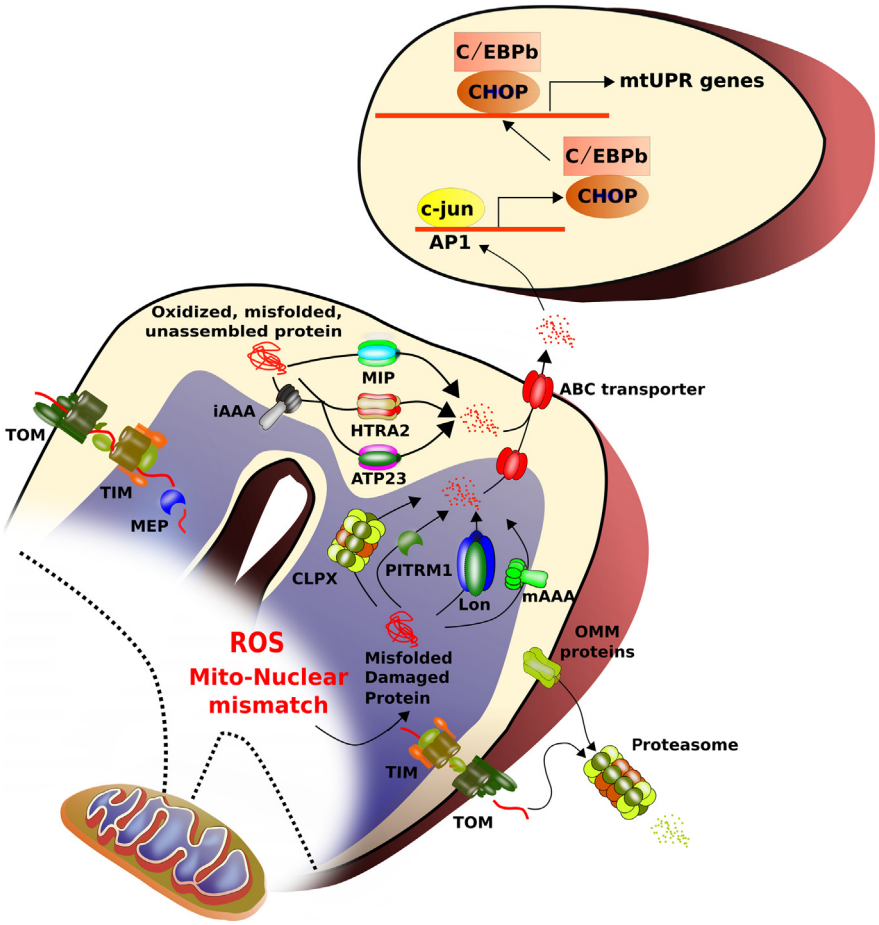

Fig. 1. Organelle quality control mediated by mitochondrial proteases and the ubiquitin-proteasome system. Mitochondrial proteases are collectively involved in the processing of newly imported polypeptides in the mitochondria during biogenesis, the degradation of non-assembled proteins that can accumulate due to mito-nuclear mismatches, and the elimination of misfolded or damaged proteins as a result of oxidative stress or other forms of proteotoxic insults. The distinct intra-mitochondrial localization of these proteases ensures compartment-specific quality control. In the matrix, proteostasis is regulated by the matrix AAA-protease (mAAA), the LON protease homologue (LONP), the ATP-dependent CLP protease proteolytic subunit (CLPP), the mitochondrial oligopeptidase (MEP), and the presequence protease PITRM1. On the inner membrane, protein surveillance is mainly exerted by the iAAA and mAAA proteases, which have their active sites oriented towards the intermembrane space or the matrix, respectively. In the intermembrane space quality control is exerted by the mitochondrial inter membrane protease Atp23 homologue (ATP23), the Ser protease HTRA2/OMI and the mitochondrial oligopeptidase (MIP). Cleavage by-products are exported to the cytosol through ABC transporters and recognized by transcription factors CHOP, C/EBPβ, and cJUN/AP1, which activate the expression of several genes of the mitochondrial unfolded protein response (mtUPR). The ubiquitin-proteasome system also plays an active role in mQC by marking and degrading proteins of the outer membrane and specific intra-mitochondrial proteins following their retrotranslocation through the TIM-TOM complex.

Building on this notion, this article focuses on mQC in the heart, where developmental metabolic reprogramming, sustained contractile activity, and multiple pathophysiological conditions pose broadly different constraints on mitochondrial biogenesis and quality control. The intention is to provide an overview of current knowledge of mitochondrial quality control systems gained from various model systems and, when data is available, to specifically discuss their potential implication in cardiac mQC under normal and diseased conditions.

2. Mitochondrial quality control: An overview

2.1. Mitochondrial proteases

Quality control by proteases and chaperones located within mitochondria constitute one of the most basic housekeeping processes (Fig. 1). Collectively, these proteases are involved in a variety of tasks, including the processing of newly imported polypeptides in the mitochondria during biogenesis, the degradation of non-assembled proteins that can accumulate due to mito-nuclear mismatches, and the elimination of misfolded or damaged proteins as a result of oxidative stress or other forms of proteotoxic insults. This protein surveillance is performed by four mitochondrial ATP-dependent proteases [15–17]: two AAA-proteases within the inner membrane (iAAA) and matrix (mAAA), along with the Lon protease homologue (LONP) and the ATP-dependent Clp protease proteolytic subunit (CLPP) that are also active in the matrix. In addition, ATP-independent proteases and oligopeptidases also participate in the process. This includes the mitochondrial inter membrane (IMM) protease Atp23 homologue (ATP23), the Ser protease HTRA2/OMI within the intermembrane space, the presequence protease PITRM1 within the matrix, and the mitochondrial oligopeptidase (MIP) within the intermembrane space [15–17]. The distinct intra-mitochondrial localization of these proteases ensures compartment-specific quality control. On the inner membrane, protein surveillance is mainly exerted by the iAAA and mAAA proteases, which have their active sites oriented towards the intermembrane space or the matrix, respectively [18]. In the matrix, LONP has a well-recognized role in the degradation of oxidized and damaged proteins, and several of its target proteins are now identified, including aconitase, cytochrome *c* oxidase subunit 4 isoform 1, steroidogenic acute regulatory protein (STAR), succinate dehydrogenase subunit 5, transcription factor A (TFAM) and glutaminase C, as well as some haeme-related enzymes such as cystathionine β-synthase, haeme oxygenase 1 and 5-aminolevulinate synthase [18–26]. In the matrix, CLPP is also involved in proteolysis by forming the CLPXP complex with the chaperone ATP-dependent CLP protease ATP-binding subunit CLPX-like (CLPX). Although its role is less well documented, CLPP is believed to play a role in the degradation of misfolded and damaged proteins [27].

Additional ATP-independent proteases also participate to the maintenance of proteostasis. In the intermembrane space, HTRA2 plays an established role in the degradation of oxidized proteins [27]. ATP23, a protease of the metallo family, is also present in the intermembrane space, but its role in mammalian cells is still unclear [28,29]. Studies in yeast suggest that it may play a role in the degradation of lipid transfer proteins in mitochondria, and the proper assembly of trans-membrane OXPHOS complexes, particularly complex V [29].

In addition to a baseline housekeeping role, mitochondrial proteases are involved in the mitochondrial unfolded protein response (mtUPR), a retrograde signaling pathway that serves to counteract mitochondrial damage (Fig. 1). This pathway is activated by mitochondrial proteotoxic stress caused by the accumulation of unfolded or unassembled proteins, by defects in components of the OXPHOS complexes, or a mitonuclear imbalance [30–32]. The mtUPR pathway has been mainly

studied in *C. elegans*, in which the process involves CLPP-mediated digestion of unfolded or unassembled matrix proteins into peptides [33,34]. These peptides are subsequently transported to the cytoplasm through mitochondrial ABC transporters (ABCme10, the homologue of Haf-1 in *C. elegans*) and recognized by transcription factors CHOP, C/EBPβ, and cJun/AP1 [33,34]. This in turn activates the expression of components of the mitochondrial protein quality control system, including mitochondrial chaperone HSP60, CLPP, LONP, mAAA (SPG7), iAAA (YME1L1), the mitochondrial processing peptidase MPPβ, and the inner membrane translocase subunit Tim17Aa [27,35]. This mtUPR response enhances the capacity of mitochondria to degrade accumulated misfolded proteins. Simultaneously, it suppresses the translation and import of several nuclear-encoded proteins until proteostasis is reestablished. In mammalian cells, the mtUPR pathway response has been mainly studied during specific proteotoxic stress induced by the expression of mutated mitochondrial proteins such as ornithine transcarbamylase (OTC) [35–37] and endonuclease G (ENDO-G) [38,39], and disruption of mitochondrial translation [40]. However, the mtUPR signaling network and its response to more physiopathologically relevant conditions remain elusive [41]. A difficulty in studying the mtUPR is to demonstrate that under the experimental conditions studied, increased expression of mitochondrial proteases and chaperones is not part of a mitochondrial biogenesis response in which case all proteins in the mitochondrial matrix would scale up together with proteases and cheperones.

2.2. Ubiquitin-proteasome

In addition to intra-organellar proteases, mitochondria also rely on the cytosolic Ubiquitin-Proteasome System (UPS) for the degradation of proteins (Fig. 1). Studies employing mitochondrial stress (*e.g.* uncoupling) show that the E3-ligase PARKIN catalyzes K63- and K47-linked polyubiquitination of mitochondrial proteins, and the recruitment of the 26S proteasome and p97, an AAA-ATPase involved in the extraction of membrane proteins [42–45]. This in turns leads to the degradation of a broad range of mitochondrial proteins, primarily located on the outer membrane including MFN1, MFN2, VDAC, FIS1 and subunits of the TOM complex [42]. Proteasomal degradation of these proteins is suggested to prime whole organelles removal through autophagy [42]. Some studies also report that matrix and inner membrane proteins can be ubiquitinated and degraded in a UPS-dependent manner. This includes OSCP, COX, UCP2, and UCP3 [46,47]. To account for this, an ERAD-like (Endoplasmic Reticulum Associated Protein Degradation) system similar to the one present in the endoplasmic reticulum has been proposed whereby matrix and inter-membrane proteins are retrotranslocated to the outer membrane for ubiquitination, and degradation by the proteasome [48]. Alternately, these proteins could also become ubiquitinated after getting stuck in the protein import channels of defective mitochondria. Identification of a specific translocation machinery will help solve this contoversy.

2.3. Mitochondrial dynamics

Mitochondrial quality control is intimately linked to its network dynamics, which results from stochastic fission and fusion events between neighboring organelles. These events are regulated by a delicate interplay between fission and fusion proteins within the double membrane system of mitochondria. Fission is triggered by the recruitment of the cytosolic cytoplasmic Dynamin-Related Protein-1 (DRP1) to the outer membrane, where it binds to human Fission protein 1 (FIS1) to form oligomeric constriction rings which allow organelle segmentation [49,50] (Fig. 2). This process is regulated by the Mitochonrial Fission Factor (MFF), which assists in the assembly of DRP1, and by the Mitochondrial Dynamics Proteins of 49 (MID49) and 51 (MID51) kDa which are suggested to play a regulatory role by recruiting DRP1 and maintaining it in an inactive state until fission is trigged by signaling events [51].

The action of these fission proteins is counterbalanced by the GTPases mitofusins (MFN1, MFN2) and Optic Atrophy Protein-1 (OPA1) which sequentially bridge the outer and inner membrane of adjacent mitochondria [52–55]. The activity of mitofusins is regulated by several post-translational modifications that affect their activity and by UPS-mediated degradation, while the activity of OPA1 is regulated through its cleavage by the zinc metalopeptidase OMA1 and AAA proteases [50,56–58], which illustrates the interplay between molecular QC by proteases and mitochondrial dynamics.

Fission and fusion have an impact on mitochondrial quality control in two major ways. First, these events are important in combining mitochondrial content between neighboring organelles, allowing for the exchange of mtDNA molecules and gene products (*e.g.* mRNA and proteins) within the mitochondrial network, for functional complementation [59–62]. When this process is inhibited, the distribution of mtDNA nucleoids [63] and respiratory chain proteins [64] is progressively altered, and mtDNA stability is reduced [65–67].

Second, the process of fission is also considered as an important mechanism by which damaged components that accumulate within an individual organelle can be segregated. This process, termed asymmetrical fission, is suggested to be an important checkpoint that determines whether mitochondria will be retained or targeted for degradation. Healthy, undamaged organelles that are able to regain their membrane potential following fission are allowed to re-integrate into the mitochondrial network, while mitochondria enriched with damaged constituents and destined to whole organelle clearance remain isolated. Loss of membrane potential and low ATP production are considered important events triggering the segregation of damaged mitochondria as both defects promote the cleavage and degradation of core fusion proteins MFNs and OPA1 [43,68]. More work is required to determine whether assymetrical fission, which has initially been described in yeast [69], is of major importance in mammalian cells and tissues. Furthermore, threshold beyond which partial loss of membrane potential and ATP flux are able to trigger the autophagic removal or defective organelles following asymetrcial fission remains unclear.

2.4. Whole organelle clearance through mitophagy

Autophagy plays a fundamental role in the degradation and recycling of cellular constituents. This ranges from protein aggregates that are too large for proteolytic dismantlement to entire organelles. This highly conserved mechanism relies on a large family of autophagy-related (ATG) proteins that activate and guide the formation of double membrane autophagosomes [70,71]. The class III PI3K complex, composed of BECLIN 1 (ATG6)/VPS34/VPS15, initiates the nucleation of the isolation membrane (vesicle nucleation step), also known as the phagophore. Subsequently, two ubiquitin-like conjugation systems, ATG12-ATG5 and ATG8/light chain 3 (LC3), guide the progressive elongation of phagophores. This promotes the interaction of soluble LC3 with phosphatidylethanolamine (PE) to form autophagosomal bound mature LC3-II [72,73]. Mature LC3-II proteins remain associated with autophagosomes where they interact with specific adaptor proteins or acceptor marks on the surface of cellular material destined for degradation. Once formed, autophagosomes fuse with lysosomes leading to the degradation of autophagosome content.

Mitochondria were originally observed in autophagosomes since the early 1960s, although the autophagic removal of mitochondria was then believed to occur non-specifically, along with other cellular material destined for degradation. However, several studies have now identified mechanisms by which mitochondria are selectively targeted for autophagy, a process which has been termed mitophagy [74,75].

2.4.1. PINK1/PARKIN-dependent mitophagy

The best characterized mechanism of mitophagy implicates the mitochondrial serine/threonine kinase PINK1 and the E3 ubiquitin-

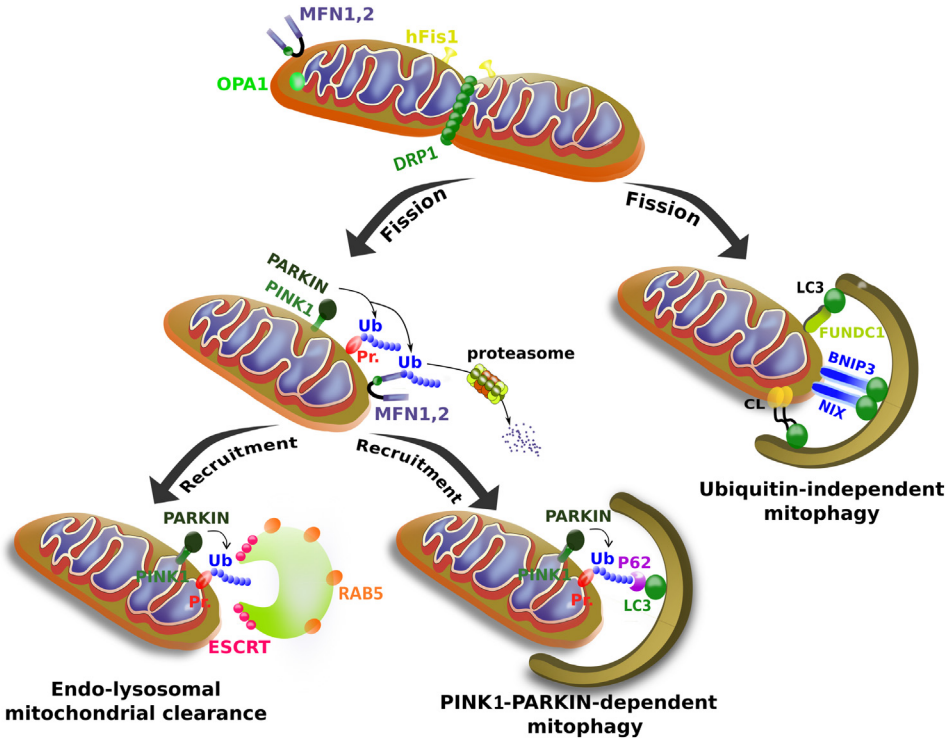

Fig. 2. Mitochondrial quality control mediated by whole organelle clearance mechanisms.
For whole organelle clearance, fission of the mitochondrial network is generally required to facilitate their engulfment. The core fission proteins Dynamin-Related Protein-1 (DRP1) controls the final step of mitochondrial fission by forming a constriction ring at ER-mitochondria contact sites, which allows to pinch off the membrane stalk between two forming daughter mitochondria. Recruitment of DRP1 is promoted by human Fission protein 1 (FIS1), a multifunctional integral protein of the outer mitochondrial membrane. Fragmented dysfunctional mitochondria are targeted for degradation through various mechanisms. 1) Ubiquitin-independent mitophagy: Several proteins that are integral (FUNDC1, FKBP8) or are recruited to the outer mitochondrial membrane (BNIP3, NIX) contain LC3 binding sites that allow direct interaction with nascent autophagosomes under conditions of stress. Similarly, cardiolipin, a phospholipid normally present in the inner membrane can be translocated to the outer membrane in response to mitochondrial dysfunction and directly bind the N-terminal region of LC3. 2) PINK1/PARKIN-dependent mitophagy: Upon loss of membrane potential, PINK1 accumulates at the surface of mitochondria where it recruits the PARKIN. Recruitment of this E3 ligase leads to the ubiquitination of several mitochondrial proteins, which in turn promote proteasomal degradation and recruitment of the core autophagy machinery, including P62 and LC3. 3) Endo-lysosomal clearance: PARKIN-dependent ubiquitination of target proteins also leads to the recruitment of RAB5-positive early endosomes which engulf mitochondria through a mechanism involving the ESCRT machinery.

ligase PARKIN [76–78] (Fig. 2). Under normal conditions, PINK1 is imported into mitochondria through the TOM import channels and rapidly degraded by the presenilin-associated rhomboid-like protein protease PARL [77]. However, in response to mitochondrial stress that leads to depolarization, typically following a sustained exposure to uncouplers, the import of PINK1 is attenuated and the full-length protein accumulates in the TOM channel with its kinase domain facing the cytosol [77]. In this conformation, PINK1 phosphorylates PARKIN which increases its ligase activity and promotes interaction with its substrates [79–82]. Several substrates are also phosphorylated by PINK1 promoting their ubiquitination by PARKIN. This is the case of MIRO, MFN1 and MFN2 [83–87] which are normally involved in mitochondrial fusion. Ubiquitination of these proteins has two important effects. First, it promotes their degradation by the proteasome, thereby reducing the capacity of defective organelles to fuse back with the mitochondrial network. Second, it promotes the recruitment of several proteins required for autophagy, including the adapter proteins BCRA1 (NBR1), sequestrosome-1 (P62/SQSTM1) and histone deacetylase-6 (HDAC6) [88–90]. BCRA1 and P62 contain ubiquitin-associated (UBA) and LC3 (LIR; LC3-interacting region) binding domains that allow them to guide the formation of nascent autophagosomes in the vicinity of defective mitochondria [88,90]. HDAC6, which also contains ubiquitin-binding activity, promotes mitochondrial clearance, possibly by promoting the fusion of autophagosomes with lysosomes [89]. Further, PARKIN can also interact with AMBRA1 (Activating Molecule in Beclin 1-Regulated Autophagy) [91] at the mitochondrial surface to force its association with the autophagy activating protein BECLIN1 [92]. Several other mitochondrial proteins have also been identified as substrates for PARKIN, including VDAC, TOM and hexokinase [93,94]. However, their role in mitophagy remains controversial [95,96].

Several cell culture studies have characterized the kinetics of PINK1/PARKIN dependent mitophagy in response to uncoupler-induced depolarization. In general, results indicate that clearance of entire mitochondria occurs between 6 and 72 h after complete depolarization [42,97–99]. While useful to compare mitophagy to other mQC mechanisms in cell culture, these estimates are difficult to translate *in vivo*, where global and sustained depolarization rarely occurs. Moreover, it is increasingly clear that the extent to which mitophagy occurs varies across cells types. In Hela cells, other cancer or transformed cell lines, in which oxidative metabolism is not essential for energy production, uncoupler-induced mitophagy leads to the complete eradication of mitochondria within 24–72 h. However, in primary cells that depend more on OXPHOS, PARKIN-dependent mitophagy occurs at much lower rates. Interestingly, if Hela cells are grown in galactose media, to force reliance on OXPHOS, PARKIN recruitment to depolarized mitochondria is drastically reduced, suggesting that the extent to which PARKIN-dependent mitophagy occurs depends in part on the bioenergetics profile of the cells [100,101].

2.4.2. *Ubiquitin-independent autophagy*

Mitophagy can also proceed through mechanisms that are independent of the PINK1/PARKIN pathway (Fig. 2). These mechanisms rely on the direct binding of LC3, present on nascent phagophores, to mitochondrial proteins and lipids. Two important proteins known to bind LC3 are BNIP3 (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3), and BNIP3L/NIX (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like protein), which are atypical members of the BCL2 protein family with BH3 domains [102–104]. Studies have shown that BNIP3 and BNIP3L/NIX are inserted in the mitochondrial outer membrane and directly bind to LC3 protein isoforms such as GABARAP (gamma-aminobutyric acid receptor-associated protein) and GABARAPL1 [105–107], which could allow the autophagic sequestration of defective mitochondria. Both sets of protein isoforms also appear to promote fission and mitophagy by stimulating the recruitment of DRP1 and PARKIN [107,108]. Importantly, prior to the discovery of their role in mitophagy, BNIP3 and BNIP3L/NIX were shown to trigger cell death [102–104] by increasing mitochondrial ROS production, and triggering opening of the permeability transition pore [104,108–110]. Therefore,

BNIP3 and BNIP3L/NIX likely trigger mitophagy indirectly by causing mitochondrial dysfunction. Similar to BNIP3 and BNIP3L/NIX, the outer mitochondrial proteins FUNDC1 (FUN14 domain containing 1) and FKBP8 (FK506 binding protein 8) also contains an LIR domain that allows direct binding to LC3. Recent studies indicate that FUNDC1 promotes mitophagy under hypoxic conditions [111–114], while FKBP8 was shown to respond to uncoupling [114]. However, the role of FUNDC1 and FKBP8 under other pro-mitophagic states remains unknown.

Ubiquitin-independent mitophagy is not strictly mediated through protein-protein interactions. Cardiolipin, a phospholipid of the mitochondrial inner membrane, can also act as a direct interactor for mitophagy [115]. Under conditions of stress such as complex I inhibition (rotenone), uncoupling (CCCP) and induction of apoptosis (staurosporine), cardiolipin molecules can relocalize to the mitochondrial outer membrane where they directly bind the N-terminal region of autophagosome-bound LC3 [115]. Interestingly, under stress conditions, oxidation of externalized cardiolipin is known to trigger the formation of pores in the outer membrane and the release of apoptotic factors including cytochrome *c* [116]. Therefore, cardiolipin relocalization and oxidation level may act as a rheostat that can induce apoptosis when mitophagic clearance of damaged organelles is overwhelmed.

2.5. Autophagy-independent mitochondrial clearance

2.5.1. Endo-lysosomal pathway

In addition to autophagy-mediated mechanisms, the endo-lysosomal pathway, which is known to play a role in cellular quality control by degrading damaged or excess plasma membrane proteins [117], was recently suggested to participate in the clearance of whole mitochondria [99] (Fig. 2). This study showed that following uncoupling with FCCP, Rab-5 positive early-endosomes are recruited to mitochondria along with the PIP3-producing VPS34 PIK3-BECLIN1 complex. Mitochondria are then internalized into endosomal membranes by the Endosomal Sorting Complexes Required for Transport (ESCRT) machinery, which is responsible for the invagination and subsequent scission of the endosomal membrane [118]. Following maturation into Rab9-positive late endosomes, mitochondria are then delivered to lysosomes for degradation.

Interestingly, the recruitment of the ESCRT machinery to mitochondria seems to depend on the ubiquitination of surface proteins by PARKIN, indicating a degree of overlap between the autophagic and the endo-lysosomal pathway of mitochondrial clearance [99]. However, PARKIN-dependent clearance of mitochondria through this pathway does not depend on the core-fission protein DRP1, suggesting that the fragmentation process required to engulf mitochondria in early endosomes occurs through other unknown mechanisms [99]. Interestingly, kinetic analysis revealed that Rab5-positive early endosomes are recruited to mitochondria 2–4 h after depolarization, compared to 8–24 h for LC3, suggesting that activation of this pathway precedes mitophagy [99].

2.5.2. Mitochondrial-derived vesicles

Bacteria commonly generate small vesicles containing specific cargos destined for transport within the bacterial colony as well as to host cells [119]. Interestingly, this capacity is conserved in eukaryotic cells as mitochondria were recently shown to generate Mitochondrial-Derived-Vesicles (MDVs) [120]. These MDVs which bud off the mitochondrial surface are 70–150 nm in size, composed of one or two membranes, and enriched with distinct mitochondrial proteins [120] (Fig. 3). Three different fates have been identified for MDVs. The first fate is the transport of cargos to peroxisomes [121], which share several functions with mitochondria including ROS and fatty acid metabolism. The second fate is the presentation of mitochondrial antigens at the cell surface, which occurs in response to mitochondrial stress and may promote auto-immunity [7]. The third fate is the incorporation of MDVs into late endosomes for lysosomal degradation, pointing to a role for this pathway in mQC [98,122] (Fig. 3).

Studies in cell models indicate that MDVs are produced at a significant rate under baseline conditions, particularly when cells are gown in galactose-containing media, which forces cellular dependence on oxidative metabolism, and therefore enhances wear and tear of mitochondrial proteins [122,123]. This suggests that MDV production may be involved in mitochondrial housekeeping under normal conditions [120]. Importantly, the production of these vesicles increases above baseline rates within minutes following induction of mitochondrial stress (*vs* hours–days for mitophagy) [42,97–99], suggesting that it acts as a first line of defense against stress [98,122,123]. This response occurs in the absence of mitochondrial depolarization and mitochondrial fragmentation [122,123] and does not require the core mitochondrial fission protein DRP1, nor the autophagy machinery [122] which indicates that MDV production is distinct from mitophagy.

The molecular mechanisms involved in the production of MDVs are not well defined [120]. *In vitro* reconstitution experiments performed on isolated mitochondria show that intra-mitochondrial and extra-mitochondrial oxidative stress leads to the formation of MDVs containing distinct protein markers arising either from the matrix or the outer-membrane, which suggest that MDV content varies according to the nature of stress [124], thereby providing capacity for tailored mQC. These experiments also indicate that MDVs are enriched with oxidized proteins compared to control mitochondrial-derived MDVs, which is compatible with a role in mQC [124]. Reconstitution assays also show that MDV production by isolated mitochondria is catalyzed by the cytosolic extract, indicating that budding depends on some factors present in the cytoplasm [124,125]. Recent studies showed that PARKIN is involved, which would point to a dual role of this protein in mQC (*i.e.* MDVs and mitophagy) [98] (Fig. 3). However, PARKIN only regulates a subset of MDVs carrying matrix cargos (*e.g.* Pyruvate Dehydrogenase [PDH^+] [98]). Furthermore, by focusing on other vesicle transport paradigms in cells, it is apparent that PARKIN likely represents the tip of the iceberg. In addition to cargo selection mechanisms and trigger signals, the MDV pathway requires mechanisms that facilitate membrane curvature, potential coating complexes, incorporation of fusion machinery, and motility factors [120]. In this regard, recent data shows that MDV formation is dependent on the presence of specific members of the sorting nexins (SNX9) and RAB protein families (RAB9), which are generally known for their role in vesicle formation [7] (Fig. 3). These studies indicate that in response to oxidative stress, SNX9 and RAB9 are recruited to mitochondria where they promote budding of MDVs [7].

3. Mitochondrial quality control in the healthy and diseased heart: Maintenance, reprogramming, and response to injury

mQC has been a topic of growing interest in the field of cardiology. While important progress has been made, specifically in the study of mitophagy, and mitochondrial dynamics, our understanding is far from complete due to a lack of information on several of the above mentioned mQC processes. Moreover, research in the area has often been guided by concepts developed using cell lines and model systems, which do not take into consideration aspects of cardiac (patho)physiology that likely have a major effect on mQC. In this section, we briefly discuss important characteristics of mitochondrial biology in the developing, healthy adult, and diseased heart to expose the different constraints imposed on mitochondrial quality control. We also discuss the potential implications of specific mQC processes during these key periods.

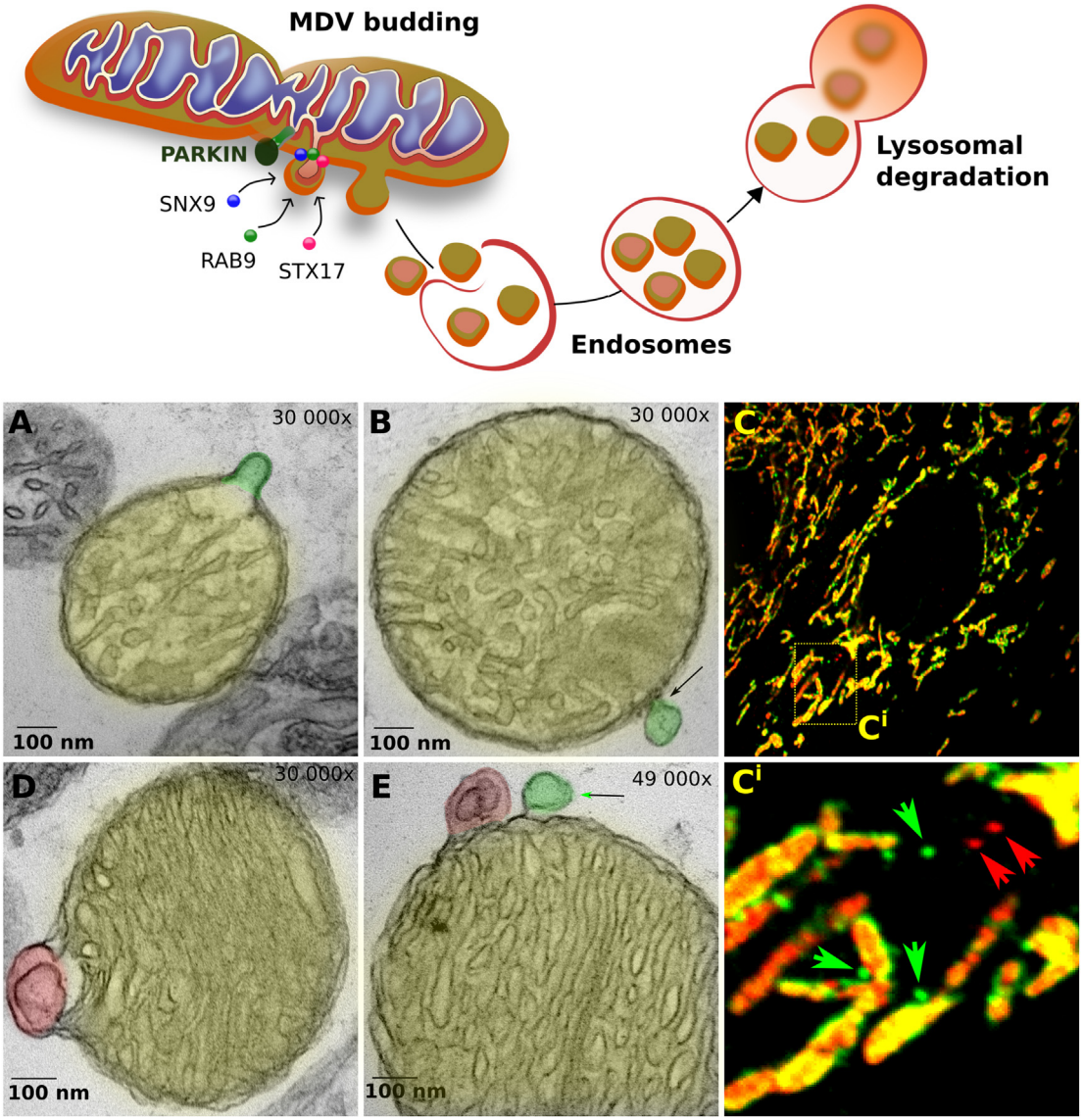

Fig. 3. Mitochondrial quality control *via* vesicle budding.
Mitochondria release small, mitochondrial-derived vesicles (MDVs) from their membranes to deliver specific mitochondrial contents to the late endosome/multivesicular bodies for subsequent lysosomal degradation. MDVs have a diameter of 70–150 nm as observed by electron microscopy and can be formed of a single membrane derived from the outer mitochondrial membrane, or a double membrane formed by the inner and outer membrane (in green and red respectively on TEM image of isolated cardiac mitochondria). These vesicles can also be distinguished from mitochondria by their selective and high enrichment for markers of the matrix such as PDH or the outer mitochondrial membrane such as TOM20 (in red and green respectively on the confocal images of H9c2 cardioblasts). Formation of MDVs involves the transient recruitment of SNX9, RAB9 and STX17, which are believed to assist the budding process. Interestingly, PARKIN regulates the production of a subset of MDVs carrying matrix cargos, suggesting a dual role of PARKIN in mitophagy and MDV production.

3.1. Cardiac cell differentiation and perinatal metabolic reprogramming

3.1.1. Mitochondrial phenotype in the developing myocardium

The heart is a unique organ in which it has a constant need for energy to sustain contractile activity, starting at early stages of embryonic development all the way through adulthood. To maintain physiological contractile demands, the adult heart requires an average of 6 kg of ATP/day, most of which is produced by oxidative phosphorylation within the mitochondria [126]. Cardiac energy metabolism, however, varies depending on the developmental stage. Pluripotent stem cells, that give rise to cardiac progenitors during fetal development, posess immature mitochondria which are characterized by low mtDNA copy number, perinuclear localization, smaller size, low number of cristae and absence of elaborate network formation [127]. This bioenergetics phenotype is maintained in immature cardiomyocytes that are less competent in oxidative metabolism and more dependent on glycolytic energy production [127–130]. This metabolic profile has been suggested to be a requisite for cardiomyocytes to maintain a proliferative potential in the fetal heart since it is compatible with high biosynthetic activity and cardiogenesis. As gestation reaches completion cardiomyocytes fully differentiate and lose their proliferative capacity. In the mouse heart this occurs just prior to birth and is accompanied by significant changes in mitochondrial morphology and function [131] with the development of complex networks of cristae-dense mitochondria (Fig. 4). This maturation of the mitochondrial network and bioenergetics shift is necessary for cardiomyocyte differentiation and is further supported by birth. Birth represents a sudden increase in oxygen saturation and shift in energy substrate availability towards fatty acids, which further favors the development of oxidative phosphorylation. Despite this sudden change in energetic environment, the cardiomyocyte and the mitochondria slowly adapt. Electron micrographs from mouse hearts show that 3 days after birth it

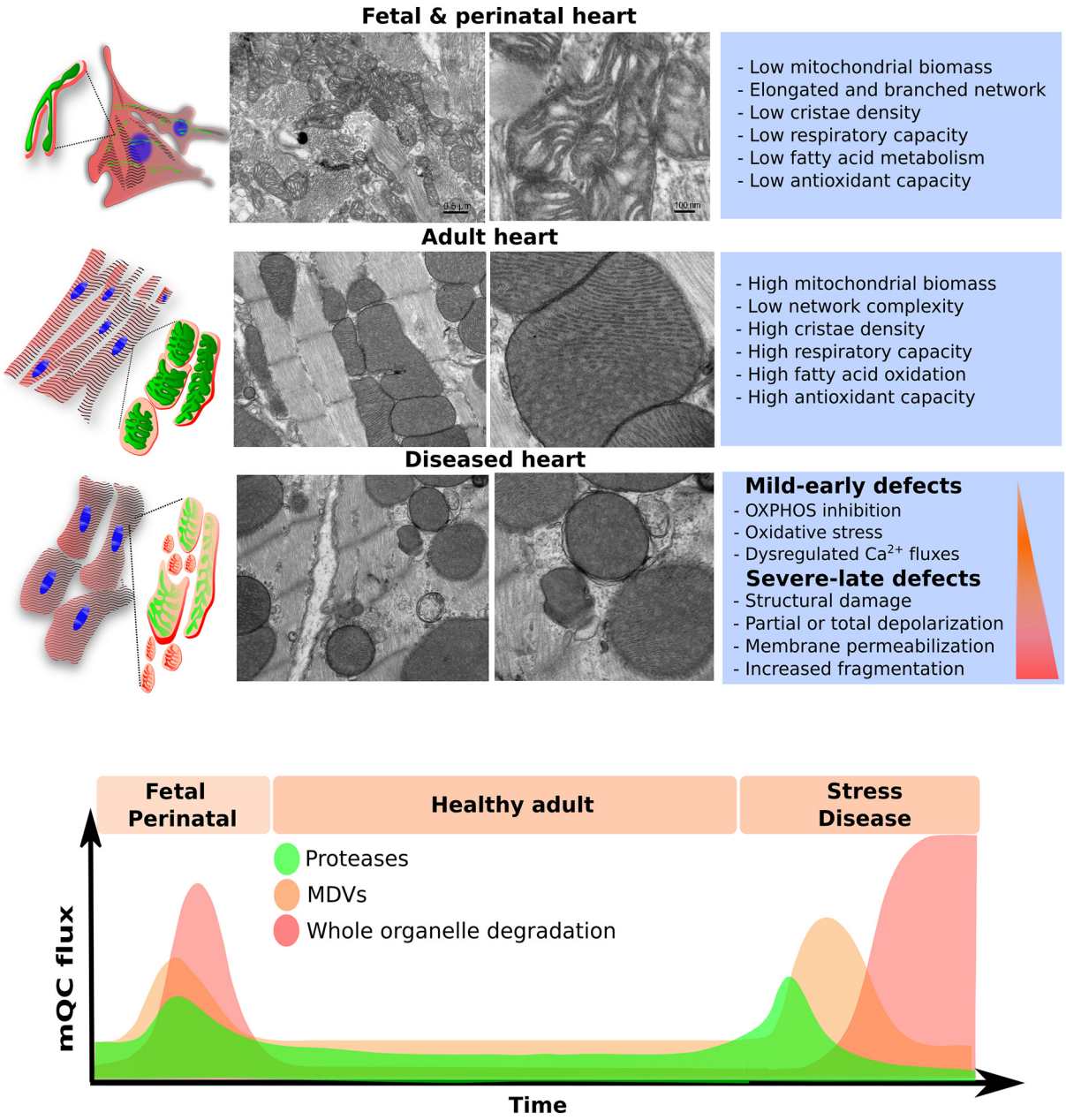

Fig. 4. Integrative view of mQC in the normal and diseased heart.
The top part presents the mitochondrial phenotype typically observed in the normal heart during the fetal/perinatal stage, and the adult period as well as the early and late stages of several cardiac diseases. This overview illustrates that broad differences exist between each of these periods, which likely impose unique constraints on the various mQC systems. The bottom part presents a view of how proteases, MDVs and whole organelle clearance mechanism may contribute to the maintenance of mitochondrial health at each of these stages and insure transition from one stage to the other. During perinatal maturation, mitochondrial fission/fusion, and upregulation of mitophagy appear to be of importance for mass-remodeling of immature mitochondria, while mitochondrial proteases, and MDV formation may play a fine-tuning role by degrading specific mitochondrial proteins. In the mature heart, where mitochondrial turnover is slow under normal situations, mitophagy, although active, likely has a more limited role in the maintenance of baseline mitochondrial homeostasis. In this condition, more specific mQC mechanisms such as protease-based systems and MDV formation likely play a significant housekeeping role. Under pathological conditions, the heart likely relies on multiple and partly overlapping strategies to limit the accumulation of mitochondrial defects.

is still possible to identify smaller, circular mitochondria with sparse cristae in cardiomyocytes with larger cytoplasmic areas [132]. By 7 days the cytoplasmic areas are reduced, interactions between myofilaments and mitochondria are increased, and cristae show higher electron density. Regular cardiomyocyte morphology is present at 21 days post birth which coincides with the onset of weaning, another marked event of change in energy substrate availability. Despite the limited data available on energy metabolism in the newborn heart, it appears that, at least in the rabbit heart, maturation of mitochondria and normal cardiac morphology is accompanied by a maturation of glucose and fatty acid oxidation pathways [133] (Fig. 4). By 21 days, the newborn rabbit heart derives ~90% of the ATP required from oxidative metabolism of lactate, glucose and fatty acids, similar to what is seen in the adult heart.

3.1.2. *Mitochondrial quality control in the developing myocardium*

The extensive remodeling of the mitochondrial network and the profound morphological and molecular adaptations that mark the

Table 1
Impact of genetic ablation of mQC-related protein on cardiac development and baseline functions.

Ref	Gene-KO	Time of KO	Viability	Cardiac phenotype
		Fission/Fusion proteins		
Song et al. [134]	DRP1 (cardiac specific)	Perinatal	Viable	Early lethal cardiomyopathy
Kageyama et al. [135]	DRP1 (cardiac specific)	Perinatal	Viable	Early lethal cardiomyopathy
Kasahara et al. [190]	MFN1 and 2 (cardiac specific)	Embryonic	Lethal	Developmental arrest
Papanicolaou et al. [136]	MFN1 and 2 (cardiac specific)	Embryonic	Lethal	Developmental arrest
		Perinatal	Viable	Early lethal cardiomyopathy
Song et al. [134]	MFN1–2 (cardiac specific)	Perinatal	Viable	Cardiac hypertrophy
Piquereau et al. [156]	OPA1 (germline)	Embryonic	Lethal	
	OPA1(germline heterozygous)	Embryonic	Viable	Cardiac and mitochondrial dysfunction at 6 months
Quiros et al. [191]	OMA1 (germline)	Embryonic	Viable	Not examined
Wai et al. [157]	YME1L1 (germline)	Embryonic	Lethal	
	YME1L1 (cardiac specific)	Embryonic	Viable	Dilated cardiomyopathy at 40 weeks
		Mitophagy related proteins		
Gong et al. [144]	PARKIN (cardiac specific)	Perinatal	Viable	Early lethal cardiomyopathy
Piquereau et al. [137]	PARKIN (germline)	Embryonic	Viable	No apparent phenotype
Kubli et al. [138]	PARKIN (germline)	Embryonic	Viable	No apparent phenotype
Billia et al. [139]	PINK1 (germline)	Embryonic	Viable	Cardiac hypertrophy at 2 Mo
Diwan et al. [140]	NIX (cardiac specific)	Embryonic	Viable	Late onset cardiomyopathy
Dorn et al. [141]	BNIP3 (germline)	Embryonic	Viable	Late onset cardiomyopathy
	BNIP3-NIX (germline-cardiac specific)	Embryonic	Viable	Late onset cardiomyopathy
		Mitochondrial proteases		
Seiferling et al. [40]	CLPP (muscle specific)	Embryonic	Viable	No apparent phenotype
Gispert et al. [150]	CLPP (germline)	Embryonic	Viable	No apparent phenotype
Martins et al. [151]	HTRA2 (germline)	Embryonic	Viable	Reduced heart weight
Song et al. [134]	DRP1 (cardiac specific)	Adult		Dilated cardiomyopathy in 8 weeks
Ikeda et al. [158]	DRP1 (cardiac specific)	Adult		Dilated cardiomyopathy in 8 weeks

transition from fetal to extra-uterine life point to a central involvement of pathways governing mitochondrial biogenesis, dynamics and mQC.

In line with this notion, cardiac specific deletion of the core fission protein DRP1 during the perinatal period is lethal [134,135]. Similarly, cardiac specific deletion of the core fusion proteins MFN1 and MFN2 in embryonic cardiac myocytes arrests cardiac development and causes embryonic lethality [136]. Loss of these fusion proteins during the perinatal period results in severe dilated cardiomyopathy accompanied by the proliferation of heterogeneously shaped mitochondria, impaired expression of PGC1α, reduced levels of mitochondrial DNA-encoded OXPHOS complexes, and accumulation of P62 and LC3-II [136]. These alterations indicate that mitochondrial dynamics is active during the embryonic and perinatal period and is essential for metabolic maturation likely by permitting morphological remodeling, effective activation of mitochondrial biogenesis, mtDNA complementation, and the removal of unwanted organelles (Table 1).

On the other hand, germline inactivation of several mitophagy regulating proteins, including PARKIN, PINK1, BNIP3 and NIX has no measurable impact on cardiac development [137–141], which would suggest that mitophagy is dispensable for metabolic maturation. However, the presence of developmental compensation has been reported in some of these mice models [137,142,143], which may contribute to underestimating the role of mitophagy. More recent studies indicate that PARKIN plays a critical role in perinatal metabolic reconfiguration. PARKIN expression is upregulated during the perinatal period [144], while its expression is constitutively low in the adult heart [145]. Furthermore, silencing of PARKIN during this critical period leads to cardiomyopathy, failed maturation of mitochondrial morphology, and perinatal mortality [144]. This phenotype is also observed in transgenic mice in which a non-phosphorylatable form of MFN2, which prevents PARKIN recruitment, is expressed perinatally [144] (Table 1). Detailed analyses performed in these transgenic mice show that the absence of morphological maturation of mitochondria is accompanied by mitochondrial dysfunction and a failure of metabolic gene reprogramming [144]. Because PARKIN is expressed at normal levels in the heart of *Mfn2* transgenic mice, the phenotype is likely attributable to defective mitophagy, and not to other functions of PARKIN. These data would therefore suggest that PARKIN-dependent mitophagy is essential to allow large scale replacement of "fetal" mitochondria by more oxidatively competent "adult" organelles [144] (Fig. 4). Alternately, these results could point to an essential role of PARKIN-dependent degradation of MFN2 in mitochondrial/metabolic remodeling independent of mitophagy, a possibility that has not formally been ruled out. It is also important to mention that PARKIN plays a permissive role for mitochondrial biogenesis by targeting PARIS, a transcriptional repressor of PGC1α, to proteasomal degradation [146]. Therefore, the upregulation of PARKIN expression during cardiac maturation may also promote metabolic reprogramming through this mechanism. Furthermore, the endo-lysosomal and MDV mQC pathways, which are both regulated by PARKIN [98,99], could play a role.

In this regard, we recently found that MDV release is substantially enhanced when H9c2 cardioblasts are forced into differentiation [123]. In H9c2 cells, differentiation is accompanied by significant reprogramming towards oxidative metabolism, which not only implies an increase in mitochondrial content and function, but also significant changes in their molecular makeup, requiring an increase in both protein biosynthesis and elimination [147–149]. Thus, increased MDV formation during H9c2 differentiation may reflect the increasing metabolic activity of mitochondria, and the subsequent need for degradation of specific subsets of proteins [69]. One hypothesis could be that mitophagy allows bulk clearance of entire organelles, while the MDV pathway could allow a fine tuning through the degradation of specific mitochondrial proteins.

Limited information is available regarding the role of mitochondrial proteases during cardiac maturation (Table 1). As discussed in section 2.2, proteomics analysis of mitochondrial protein turnover in adult hearts point to an important role of the protease system in the turnover of OXPHOS components, which suggest that they may also be important for metabolic maturation. However, unequivocal evidence from genetic mouse models of mitochondrial protease deficiency are still missing. Indeed, recent studies show that mitochondrial respiratory function and overall cardiac phenotype is normal in mice harboring ubiquitous or cardiomyocyte specific disruption of CLPP [40,150]. The only defect reported is a mild reduction in mitochondrial supercomplexes [150], but this is not consistent [40]. Interestingly, proteins and/or transcript levels for mitochondrial chaperones and proteases, including HSP60,

LONP1, AFG3L2, and YME1l1 are unchanged in CLPP-deficient hearts [40], suggesting that there is no compensatory upregulation of the mtUPR pathway. It should however be mentioned that CLPP is acting mainly on soluble matrix proteins, which may explain the relatively mild effect of CLPP inactivation on OXPHOS complexes and bioenergetics integrity. Results from another study indicate that germline deletion of the iAAA protease HTRA2 results in a reduction in the weight of several organs including the heart [151]. The cardiac phenotype was not studied further as mice died at 4 weeks of age from a severe neurological phenotype. Therefore, it remains unclear whether reduced cardiac mass was due to failed mQC, or altered regulation of apoptosis, which is another well-established function of HTRA2 [152]. Overall, the results available suggest that CLPP is dispensable for normal metabolic/mitochondrial maturation of the heart, perhaps because during this intense period of metabolic remodeling, bulk removal of whole organelles plays a predominant role compared to specific molecular quality control processes. Further studies are required to delineate the role and importance of mitochondrial proteases for cardiac maturation. The use of inducible cardiac specific knockout approaches during the perinatal period may again be required to limit opportunities for developmental compensation or severe systemic phenotypes.

3.2. Mitochondrial maintenance in the healthy adult heart

3.2.1. Mitochondrial phenotype in the healthy adult myocardium

In the normal, adult heart mitochondria are more abundant than during fetal life and account to more than 30% of cardiomyocyte volume [132]. The transition from fetal to adult is characterized by an increase in mitochondrion size and cristae density, and accompanied by the optimization of oxidative phosphorylation through the expression of specific sets of proteins (Fig. 4) to sustain increasing energetic requirements. Fatty-acid oxidation enzymes and transporters are highly expressed compared to fetal mitochondria [133], and their phosphorylation machinery is tightly coupled to SR and myosin ATPases through the expression of mitochondrial creatine kinase [132], which is not present during the fetal and perinatal period. Furthermore, adult organelles are endowed with a greater anti-oxidant capacity that matches the higher density of ROS producing ETC complexes. This provides enhanced resistance to oxidative stress, which is important considering that cardiac mitochondrial proteins are turned over at a rate (average half-life = 17 days) that is four fold lower than in other organs such as the liver (average half-life = 4 days) [153].

3.2.2. Mitochondrial quality control in the healthy adult myocardium

Routine maintenance of such a large pool of highly specialized and metabolically active organelles must rely on efficient quality control mechanisms.

Several pieces of evidence point to an important role of mitochondrial dynamics in the maintenance of baseline mitochondrial health (Table 1). Despite important cytoarchitectural constraints limiting mitochondrial mobility, fission and fusion between neighboring organelles is frequent in adult cardiomyocytes, and is coordinated with SR Ca^{2+} release and re-uptake [154]. In line with the importance of this process, partial loss of the inner membrane fission protein OPA1 in heterozygous *Opa1*$^{+/-}$ mice causes mild contractile dysfunction, accumulation of small fragmented mitochondria with abnormal cristae morphology, reduced respiratory capacity, enhanced ROS production, and susceptibility to PTP opening [155,156]. Accumulation of small fragmented mitochondria is also observed following genetic activation of OMA1, the protease involved in OPA1 processing in the heart [157]. This study shows that ablation of YME1L1, which increases the activity of OMA1, results in greater processing of the long OPA1 form and enhanced mitochondrial fragmentation, a switch towards fetal metabolic profile and the development of heart failure by 40 weeks of age [157]. Interestingly, these alterations occur despite *in vitro* mitochondrial respiration and ETC enzyme activities being normal, which suggest that unopposed fission and subtle mitochondrial mechanisms other than overt disruption of the OXPHOS machinery likely underlie the development of heart failure in absence of YME1L1.

Cardiac specific deletion of the core fission protein DRP1 during adulthood is also detrimental and causes dilated cardiomyopathy and heart failure within 7–8 weeks [134,158]. *Drp1*-KO hearts display increased susceptibility to PTP opening [134,158], reduced ETC complex activity [158], lower ATP production [158], impaired respiration [135,158], and enhanced ROS production [134]. Combined with the above mentioned studies, these data convincingly show that fission and fusion are important for the maintenance of baseline mitochondrial function in the adult heart. However, whether this is due to impaired functional complementation, aberrant cristae remodeling, failed mitophagy, or other unknown mechanisms that depend on competent fission/fusion remains unclear.

Several studies performed in the adult heart have also focused on mitophagy, but considerable debate exists regarding the underlying pathways and the quantitative importance of this process for baseline mitochondrial housekeeping (Table 1). The emerging view is however that mitophagy does not play a major role under normal conditions. Indeed, quantitative TEM imaging indicate that autophagosomes containing only mitochondria are extremely rare in the healthy heart [123,137]. Recent studies using transgenic mice that express fluorescent mitophagy reporters (*e.g.* mQC [mitochondria-targeted mCherry-GFP] or mitoKeima) report more frequent mitophagy events compared to TEM imaging [159–161]. However, direct comparison across tissues show that the mitophagy index measured with this method is three to ten fold lower in the heart than in most other tissues analyzed including retina, pancreas, cerebral vasculature, microglia, and midbrain region [159].

Additional evidence against a central role of mitophagy in baseline mQC is the fact that mice with a germline or cardiac-specific knockout of PARKIN [137,138,145], or BNIP3 [140] do not develop spontaneous cardiac phenotypes, and have little or no mitochondrial defects at baseline. Mice harboring a germline deletion of PINK1 do develop spontaneous cardiomyopathy by 6–8 months of age, which is accompanied by mitochondrial dysfunction [139]. However, a recent study using the mQC reporter mouse has shown that baseline mitophagy is similar in WT and *Pink*$^{-/-}$ mice, suggesting that the cardiac and mitochondrial phenotype in these mice is not related to impaired mitophagy [159]. Redundancy in the regulation of mitophagy may account in part for the relatively mild phenotype observed at baseline. This redundancy has been observed in germline PARKIN [137] and PINK1 [159] knockout mice. It was also suggested as a potential explanation for why mitochondrial dysfunction is absent in the heart of BNIP3 knockout mice unless NIX is also inactivated [141].

Convincing evidence against a major role for mitophagy in baseline cardiac mQC also comes from protein turnover studies using Silac or Heavy Water labeling. These proteomic studies show that the half-life of individual mitochondrial proteins in the healthy adult heart varies from a few hours up to two months [153,162]. Moreover, specific analysis of the OXPHOS machinery reveals that the turnover of respiratory chain subunits varies considerably (up to 7-fold) within each complex [163]. Interestingly this variation is dependent on the location of subunits (matrix facing *vs* membrane integral), their evolutionary origin, the location of protein encoding (mtDNA *vs* nDNA), and the ubiquitination level [163]. In addition to challenging the notion that mitochondria are turned-over a single units through mitophagy, these data therefore suggest that there are common rules governing the differential clearance of mitochondrial proteins through proteolysis, and possibly through MDV formation.

Beyond two studies performed in germline CLPP deficient mice [40,150], little information is yet available regarding the role of specific mitochondrial proteases in baseline mQC in the adult heart (Table 1). These studies report that the absence of CLPP leads to a progressive impairment in the assembly of supercomplexes between 6 and 16 weeks

Table 2
Impact of genetic ablation of mQC-related proteins on the cardiac response to stress.

Reference	Gene-KO	Insult	Cardiac phenotype
		Fission/Fusion proteins	
Ikeda et al. [158]	DRP1 (Cardiac specific)	Ischemia-reperfusion	Enhanced dysfunction and infarct size
Piquereau et al. [156]	OPA1 (germline, heterozygous)	Thoracic aortic constriction	Enhanced hypertrophy and cardiac dysfunction
		Mitophagy related proteins	
Kubli et al. [138]	PARKIN (germline)	Infarction	Enhanced cardiac injury and mitochondrial dysfunction
Piquereau et al. [137]	PARKIN (germline)	Sepsis with *E. coli* LPS	Impaired recovery of cardiac and mitochondrial functions
Hoshino et al. [175]	PARKIN (germline)	Doxorubicin	Enhanced cardiac dysfunction and mitochondrial abnormalities
Shirakabe et al. [176]	DRP1 (germline heterozygous)	Thoracic aortic constriction	Enhanced cardiac dysfunction with impaired mitophagy

of age, and a mild reduction of OXPHOS capacity in presence of complex I but not complex II substrates [150]. These data are compatible with a role of CLPP in the maintenance of OXPHOS complexes that require a balance between mtDNA and nuclear-encoded subunits for proper assembly. It should be mentioned that these studies did not determine whether mitophagy or other mQC mechanisms were upregulated in CLPP-deficient mice, which could contribute to the observed mild phenotype. As mentioned previously, the mild phenotype may also be explained by the fact that CLPP is acting mainly on soluble matrix proteins and therefore does not overtly disrupt the integrity of OXPHOS complexes. Future studies targeting other mitochondrial proteases will likely confirm the central role of the protease system for basal cardiac mQC.

Very little is known on the role of MDVs in the heart. However, in a recent study our laboratory found that MDVs enriched with cargos destined to lysosomes are constitutively generated under baseline conditions in H9c2 cardioblasts [123]. The rate at which these vesicles are produced is enhanced when glucose is replaced by galactose in the culture media, indicating that the production of these vesicles is increased under conditions of sustained oxidative metabolism, which causes accelerated wear and tear of the mitochondrial machinery. This study also used electron microscopy approaches including quantitative TEM, immunogold-labeling, and electron tomography to visualize and quantify MDV budding in the adult heart. This analysis, performed on entire reconstituted myocytes from multiple hearts, showed that mitochondria commonly shed vesicles that display all of the characteristics of MDVs, namely a diameter of 70–150 nm, a single or a double membrane, and immuno-gold labeling for PDH and TOM20, two markers of MDVs destined for lysosomal degradation [123]. Interestingly, MDV budding events were far more numerous than mitochondria-containing autophagosomes [123], which is in line with the notion that the MDV pathway is constitutively active, and may play a role in baseline mQC.

3.3. Response during cardiac stress

3.3.1. Mitochondrial dysfunction in the diseased myocardium

Mitochondrial dysfunction is implicated in the pathogenesis of numerous cardiac diseases which commonly progress towards heart failure [3,4,10]. This includes overload-induced hypertrophy [164], diabetic cardiomyopathy [165], ischemia-reperfusion injury [3,4,166], post-infarction remodeling [103], sepsis-induced myocardial dysfunction [137,167,168], and cardiotoxicity induced by chemotherapeutic drugs such as anthracyclines, and tyrosine kinase inhibitors [169–171]. Despite their heterogeneous etiology, the development of these cardiac disorders is linked to common mitochondrial mechanisms (Fig. 4). Neuro-humoral activation, chronic exposure to inflammatory cytokines, hypoxia or toxic agents can all lead to OXPHOS inhibition, dysregulation of Ca^{2+} fluxes, and increased generation of ROS which damages mitochondrial DNA, proteins and membrane lipids [3]. Damage to these components can in turn create a vicious cycle of increasing organelle dysfunction, which leads to overt defects that include structural alterations, partial or total loss of membrane potential, and membrane permeabilization of mitochondria, along with activation of inflammation, apoptosis and necrotic cell death [3–5,172]. In most pathologies, this vicious cycle develops progressively in a limited number of vulnerable organelles and is initially contained by mQC processes. However, if the level of stress is overwhelming and mQC processes are insufficient or fail then damage spreads to a larger proportion of the mitochondrial population, which likely explains why the accumulation of overt mitochondrial defects is a general characteristic of late stage cardiac diseases [3,164].

3.3.2. Mitochondrial quality control in the diseased myocardium

Mounting an adequate mQC response to stress therefore represents a major determinant of how the mitochondrial population will fare under conditions of cardiac disease. Although much is currently unexplored, evidence indicates that multiple mQC mechanisms are activated in these conditions.

Several studies indicate that cardiac mitophagy is stimulated in response to various stressors (Table 2). This is observed in cell models in response to uncoupling, complex I inhibition with rotenone [173], and chemical simulated ischemia-reperfusion [174]. It is also observed in the intact heart in response to doxorubicin administration [123], ischemia-reperfusion (I-R) [138], and sepsis [137]. The PINK1-PARKIN pathway appears to play an important role in triggering stress-induced mitophagy. This is supported by data showing that myocardial PARKIN expression and localization to the mitochondria, normally very low under baseline conditions, are strongly upregulated when the heart is exposed to adverse conditions [137,138,145]. Furthermore, several studies show that germline or cardiac specific deletion of PARKIN or PINK1 exacerbates mitochondrial and cardiac dysfunction induced by pathological stress [137,138,175]. Similar observations were made following downregulation of the core fission protein DRP1, which lies immediately upstream of PINK1. Reduced levels of DRP1 blunts mitophagy and exacerbates cardiac dysfunction following I-R or pressure overload [158,176].

In contrast to cardiac stressors that result in energetic starvation, energetic overload (*i.e.* lipotoxicity, hyperglycemia, diabetes) triggers a distinct mQC response. Hyperglycemia and lipid overload induce DRP1-mediated mitochondrial fission accompanied by mitochondrial dysfunction that can be rescued by overexpression of dominant-negative DRP1-K38E [177,178]. Unfortunately, the impact of mitochondrial dynamics on mitophagy was not reported in these studies. Despite these reports on mitochondrial dynamics, little is known about the impact of metabolic diseases on mitochondrial clearance and overall mQC in the heart. The few available reports suggest that in the diabetic heart mitophagy is inhibited due to reduced PINK1/PARKIN expression (for review see [179]) Further studies are necessary to elucidate the interplay between energy substrate, mitochondria dynamics and mitophagy in the heart.

While the role of PARKIN in the heart is established, it should be noted that stress-induced mitophagy can still occur in the absence of PARKIN [137,138], indicating the presence of redundant mechanisms. Data from our laboratory shows that BNIP3, NIX and the core autophagy machinery is recruited to mitochondria in wild type, and PARKIN

deficient mice during sepsis [137]. Other studies also show that BNIP3 is recruited to mitochondria in response to I-R, where it stimulates mitophagy [180,181]. These data thus suggest that ubiquitin-independent mitophagy can be activated in parallel to the PINK1-PARKIN pathway to clear damaged organelles. Of note, recruitment of BNIP3 and NIX to mitochondria during stress can also trigger cell death through mitochondrial membrane permeabilization [102,104,182], which suggests that these proteins may cause dysfunction of individual organelles, and simultaneously promote their clearance. Other mechanisms that could contribute to PARKIN-independent mitophagy are the externalization of cardiolipin on the mitochondrial outer membrane [115], as well as binding of the mitophagy adaptor proteins FUNDC1 and FKBP8 to LC3 [111,112,114,183]. Although alterations in cardiolipin content and oxidation status occur in several heart diseases [184], the role of this phospholipid, and of FUNDC1 or FKBP8 in cardiac mitophagy remains to be elucidated.

The possibility that excessive autophagy contributes to disease progression has been raised in the literature. Autophagic cell death, also called autosis, has initially been described in the context of high levels of autophagy triggered by an autophagy-inducing peptides [185]. Evidence indicate that excessive activation of macro-autophagy can occur in the heart in response to severe hemodynamic stress [186] and during reperfusion following ischemia [187]. Studies suggest that Beclin1 is an important regulator of this maladaptive response since knockdown of this protein decreases cardiomyocyte autophagy and cell death, while overexpression of Beclin1 exacerbates the response. However, we are not aware of any study in which excessive mitophagy was observed. Therefore, most evidence to date suggest that activation of mitophagy is a protective response to cardiac stress.

In addition to mitophagy, recent data suggest that in cardiac cells, mitochondrial elimination following simulated I-R is also mediated by the endo-lysosomal pathway [99]. An intriguing aspect of this mechanism is that although it requires PARKIN to proceed, its activation in response to depolarization of the mitochondrial population is more rapid than mitophagy, suggesting a partial overlap of the two pathways [99]. However, the implication of the endo-lysosomal pathway in cardiac mQC *in vivo* remains to be assessed.

Recent data indicate that in the cardiac system, upregulation of MDV production occurs in response to stress [123,124]. In H9c2 cardioblasts, MDV production is rapidly increased within 30–60 min of exposure to antimycin-A or Xanthine/Xanthine Oxidase [123]. A similar response is also observed when cells are incubated with the cardiotoxic chemotherapy compound doxorubicin [123]. Quantitative analysis of mitochondrial morphology shows that this MDV response is maximal within minutes-hours, prior to bioenergetic dysfunction and fission of the mitochondrial network [123]. This is consistent with data reporting that the activation of the MDV pathway is independent of mitophagy, and occurs on a significantly shorter time scale (*i.e.* minutes-hours *vs* hours-days), making this pathway a possible first line of defense against mitochondrial stress [98]. Importantly, this study also shows that the MDV budding rate more than doubles in isolated mitochondria of mice heart following acute administration of doxorubicin [123], suggesting that the pathway is active and dynamic *in vivo* [123].

Little information is currently available on the involvement of mitochondrial proteases in the cardiac response to stress. A recent study showed that LON expression is increased in the heart following I-R, where it drives the degradation of selected COX subunits [188]. The targeting mechanism likely involves post-translational modifications of COX subunits, such as PKA-mediated phosphorylation [188]. Another study reported that LON and CLPP expression is upregulated in mice harboring a cardiac-specific deletion of Frataxin, a protein involved in the assembly of iron-sulfur cluster-dependent proteins, which include CI, CII, CIII, and aconitase [189]. In this model, the triggering signal is likely loss of proteostasis, secondary to failed assembly of these enzymes. Although much remains to be learned about these mitochondrial proteases, an interesting hypothesis is that during stress these proteases may initiate mtUPR signaling and perhaps prime mitochondria for MDV formation by partially degrading proteins.

4. Conclusion

Mitochondrial quality control is a burgeoning field of research. In the coming years, studies in cellular models will undoubtedly increase our understanding of the triggers and molecular mechanisms involved in cardiac mQC. A major challenge will be to consolidate these mechanistic insights into a knowledge framework that integrates the unique properties of mitochondrial biology and the dynamic physiology of cardiac development throughout life. Thus far, current knowledge suggests that maintenance of cardiac mitochondrial health relies on multiple overlapping mechanisms that each play a role according to the developmental stage, the physiological state, and the type of pathological stress (Fig. 4).

The majority of individual mQC mechanisms appear to be tightly associated with the specific cardiac developmental stage. During embryonic development and perinatal maturation, mitochondrial fission/fusion and mitophagy are of vital importance for extensive remodeling of fetal mitochondria, while mitochondrial proteases and MDV formation may fine tune mQC by degrading specific mitochondrial proteins. In contrast, as the heart metabolically matures, mitochondrial turnover and mitophagy slow down and more specific mQC mechanisms, such as MDV and protease-based systems, likely play an active housekeeping role. Under pathological conditions, the heart appears to be capable of enhancing these partly overlapping processes to limit the accumulation of mitochondrial defects. Available data indicates that such processes might involve upregulation of MDV production and LON activity, as well as stimulation of whole organelle clearance through the endo-lysosomal and various mitophagy pathways. Interestingly, these systems are activated by distinct mechanisms, and operate over different time scales, suggesting a hierarchy in the mQC response to stress.

The development of more advanced experimental strategies, namely the use of conditional transgenic animal models, enhanced imaging techniques and importantly, measurement of individual protein turnover rate, will be essential for the true understanding of the role of mQC in cardiac pathophysiology.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgements

We apologize in advance for not being able to cite all important primary work that has contributed to our understanding of mitochondrial quality control mechanisms and their implication in cardiac physiology and pathophysiology.

We thank the Facility for Electron Microscopy Research (FEMR), McGill University, in particular Jeannie Mui for technical support and TEM imaging. We thank Dr. Jérôme Piquereau for providing the TEM images of neonatal cardiac mitochondria presented in Fig. 4. This work was funded by grants from the Canadian Institutes of Health Research (MOP 136999), and the Natural Sciences and Engineering Research Council of Canada (RGPIN 2016-09932) to YB. YB is a University of Ottawa Chair in Integrative Mitochondrial Biology.

References

[1] D.C. Wallace, W. Fan, Energetics, epigenetics, mitochondrial genetics, Mitochondrion 10 (2010) 12–31, https://doi.org/10.1016/j.mito.2009.09.006.
[2] H. Lemieux, C.L. Hoppel, Mitochondria in the human heart, J. Bioenerg. Biomembr. 41 (2009) 99–106, https://doi.org/10.1007/s10863-009-9211-0.
[3] Y. Burelle, M. Khairallah, A. Ascah, B.G. Allen, C.F. Deschepper, B.J. Petrof, et al., Alterations in mitochondrial function as a harbinger of cardiomyopathy: lessons

from the dystrophic heart, J. Mol. Cell. Cardiol. 48 (2010) 310–321, https://doi.org/10.1016/j.yjmcc.2009.09.004.
[4] J. Marín-García, M.J. Goldenthal, Mitochondrial centrality in heart failure, Heart Fail. Rev. 13 (2008) 137–150, https://doi.org/10.1007/s10741-007-9079-1.
[5] T. Oka, S. Hikoso, O. Yamaguchi, M. Taneike, T. Takeda, T. Tamai, et al., Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure, Nature 485 (2012) 251–255, https://doi.org/10.1038/nature10992.
[6] Z. Kaya, C. Leib, H.A. Katus, Autoantibodies in heart failure and cardiac dysfunction, Circ. Res. 110 (2012) 145–158, https://doi.org/10.1161/CIRCRESAHA.111.243360.
[7] D. Matheoud, A. Sugiura, A. Bellemare-Pelletier, A. Laplante, C. Rondeau, M. Chemali, et al., Parkinson's disease-related proteins PINK1 and Parkin repress mitochondrial antigen presentation, Cell 166 (2016) 314–327, https://doi.org/10.1016/j.cell.2016.05.039.
[8] M. Crow, K. Mani, Y.J. Nam, R.N. Kitsis, The mitochondrial death pathway and cardiac myocyte apoptosis, Circ. Res. 95 (2004) 957–970.
[9] D.A. Hood, Invited review: contractile activity-induced mitochondrial biogenesis in skeletal muscle, J. Appl. Physiol. 90 (2001) 1137–1157.
[10] M.P. Mattson, G. Kroemer, Mitochondria in cell death: novel targets for neuroprotection and cardioprotection, Trends Mol. Med. 9 (2003) 196–205.
[11] C.D. Moyes, D.A. Hood, Origins andc onsequences ofm itochondrialv ariation inv ertebratem uscle, Annu. Rev. Physiol. 65 (2003) 177–201, https://doi.org/10.1146/annurev.physiol.65.092101.142705.
[12] D.R. Green, The pathophysiology of mitochondrial cell death, Science 305 (2004) 626–629, https://doi.org/10.1126/science.1099320.
[13] D.P. Kelly, Transcriptional regulatory circuits controlling mitochondrial biogenesis and function, Genes Dev. 18 (2004) 357–368, https://doi.org/10.1101/gad.1177604.
[14] R.A. Gottlieb, R.S. Carreira, Autophagy in health and disease. 5. Mitophagy as a way of life, Am. J. Physiol. Cell Physiol. 299 (2010) C203–C210, https://doi.org/10.1152/ajpcell.00097.2010.
[15] T. Tatsuta, T. Langer, Quality control of mitochondria: protection against neurodegeneration and ageing, EMBO J. 27 (2008) 306–314, https://doi.org/10.1038/sj.emboj.7601972.
[16] P.M. Quirós, T. Langer, C. López-Otín, New roles for mitochondrial proteases in health, ageing and disease, Nat. Rev. Mol. Cell Biol. 16 (2015) 345–359, https://doi.org/10.1038/nrm3984.
[17] E.I. Rugarli, T. Langer, Focus ReviewMitochondrial quality control: a matter of life and death for neurons, EMBO J. 31 (2012) 1336–1349, https://doi.org/10.1038/emboj.2012.38.
[18] F. Gerdes, T. Tatsuta, T. Langer, Mitochondrial AAA proteases — towards a molecular understanding of membrane-bound proteolytic machines, Biochim. Biophys. Acta, Mol. Cell Res. 1823 (2012) 49–55, https://doi.org/10.1016/j.bbamcr.2011.09.015.
[19] H. Teng, B. Wu, K. Zhao, G. Yang, L. Wu, R. Wang, Oxygen-sensitive mitochondrial accumulation of cystathionine β-synthase mediated by Lon protease, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 12679–12684, https://doi.org/10.1073/pnas.1308487110.
[20] A. Bezawork-Geleta, T. Saiyed, D.A. Dougan, K.N. Truscott, Mitochondrial matrix proteostasis is linked to hereditary paraganglioma: LON-mediated turnover of the human flavinylation factor SDH5 is regulated by its interaction with SDHA, FASEB J. 28 (2014) 1794–1804, https://doi.org/10.1096/fj.13-242420.
[21] K. Kita, T. Suzuki, T. Ochi, Diphenylarsinic acid promotes degradation of glutaminase C by mitochondrial Lon protease, J. Biol. Chem. 287 (2012) 18163–18172, https://doi.org/10.1074/jbc.M112.362699.
[22] R. Fukuda, H. Zhang, J.-W. Kim, L. Shimoda, C.V. Dang, G.L. Semenza, HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells, Cell 129 (2007) 111–122, https://doi.org/10.1016/j.cell.2007.01.047.
[23] D.A. Bota, K.J.A. Davies, Lon protease preferentially degrades oxidized mitochondrial aconitase by an ATP-stimulated mechanism, Nat. Cell Biol. 4 (2002) 674–680, https://doi.org/10.1038/ncb836.
[24] Z. Granot, O. Kobiler, N. Melamed-Book, S. Eimerl, A. Bahat, B. Lu, et al., Turnover of mitochondrial steroidogenic acute regulatory (StAR) protein by lon protease: the unexpected effect of proteasome inhibitors, Mol. Endocrinol. 21 (2007) 2164–2177, https://doi.org/10.1210/me.2005-0458.
[25] Q. Tian, T. Li, W. Hou, J. Zheng, L.W. Schrum, H.L. Bonkovsky, Lon Peptidase 1 (LONP1)-dependent breakdown of mitochondrial 5-aminolevulinic acid synthase protein by heme in human liver cells, J. Biol. Chem. 286 (2011) 26424–26430, https://doi.org/10.1074/jbc.M110.215772.
[26] Y. Matsushima, Y.-I. Goto, L.S. Kaguni, Mitochondrial Lon protease regulates mitochondrial DNA copy number and transcription by selective degradation of mitochondrial transcription factor A (TFAM), Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 18410–18415, https://doi.org/10.1073/pnas.1008924107.
[27] C.M. Haynes, K. Petrova, C. Benedetti, Y. Yang, D. Ron, ClpP mediates activation of a mitochondrial unfolded protein response in *C. elegans*, Dev. Cell 13 (2007) 467–480, https://doi.org/10.1016/j.devcel.2007.07.016.
[28] C. Potting, C. Wilmes, T. Engmann, C. Osman, T. Langer, Regulation of mitochondrial phospholipids by Ups1/PRELI-like proteins depends on proteolysis and Mdm35, EMBO J. 29 (2010) 2888–2898, https://doi.org/10.1038/emboj.2010.169.
[29] C. Osman, C. Wilmes, T. Tatsuta, T. Langer, Prohibitins interact genetically with Atp23, a novel processing peptidase and chaperone for the F1Fo-ATP synthase, Mol. Biol. Cell 18 (2007) 627–635, https://doi.org/10.1091/mbc.E06-09-0839.
[30] R.H. Houtkooper, L. Mouchiroud, D. Ryu, N. Moullan, E. Katsyuba, G. Knott, et al., Mitonuclear protein imbalance as a conserved longevity mechanism, Nature 497 (2013) 451–457, https://doi.org/10.1038/nature12188.
[31] E.D. Runkel, S. Liu, R. Baumeister, E. Schulze, Surveillance-activated defenses block the ROS-induced mitochondrial unfolded protein response, PLoS Genet. 9 (2013) e1003346, , https://doi.org/10.1371/journal.pgen.1003346.
[32] Q. Zhao, J. Wang, I.V. Levichkin, S. Stasinopoulos, M.T. Ryan, N.J. Hoogenraad, A mitochondrial specific stress response in mammalian cells, EMBO J. 21 (2002) 4411–4419, https://doi.org/10.1093/emboj/cdf445.
[33] C.M. Haynes, Y. Yang, S.P. Blais, T.A. Neubert, D. Ron, The matrix peptide exporter HAF-1 signals a mitochondrial UPR by activating the transcription factor ZC376.7 in *C. elegans*, Mol. Cell 37 (2010) 529–540, https://doi.org/10.1016/j.molcel.2010.01.015.
[34] C.M. Haynes, D. Ron, The mitochondrial UPR - protecting organelle protein homeostasis, J. Cell Sci. 123 (2010) 3849–3855, https://doi.org/10.1242/jcs.075119.
[35] J.E. Aldridge, T. Horibe, N.J. Hoogenraad, Discovery of genes activated by the mitochondrial unfolded protein response (mtUPR) and cognate promoter elements, PLoS One 2 (2007) e874, , https://doi.org/10.1371/journal.pone.0000874.s004.
[36] T. Horibe, N.J. Hoogenraad, The chop gene contains an element for the positive regulation of the mitochondrial unfolded protein response, PLoS One 2 (2007) e835, , https://doi.org/10.1371/journal.pone.0000835.g003.
[37] E. Rath, E. Berger, A. Messlik, T. Nunes, B. Liu, S.C. Kim, et al., Induction of dsRNA-activated protein kinase links mitochondrial unfolded protein response to the pathogenesis of intestinal inflammation, Gut 61 (2012) 1269–1278, https://doi.org/10.1136/gutjnl-2011-300767.
[38] L. Papa, D. Germain, Estrogen receptor mediates a distinct mitochondrial unfolded protein response, J. Cell Sci. 124 (2011) 1396–1402, https://doi.org/10.1242/jcs.078220.
[39] L. Papa, D. Germain, SirT3 regulates the mitochondrial unfolded protein response, Mol. Cell. Biol. 34 (2014) 699–710, https://doi.org/10.1128/MCB.01337-13.
[40] D. Seiferling, K. Szczepanowska, C. Becker, K. Senft, S. Hermans, P. Maiti, et al., Loss of CLPP alleviates mitochondrial cardiomyopathy without affecting the mammalian UPR mt, EMBO Rep. 17 (2016) 953–964, https://doi.org/10.15252/embr.201642077.
[41] V. Jovaisaite, J. Auwerx, The mitochondrial unfolded protein response—synchronizing genomes, Curr. Opin. Cell Biol. 33 (2015) 74–81, https://doi.org/10.1016/j.ceb.2014.12.003.
[42] N.C. Chan, A.M. Salazar, A.H. Pham, M.J. Sweredoski, N.J. Kolawa, R.L.J. Graham, et al., Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy, Hum. Mol. Genet. 20 (2011) 1726–1737, https://doi.org/10.1093/hmg/ddr048.
[43] A. Tanaka, M.M. Cleland, S. Xu, D.P. Narendra, D.-F. Suen, M. Karbowski, et al., Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin, J. Cell Biol. 191 (2010) 1367–1380, https://doi.org/10.1083/jcb.201007013.
[44] N. Nakamura, S. Hirose, Regulation of mitochondrial morphology by USP30, a deubiquitinating enzyme present in the mitochondrial outer membrane, Mol. Biol. Cell 19 (2008) 1903–1911, https://doi.org/10.1091/mbc.E07-11-1103.
[45] S. Xu, G. Peng, Y. Wang, S. Fang, M. Karbowski, The AAA-ATPase p97 is essential for outer mitochondrial membrane protein turnover, Mol. Biol. Cell 22 (2011) 291–300, https://doi.org/10.1091/mbc.E10-09-0748.
[46] V. Azzu, M.D. Brand, Degradation of an intramitochondrial protein by the cytosolic proteasome, J. Cell Sci. 123 (2010) 578–585, https://doi.org/10.1242/jcs.081976.
[47] V. Azzu, S.A. Mookerjee, M.D. Brand, Rapid turnover of mitochondrial uncoupling protein 3, Biochem. J. 426 (2010) 13–17, https://doi.org/10.1042/BJ20091321.
[48] E.B. Taylor, J. Rutter, Mitochondrial quality control by the ubiquitin–proteasome system, Biochem. Soc. Trans. 39 (2011) 1509–1513, https://doi.org/10.1083/jcb.201008084.
[49] S.A. Detmer, D.C. Chan, Functions and dysfunctions of mitochondrial dynamics, Nat. Rev. Mol. Cell Biol. 8 (2007) 870–879, https://doi.org/10.1038/nrm2275.
[50] H. McBride, V. Soubannier, Mitochondrial function: OMA1 and OPA1, the grandmasters of mitochondrial health, Curr. Biol. 20 (2010) R274–R276, https://doi.org/10.1016/j.cub.2010.02.011.
[51] O.C. Losón, Z. Song, H. Chen, D.C. Chan, Fis1, Mff, MiD49, and MiD51 mediate Drp1 recruitment in mitochondrial fission, Mol. Biol. Cell 24 (2013) 659–667, https://doi.org/10.1091/mbc.E12-10-0721.
[52] T. Koshiba, S.A. Detmer, J.T. Kaiser, H. Chen, J.M. McCaffery, D.C. Chan, Structural basis of mitochondrial tethering by mitofusin complexes, Science 305 (2004) 858–862, https://doi.org/10.1126/science.1099793.
[53] N. Ishihara, Mitofusin 1 and 2 play distinct roles in mitochondrial fusion reactions via GTPase activity, J. Cell Sci. 117 (2004) 6535–6546, https://doi.org/10.1242/jcs.01565.
[54] M. Sardiello, M. Palmieri, A. di Ronza, D.L. Medina, M. Valenza, V.A. Gennarino, et al., A gene network regulating lysosomal biogenesis and function, Science 325 (2009) 473–477, https://doi.org/10.1126/science.1174447.
[55] J.R. Friedman, L.L. Lackner, M. West, J.R. Dibenedetto, J. Nunnari, G.K. Voeltz, ER tubules mark sites of mitochondrial division, Science 334 (2011) 358–362, https://doi.org/10.1126/science.1207385.
[56] M. Käser, M. Kambacheld, B. Kisters-Woike, T. Langer, Oma1, a novel membrane-bound metallopeptidase in mitochondria with activities overlapping with the m-AAA protease, J. Biol. Chem. 278 (2003) 46414–46423, https://doi.org/10.1074/jbc.M305584200.
[57] T.D.B. MacVicar, J.D. Lane, Impaired OMA1-dependent cleavage of OPA1 and reduced DRP1 fission activity combine to prevent mitophagy in cells that are dependent on oxidative phosphorylation, J. Cell Sci. 127 (2014) 2313–2325,

https://doi.org/10.1242/jcs.144337.
[58] R. Anand, T. Wai, M.J. Baker, N. Kladt, A.C. Schauss, E. Rugarli, et al., The i-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission, J. Cell Biol. 204 (2014) 919–929, https://doi.org/10.1083/jcb.201308006.
[59] T. Ono, K. Isobe, K. Nakada, J.I. Hayashi, Human cells are protected from mitochondrial dysfunction by complementation of DNA products in fused mitochondria, Nat. Genet. 28 (2001) 272–275, https://doi.org/10.1038/90116.
[60] K. Nakada, K. Inoue, T. Ono, K. Isobe, A. Ogura, Y.I. Goto, et al., Inter-mitochondrial complementation: mitochondria-specific system preventing mice from expression of disease phenotypes by mutant mtDNA, Nat. Med. 7 (2001) 934–940, https://doi.org/10.1038/90976.
[61] Y.-J. Lee, S.-Y. Jeong, M. Karbowski, C.L. Smith, R.J. Youle, Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis, Mol. Biol. Cell 15 (2004) 5001–5011, https://doi.org/10.1091/mbc.E04-04-0294.
[62] H. Chen, D.C. Chan, Physiological functions of mitochondrial fusion, Ann. N. Y. Acad. Sci. 1201 (2010) 21–25, https://doi.org/10.1111/j.1749-6632.2010.05615.x.
[63] H. Chen, J.M. McCaffery, D.C. Chan, Mitochondrial fusion protects against neurodegeneration in the cerebellum, Cell 130 (2007) 548–562, https://doi.org/10.1016/j.cell.2007.06.026.
[64] H. Chen, M. Vermulst, Y.E. Wang, A. Chomyn, T.A. Prolla, J.M. McCaffery, et al., Mitochondrial fusion is required for mtdna stability in skeletal muscle and tolerance of mtDNA mutations, Cell 141 (2010) 280–289, https://doi.org/10.1016/j.cell.2010.02.026.
[65] C. Rouzier, S. Bannwarth, A. Chaussenot, A. Chevrollier, A. Verschueren, N. Bonello-Palot, et al., The MFN2 gene is responsible for mitochondrial DNA instability and optic atrophy "plus" phenotype, Brain 135 (2012) 23–34, https://doi.org/10.1093/brain/awr323.
[66] P. Yu-Wai-Man, K.S. Sitarz, D.C. Samuels, P.G. Griffiths, A.K. Reeve, L.A. Bindoff, et al., OPA1 mutations cause cytochrome c oxidase deficiency due to loss of wild-type mtDNA molecules, Hum. Mol. Genet. 19 (2010) 3043–3052, https://doi.org/10.1093/hmg/ddq209.
[67] P. Amati-Bonneau, M.L. Valentino, P. Reynier, M.E. Gallardo, B. Bornstein, A. Boissiere, et al., OPA1 mutations induce mitochondrial DNA instability and optic atrophy "plus" phenotypes, Brain 131 (2008) 338–351, https://doi.org/10.1093/brain/awm298.
[68] G. Twig, A. Elorza, A.J.A. Molina, H. Mohamed, J.D. Wikstrom, G. Walzer, et al., Fission and selective fusion govern mitochondrial segregation and elimination by autophagy, EMBO J. 27 (2008) 433–446, https://doi.org/10.1038/sj.emboj.7601963.
[69] P. Katajisto, J. Dohla, C.L. Chaffer, N. Pentinmikko, N. Marjanovic, S. Iqbal, et al., Asymmetric apportioning of aged mitochondria between daughter cells is required for stemness, Science 348 (2015) 340–343, https://doi.org/10.1126/science.1260384.
[70] Y. Dong, V.V. Undyala, R.A. Gottlieb, R.M. Mentzer, K. Przyklenk, Review: autophagy: definition, molecular machinery, and potential role in myocardial ischemia-reperfusion injury, J. Cardiovasc. Pharmacol. Ther. 15 (2010) 220–230, https://doi.org/10.1177/1074248410370327.
[71] R.A. Gottlieb, R.M. Mentzer Jr., Autophagy during cardiac stress: joys and frustrations of autophagy, Annu. Rev. Physiol. 72 (2010) 45–59, https://doi.org/10.1146/annurev-physiol-021909-135757.
[72] Y. Kabeya, N. Mizushima, T. Ueno, A. Yamamoto, T. Kirisako, T. Noda, et al., LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing, EMBO J. 19 (2000) 5720–5728, https://doi.org/10.1093/emboj/19.21.5720.
[73] T. Kirisako, Y. Ichimura, H. Okada, Y. Kabeya, N. Mizushima, T. Yoshimori, et al., The reversible modification regulates the membrane-binding state of Apg8/Aut7 essential for autophagy and the cytoplasm to vacuole targeting pathway, J. Cell Biol. 151 (2000) 263–276.
[74] R.J. Youle, D.P. Narendra, Mechanisms of mitophagy, Nat. Rev. Mol. Cell Biol. 12 (2011) 9–14, https://doi.org/10.1038/nrm3028.
[75] T. Saito, J. Sadoshima, Molecular mechanisms of mitochondrial autophagy/mitophagy in the heart, Circ. Res. 116 (2015) 1477–1490, https://doi.org/10.1161/CIRCRESAHA.116.303790.
[76] D.P. Narendra, S.M. Jin, A. Tanaka, D.-F. Suen, C.A. Gautier, J. Shen, et al., PINK1 is selectively stabilized on impaired mitochondria to activate Parkin, PLoS Biol. 8 (2010) e1000298, , https://doi.org/10.1371/journal.pbio.1000298.s013.
[77] S.M. Jin, M. Lazarou, C. Wang, L.A. Kane, D.P. Narendra, R.J. Youle, Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL, J. Cell Biol. 191 (2010) 1509–1513, https://doi.org/10.1083/jcb.201008084.dv.
[78] S.M. Jin, R.J. Youle, The accumulation of misfolded proteins in the mitochondrial matrix is sensed by PINK1 to induce PARK2/Parkin-mediated mitophagy of polarized mitochondria, Autophagy 9 (2014) 1750–1757, https://doi.org/10.4161/auto.26122.
[79] Y. Kim, J. Park, S. Kim, S. Song, S.-K. Kwon, S.-H. Lee, et al., PINK1 controls mitochondrial localization of Parkin through direct phosphorylation, Biochem. Biophys. Res. Commun. 377 (2008) 975–980, https://doi.org/10.1016/j.bbrc.2008.10.104.
[80] L.A. Kane, M. Lazarou, A.I. Fogel, Y. Li, K. Yamano, S.A. Sarraf, et al., PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity, J. Cell Biol. 205 (2014) 143–153, https://doi.org/10.1083/jcb.201402104.
[81] F. Koyano, K. Okatsu, H. Kosako, Y. Tamura, E. Go, M. Kimura, et al., Ubiquitin is phosphorylated by PINK1 to activate Parkin, Nature 510 (2014) 162–166, https://doi.org/10.1038/nature13392.
[82] A. Kazlauskaite, V. Kelly, C. Johnson, C. Baillie, C.J. Hastie, M. Peggie, et al., Phosphorylation of Parkin at Serine65 is essential for activation: elaboration of a Miro1 substrate-based assay of Parkin E3 ligase activity, Open Biol. 4 (2014) 130213, https://doi.org/10.1098/rsob.130213.
[83] L.A. Kane, R.J. Youle, PINK1 and Parkin flag miro to direct mitochondrial traffic, Cell 147 (2011) 721–723, https://doi.org/10.1016/j.cell.2011.10.028.
[84] Y. Chen, G.W. Dorn, PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria, Science 340 (2013) 471–475, https://doi.org/10.1126/science.1231031.
[85] M.E. Gegg, J.M. Cooper, K.Y. Chau, M. Rojo, A.H.V. Schapira, J.W. Taanman, Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/Parkin-dependent manner upon induction of mitophagy, Hum. Mol. Genet. 19 (2010) 4861–4870, https://doi.org/10.1093/hmg/ddq419.
[86] A.C. Poole, R.E. Thomas, S. Yu, E.S. Vincow, L. Pallanck, The mitochondrial fusion-promoting factor mitofusin is a substrate of the PINK1/Parkin pathway, PLoS One 5 (2010) e10054, , https://doi.org/10.1371/journal.pone.0010054.s001.
[87] L. Glauser, S. Sonnay, K. Stafa, D.J. Moore, Parkin promotes the ubiquitination and degradation of the mitochondrial fusion factor mitofusin 1, J. Neurochem. 118 (2011) 636–645, https://doi.org/10.1111/j.1471-4159.2011.07318.x.
[88] V. Kirkin, T. Lamark, Y.-S. Sou, G. Bjoslashrkoslashy, J.L. Nunn, J.-A. Bruun, et al., A role for NBR1 in autophagosomal degradation of ubiquitinated substrates, Mol. Cell 33 (2009) 505–516, https://doi.org/10.1016/j.molcel.2009.01.020.
[89] J.-Y. Lee, Y. Nagano, J.P. Taylor, K.L. Lim, T.-P. Yao, Disease-causing mutations in Parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy, J. Cell Biol. 189 (2010) 671–679, https://doi.org/10.1083/jcb.201001039.
[90] S. Pankiv, T.H. Clausen, T. Lamark, A. Brech, J.-A. Bruun, H. Outzen, et al., p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy, J. Biol. Chem. 282 (2007) 24131–24145, https://doi.org/10.1074/jbc.M702824200.
[91] C. Van Humbeeck, T. Cornelissen, H. Hofkens, W. Mandemakers, K. Gevaert, B. De Strooper, et al., Parkin interacts with ambra1 to induce mitophagy, J. Neurosci. 31 (2011) 10249–10261, https://doi.org/10.1523/JNEUROSCI.1917-11.2011.
[92] S. Michiorri, V. Gelmetti, E. Giarda, F. Lombardi, F. Romano, R. Marongiu, et al., The Parkinson-associated protein PINK1 interacts with Beclin1 and promotes autophagy, Cell Death Differ. 17 (2010) 962–974, https://doi.org/10.1038/cdd.2009.200.
[93] S.A. Sarraf, M. Raman, V. Guarani-Pereira, M.E. Sowa, E.L. Huttlin, S.P. Gygi, et al., Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization, Nature 496 (2013) 372–376, https://doi.org/10.1038/nature12043.
[94] K. Okatsu, S.-I. Iemura, F. Koyano, E. Go, M. Kimura, T. Natsume, et al., Mitochondrial hexokinase HKI is a novel substrate of the Parkin ubiquitin ligase, Biochem. Biophys. Res. Commun. 428 (2012) 197–202, https://doi.org/10.1016/j.bbrc.2012.10.041.
[95] D. Narendra, L.A. Kane, D.N. Hauser, I.M. Fearnley, R.J. Youle, p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both, Autophagy 6 (2014) 1090–1106, https://doi.org/10.4161/auto.6.8.13426.
[96] S. Geisler, K.M. Holmström, D. Skujat, F.C. Fiesel, O.C. Rothfuss, P.J. Kahle, et al., PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1, Nat. Cell Biol. 12 (2010) 119–131, https://doi.org/10.1038/ncb2012.
[97] S.R. Yoshii, C. Kishi, N. Ishihara, N. Mizushima, Parkin mediates proteasome-dependent protein degradation and rupture of the outer mitochondrial membrane, J. Biol. Chem. 286 (2011) 19630–19640, https://doi.org/10.1074/jbc.M110.209338.
[98] G.-L. McLelland, V. Soubannier, C.X. Chen, H.M. McBride, E.A. Fon, Parkin and PINK1 function in a vesicular trafficking pathway regulating mitochondrial quality control, EMBO J. 33 (2014) 282–295, https://doi.org/10.1002/embj.201385902.
[99] B.C. Hammerling, R.H. Najor, M.Q. Cortez, S.E. Shires, L.J. Leon, E.R. Gonzalez, et al., A Rab5 endosomal pathway mediates Parkin-dependent mitochondrial clearance, Nat. Commun. 8 (2017) 1–16, https://doi.org/10.1038/ncomms14050.
[100] V.S. Van Laar, B. Arnold, S.J. Cassady, C.T. Chu, E.A. Burton, S.B. Berman, Bioenergetics of neurons inhibit the translocation response of Parkin following rapid mitochondrial depolarization, Hum. Mol. Genet. 20 (2011) 927–940, https://doi.org/10.1093/hmg/ddq531.
[101] Q. Cai, H.M. Zakaria, A. Simone, Z.-H. Sheng, Spatial Parkin translocation and degradation of damaged mitochondria via mitophagy in live cortical neurons, Curr. Biol. 22 (2012) 545–552, https://doi.org/10.1016/j.cub.2012.02.005.
[102] A.B. Gustafsson, Bnip3 as a dual regulator of mitochondrial turnover and cell death in the myocardium, Pediatr. Cardiol. 32 (2011) 267–274, https://doi.org/10.1007/s00246-010-9876-5.
[103] G.W. Dorn, L.A. Kirshenbaum, Cardiac reanimation: targeting cardiomyocyte death by BNIP3 and NIX/BNIP3L, Oncogene 27 (2008) S158–S167, https://doi.org/10.1038/onc.2009.53.
[104] Y. Chen, W. Lewis, A. Diwan, E.H.-Y. Cheng, S.J. Matkovich, G.W. Dorn, Dual autonomous mitochondrial cell death pathways are activated by Nix/BNip3L and induce cardiomyopathy, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 9035–9042, https://doi.org/10.1073/pnas.0914013107/-/DCSupplemental.
[105] I. Novak, V. Kirkin, D.G. McEwan, J. Zhang, P. Wild, A. Rozenknop, et al., Nix is a selective autophagy receptor for mitochondrial clearance, EMBO Rep. 11 (2009) 45–51, https://doi.org/10.1038/embor.2009.256.
[106] F.Z. Chakrama, S. Seguin-Py, J.N. Le Grand, A. Fraichard, R. Delage-Mourroux, G. Despouy, et al., GABARAPL1 (GEC1) associates with autophagic vesicles, Autophagy 6 (2014) 495–505, https://doi.org/10.4161/auto.6.4.11819.

[107] R.A. Hanna, M.N. Quinsay, A.M. Orogo, K. Giang, S. Rikka, A.B. Gustafsson, Microtubule-associated protein 1 light chain 3 (LC3) interacts with bnip3 protein to selectively remove endoplasmic reticulum and mitochondria via autophagy, J. Biol. Chem. 287 (2012) 19094–19104, https://doi.org/10.1074/jbc.M111.322933.
[108] W.X. Ding, H.M. Ni, M. Li, Y. Liao, X. Chen, D.B. Stolz, et al., Nix is critical to two distinct phases of mitophagy, reactive oxygen species-mediated autophagy induction and Parkin-ubiquitin-p62-mediated mitochondrial priming, J. Biol. Chem. 285 (2010) 27879–27890, https://doi.org/10.1074/jbc.M110.119537.
[109] C.V. Velde, J. Cizeau, D. Dubik, J. Alimonti, T. Brown, S. Israels, et al., BNIP3 and genetic control of necrosis-like cell death through the mitochondrial permeability transition pore, Mol. Cell. Biol. 20 (2000) 5454–5468.
[110] S. Rikka, M.N. Quinsay, R.L. Thomas, D.A. Kubli, X. Zhang, A.N. Murphy, et al., Bnip3 impairs mitochondrial bioenergetics and stimulates mitochondrial turnover, Cell Death Differ. 18 (2010) 721–731, https://doi.org/10.1038/cdd.2010.146.
[111] L. Liu, G. Chen Du Feng, M. Chen, Q. Zheng, P. Song, et al., Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells, Nat. Cell Biol. 14 (2012) 177–185, https://doi.org/10.1038/ncb2422.
[112] Z. Chen, L. Liu, Q. Cheng, Y. Li, H. Wu, W. Zhang, et al., Mitochondrial E3 ligase MARCH5 regulates FUNDC1 to fine-tune hypoxic mitophagy, EMBO Rep. 18 (2017) 495–509, https://doi.org/10.15252/embr.201643309.
[113] W. Zhang, S. Siraj, R. Zhang, Q. Chen, Mitophagy receptor FUNDC1 regulates mitochondrial homeostasis and protects the heart from I/R injury, Autophagy 13 (2017) 1–2, https://doi.org/10.1080/15548627.2017.1300224.
[114] Z. Bhujabal, Å.B. Birgisdottir, E. Sjøttem, H.B. Brenne, A. Øvervatn, S. Habisov, et al., FKBP8 recruits LC3A to mediate Parkin-independent mitophagy, EMBO Rep. 18 (2017) 947–961, https://doi.org/10.15252/embr.201643147.
[115] C.T. Chu, J. Ji, R.K. Dagda, J.F. Jiang, Y.Y. Tyurina, A.A. Kapralov, et al., Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells, Nat. Cell Biol. 15 (2013) 1197–1205, https://doi.org/10.1038/ncb2837.
[116] X.-X. Li, B. Tsoi, Y.-F. Li, H. Kurihara, R.-R. He, Cardiolipin and its different properties in mitophagy and apoptosis, J. Histochem. Cytochem. 63 (2015) 301–311, https://doi.org/10.1369/0022155415574818.
[117] A. Marchese, M.M. Paing, B.R.S. Temple, J. Trejo, G protein–coupled receptor sorting to endosomes and lysosomes, Annu. Rev. Pharmacol. Toxicol. 48 (2008) 601–629, https://doi.org/10.1146/annurev.pharmtox.48.113006.094646.
[118] C. Campsteijn, M. Vietri, H. Stenmark, ScienceDirect Novel ESCRT functions in cell biology: spiraling out of control? Curr. Opin. Cell Biol. 41 (2016) 1–8, https://doi.org/10.1016/j.ceb.2016.03.008.
[119] M.A. Andrade-Navarro, L. Sanchez-Pulido, H.M. McBride, Mitochondrial vesicles: an ancient process providing new links to peroxisomes, Curr. Opin. Cell Biol. 21 (2009) 560–567, https://doi.org/10.1016/j.ceb.2009.04.005.
[120] A. Sugiura, G.L. McLelland, E.A. Fon, H.M. McBride, A new pathway for mitochondrial quality control: mitochondrial-derived vesicles, EMBO J. 33 (2014) 2142–2156, https://doi.org/10.15252/embj.201488104.
[121] M. Neuspiel, A.C. Schauss, E. Braschi, R. Zunino, P. Rippstein, R.A. Rachubinski, et al., Cargo-selected transport from the mitochondria to peroxisomes is mediated by vesicular carriers, Curr. Biol. 18 (2008) 102–108, https://doi.org/10.1016/j.cub.2007.12.038.
[122] V. Soubannier, G.-L. McLelland, R. Zunino, E. Braschi, P. Rippstein, E.A. Fon, et al., A vesicular transport pathway shuttles cargo from mitochondria to lysosomes, Curr. Biol. 22 (2012) 135–141, https://doi.org/10.1016/j.cub.2011.11.057.
[123] V.J.J. Cadete, S. Deschenes, A. Cuillerier, F. Brisebois, A. Sugiura, A. Vincent, et al., Formation of mitochondrial-derived vesicles is an active and physiologically relevant mitochondrial quality control process in the cardiac system, J. Physiol. Lond. 594 (2016) 5343–5362, https://doi.org/10.1113/JP272703.
[124] V. Soubannier, P. Rippstein, B.A. Kaufman, E.A. Shoubridge, H.M. McBride, Reconstitution of mitochondria derived vesicle formation demonstrates selective enrichment of oxidized cargo, PLoS One 7 (2012) e52830, , https://doi.org/10.1371/journal.pone.0052830.s001.
[125] G.-L. McLelland, S.A. Lee, H.M. McBride, E.A. Fon, Syntaxin-17 delivers PINK1/Parkin-dependent mitochondrial vesicles to the endolysosomal system, J. Cell Biol. 214 (2016) 275–291, https://doi.org/10.1016/j.bbamcr.2015.01.013.
[126] A.R. Hall, N. Burke, R.K. Dongworth, D.J. Hausenloy, Mitochondrial fusion and fission proteins: novel therapeutic targets for combating cardiovascular disease, Br. J. Pharmacol. 171 (2014) 1890–1906, https://doi.org/10.1111/bph.12516.
[127] S. Chung, P.P. Dzeja, R.S. Faustino, C. Perez-Terzic, A. Behfar, A. Terzic, Mitochondrial oxidative metabolism is required for the cardiac differentiation of stem cells, Nat. Clin. Pract. Cardiovasc. Med. 4 (2007) S60–S67, https://doi.org/10.1038/ncpcardio0766.
[128] S. Chung, D.K. Arrell, R.S. Faustino, A. Terzic, P.P. Dzeja, Glycolytic network restructuring integral to the energetics of embryonic stem cell cardiac differentiation, J. Mol. Cell. Cardiol. 48 (2010) 725–734, https://doi.org/10.1016/j.yjmcc.2009.12.014.
[129] Y.M. Cho, S. Kwon, Y.K. Pak, H.W. Seol, Y.M. Choi, D.J. Park, et al., Dynamic changes in mitochondrial biogenesis and antioxidant enzymes during the spontaneous differentiation of human embryonic stem cells, Biochem. Biophys. Res. Commun. 348 (2006) 1472–1478, https://doi.org/10.1016/j.bbrc.2006.08.020.
[130] J.C. St John, J. Ramalho-Santos, H.L. Gray, P. Petrosko, V.Y. Rawe, C.S. Navara, et al., The expression of mitochondrial DNA transcription factors during early cardiomyocyte in vitro differentiation from human embryonic stem cells, Cloning Stem Cells 7 (2005) 141–153, https://doi.org/10.1089/clo.2005.7.141.
[131] C.D.L. Folmes, A. Terzic, Metabolic determinants of embryonic development and stem cell fate, Reprod. Fertil. Dev. 27 (2015), https://doi.org/10.1071/RD14383 82–12.
[132] J. Piquereau, M. Novotova, D. Fortin, A. Garnier, R. Ventura-Clapier, V. Veksler, et al., Postnatal development of mouse heart: formation of energetic microdomains, J. Physiol. Lond. 588 (2010) 2443–2454, https://doi.org/10.1113/jphysiol.2010.189670.
[133] T. Oka, V.H. Lam, L. Zhang, W. Keung, V.J.J. Cadete, V. Samokhvalov, et al., Cardiac hypertrophy in the newborn delays the maturation of fatty acid β-oxidation and compromises postischemic functional recovery, Am. J. Physiol. Heart Circ. Physiol. 302 (2012) H1784–H1794, https://doi.org/10.1152/ajpheart.00804.2011.
[134] M. Song, K. Mihara, Y. Chen, L. Scorrano, G.W. Dorn, Mitochondrial fission and fusion factors reciprocally orchestrate mitophagic culling in mouse hearts and cultured fibroblasts, Cell Metab. 21 (2015) 273–285, https://doi.org/10.1016/j.cmet.2014.12.011.
[135] Y. Kageyama, M. Hoshijima, K. Seo, D. Bedja, P. Sysa Shah, S.A. Andrabi, et al., Parkin-independent mitophagy requires Drp1 and maintains the integrity of mammalian heart and brain, EMBO J. 33 (2014) 2798–2813, https://doi.org/10.15252/embj.201488658.
[136] K.N. Papanicolaou, R. Kikuchi, G.A. Ngoh, K.A. Coughlan, I. Dominguez, W.C. Stanley, et al., Mitofusins 1 and 2 are essential for postnatal metabolic remodeling in heart, Circ. Res. 111 (2012) 1012–1026, https://doi.org/10.1161/CIRCRESAHA.112.274142.
[137] J. Piquereau, R. Godin, S. Deschenes, V.L. Bessi, M. Mofarrahi, S.N. Hussain, et al., Protective role of PARK2/Parkin in sepsis-induced cardiac contractile and mitochondrial dysfunction, Autophagy 9 (2013) 1837–1851, https://doi.org/10.4161/auto.26502.
[138] D.A. Kubli, X. Zhang, Y. Lee, R.A. Hanna, M.N. Quinsay, C.K. Nguyen, et al., Parkin protein deficiency exacerbates cardiac injury and reduces survival following myocardial infarction, J. Biol. Chem. 288 (2013) 915–926, https://doi.org/10.1074/jbc.M112.411363.
[139] F. Billia, L. Hauck, F. Konecny, V. Rao, J. Shen, T.W. Mak, PTEN-inducible kinase 1 (PINK1)/Park6 is indispensable for normal heart function, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 9572–9577, https://doi.org/10.1073/pnas.1106291108.
[140] A. Diwan, J. Wansapura, F.M. Syed, S.J. Matkovich, J.N. Lorenz, G.W. Dorn, Nix-mediated apoptosis links myocardial fibrosis, cardiac remodeling, and hypertrophy decompensation, Circulation 117 (2008) 396–404, https://doi.org/10.1161/CIRCULATIONAHA.107.727073.
[141] G.W. Dorn, Mitochondrial pruning by nix and bnip3: an essential function for cardiac-expressed death factors, J. Cardiovasc. Transl. Res. 3 (2010) 374–383, https://doi.org/10.1007/s12265-010-9174-x.
[142] L.A. Scarffe, D.A. Stevens, V.L. Dawson, T.M. Dawson, Parkin and PINK1: much more than mitophagy, Trends Neurosci. 37 (2014) 315–324, https://doi.org/10.1016/j.tins.2014.03.004.
[143] G.W. Dorn II, Accepted manuscript, J. Mol. Cell. Cardiol. (2015) 1–20, https://doi.org/10.1016/j.yjmcc.2015.11.023.
[144] G. Gong, M. Song, G. Csordas, D.P. Kelly, S.J. Matkovich, G.W. Dorn, Parkin-mediated mitophagy directs perinatal cardiac metabolic maturation in mice, Sciences 350 (2015), https://doi.org/10.1126/science.aad2459 aad2459–aad2459.
[145] M. Song, G. Gong, Y. Burelle, A.B. Gustafsson, R.N. Kitsis, S.J. Matkovich, et al., Interdependence of Parkin-mediated mitophagy and mitochondrial fission in adult mouse hearts, Circ. Res. 117 (2015) 346–351, https://doi.org/10.1161/CIRCRESAHA.117.306859.
[146] J.-H. Shin, H.S. Ko, H. Kang, Y. Lee, Y.-I. Lee, O. Pletinkova, et al., PARIS (ZNF746) repression of PGC-1α contributes to neurodegeneration in Parkinson's disease, Cell 144 (2011) 689–702, https://doi.org/10.1016/j.cell.2011.02.010.
[147] E. Bisetto, M. Comelli, A.M. Salzano, P. Picotti, A. Scaloni, G. Lippe, et al., Biochimica et biophysica acta, Biochim. Biophys. Acta Bioenerg. 1827 (2013) 807–816, https://doi.org/10.1016/j.bbabio.2013.04.002.
[148] A.F. Branco, S.P. Pereira, S. Gonzalez, O. Gusev, A.A. Rizvanov, P.J. Oliveira, Gene expression profiling of H9c2 myoblast differentiation towards a cardiac-like phenotype, PLoS One 10 (2015) e0129303, , https://doi.org/10.1371/journal.pone.0129303.t002.
[149] A.V. Kuznetsov, S. Javadov, S. Sickinger, S. Frotschnig, M. Grimm, H9c2 and HL-1 cells demonstrate distinct features of energy metabolism, mitochondrial function and sensitivity to hypoxia-reoxygenation, Biochim. Biophys. Acta, Mol. Cell Res. 1853 (2015) 276–284, https://doi.org/10.1016/j.bbamcr.2014.11.015.
[150] S. Gispert, D. Parganlija, M. Klinkenberg, S. Dröse, I. Wittig, M. Mittelbronn, et al., Loss of mitochondrial peptidase Clpp leads to infertility, hearing loss plus growth retardation via accumulation of CLPX, mtDNA and inflammatory factors, Hum. Mol. Genet. 22 (2013) 4871–4887, https://doi.org/10.1093/hmg/ddt338.
[151] L.M. Martins, A. Morrison, K. Klupsch, V. Fedele, N. Moisoi, P. Teismann, et al., Neuroprotective role of the Reaper-related serine protease HtrA2/Omi revealed by targeted deletion in mice, Mol. Cell. Biol. 24 (2004) 9848–9862, https://doi.org/10.1128/MCB.24.22.9848-9862.2004.
[152] L. Vande Walle, M. Lamkanfi, P. Vandenabeele, The mitochondrial serine protease HtrA2/Omi: an overview, Cell Death Differ. 15 (2008) 453–460, https://doi.org/10.1038/sj.cdd.4402291.
[153] T.Y. Kim, D. Wang, A.K. Kim, E. Lau, A.J. Lin, D.A. Liem, et al., Metabolic labeling reveals proteome dynamics of mouse mitochondria, Mol. Cell. Proteomics 11 (2012) 1586–1594, https://doi.org/10.1074/mcp.M112.021162.
[154] V. Eisner, R.R. Cupo, E. Gao, G. Csordás, W.S. Slovinsky, M. Paillard, et al., Mitochondrial fusion dynamics is robust in the heart and depends on calcium oscillations and contractile activity, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) E859–E868, https://doi.org/10.1073/pnas.1617288114.
[155] L. Chen, T. Liu, A. Tran, X. Lu, A.A. Tomilov, V. Davies, et al., OPA1 mutation and

late-onset cardiomyopathy: mitochondrial dysfunction and mtDNA instability, J. Am. Heart Assoc. 1 (2012) e003012, https://doi.org/10.1161/JAHA.112.003012.
[156] J. Piquereau, F. Caffin, M. Novotova, A. Prola, A. Garnier, P. Mateo, et al., Down-regulation of OPA1 alters mouse mitochondrial morphology, PTP function, and cardiac adaptation to pressure overload, Cardiovasc. Res. 94 (2012) 408–417, https://doi.org/10.1093/cvr/cvs117.
[157] T. Wai, J. Garcia-Prieto, M.J. Baker, C. Merkwirth, P. Benit, P. Rustin, et al., Imbalanced OPA1 processing and mitochondrial fragmentation cause heart failure in mice, Science 350 (2015) aad0116, https://doi.org/10.1126/science.aad0116.
[158] Y. Ikeda, A. Shirakabe, Y. Maejima, P. Zhai, S. Sciarretta, J. Toli, et al., Endogenous Drp1 mediates mitochondrial autophagy and protects the heart against energy stress, Circ. Res. 116 (2015) 264–278, https://doi.org/10.1161/CIRCRESAHA.116.303356.
[159] T.G. McWilliams, A.R. Prescott, L. Montava-Garriga, G. Ball, F. Singh, E. Barini, et al., Basal mitophagy occurs independently of PINK1 in mouse tissues of high metabolic demand, Cell Metab. 27 (2018), https://doi.org/10.1016/j.cmet.2017.12.008 439–449.e5..
[160] T.G. McWilliams, A.R. Prescott, G.F.G. Allen, J. Tamjar, M.J. Munson, C. Thomson, et al., mito-QC illuminates mitophagy and mitochondrial architecture in vivo, J. Cell Biol. 214 (2016) 333–345, https://doi.org/10.1083/jcb.201603039dv.
[161] D. Malide, J. Liu, I.I. Rovira, C.A. Combs, N. Sun, T. Finkel, A fluorescence-based imaging method to measure in vitro and in vivo mitophagy using mt-Keima, Nat. Protoc. 12 (2016) 1576–1587, https://doi.org/10.1038/nprot.2017-060.
[162] E. Lau, D. Huang, Q. Cao, T.U. Dincer, C.M. Black, A.J. Lin, et al., Spatial and temporal dynamics of the cardiac mitochondrial proteome, Expert Rev. Proteomics 12 (2015) 133–146, https://doi.org/10.1586/14789450.2015.1024227.
[163] P.P. Karunadharma, N. Basisty, Y.A. Chiao, D.-F. Dai, R. Drake, N. Levy, et al., Respiratory chain protein turnover rates in mice are highly heterogeneous but strikingly conserved across tissues, ages, and treatments, FASEB J. 29 (2015) 3582–3592, https://doi.org/10.1096/fj.15-272666.
[164] M. Marcil, A. Ascah, J. Matas, S. Belanger, C.F. Deschepper, Y. Burelle, Compensated volume overload increases the vulnerability of heart mitochondria without affecting their functions in the absence of stress, J. Mol. Cell. Cardiol. 41 (2006) 998–1009, https://doi.org/10.1016/j.yjmcc.2006.08.117.
[165] H. Bugger, E.D. Abel, Molecular mechanisms of diabetic cardiomyopathy, Diabetologia 57 (2014) 660–671, https://doi.org/10.1007/s00125-014-3171-6.
[166] J. Huss, D. Kelly, Mitochondrial energy metabolism in heart failure: a question of balance, J. Clin. Invest. 115 (2005) 547–555.
[167] E.D. Crouser, Mitochondrial dysfunction in septic shock and multiple organ dysfunction syndrome, Mitochondrion 4 (2004) 729–741, https://doi.org/10.1016/j.mito.2004.07.023.
[168] E.D. Crouser, M.W. Julian, D.V. Blaho, D.R. Pfeiffer, Endotoxin-induced mitochondrial damage correlates with impaired respiratory activity, Crit. Care Med. 30 (2002) 276–284.
[169] K.B. Wallace, Doxorubicin-induced cardiac mitochondrionopathy, Pharmacol. Toxicol. 93 (2003) 105–115.
[170] C.M. Palmeira, J. Serrano, D.W. Kuehl, K.B. Wallace, Preferential oxidation of cardiac mitochondrial DNA following acute intoxication with doxorubicin, Biochim. Biophys. Acta 1321 (1997) 101–106.
[171] S. Zhou, C.M. Palmeira, K.B. Wallace, Doxorubicin-induced persistent oxidative stress to cardiac myocytes, Toxicol. Lett. 121 (2001) 151–157.
[172] C. Baines, J. Molkentin, STRESS signaling pathways that modulate cardiac myocyte apoptosis, J. Mol. Cell. Cardiol. 38 (2005) 47–62, https://doi.org/10.1016/j.yjmcc.2004.11.004.
[173] D.A. Kubli, M.Q. Cortez, A.G. Moyzis, R.H. Najor, Y. Lee, A.B. Gustafsson, PINK1 is dispensable for mitochondrial recruitment of Parkin and activation of mitophagy in cardiac myocytes, PLoS One 10 (2015) e0130707–e0130716, https://doi.org/10.1371/journal.pone.0130707.
[174] C. Huang, A.M. Andres, E.P. Ratliff, G. Hernandez, P. Lee, R.A. Gottlieb, Preconditioning involves selective mitophagy mediated by Parkin and p62/SQSTM1, PLoS One 6 (2011) e20975, , https://doi.org/10.1371/journal.pone.0020975.g010.
[175] A. Hoshino, Y. Mita, Y. Okawa, M. Ariyoshi, E. Iwai-Kanai, T. Ueyama, et al., Cytosolic p53 inhibits Parkin-mediated mitophagy and promotes mitochondrial dysfunction in the mouse heart, Nat. Commun. 4 (2013) 2308, https://doi.org/10.1038/ncomms3308.
[176] A. Shirakabe, P. Zhai, Y. Ikeda, T. Saito, Y. Maejima, C.-P. Hsu, et al., Drp1-dependent mitochondrial autophagy plays a protective role against pressure overload-induced mitochondrial dysfunction and heart failure, Circulation 133 (2016) 1249–1263, https://doi.org/10.1161/CIRCULATIONAHA.115.020502.
[177] T. Yu, S.S. Sheu, J.L. Robotham, Y. Yoon, Mitochondrial fission mediates high glucose-induced cell death through elevated production of reactive oxygen species, Cardiovasc. Res. 79 (2008) 341–351, https://doi.org/10.1093/cvr/cvn104.
[178] K. Tsushima, H. Bugger, A.R. Wende, J. Soto, G.A. Jenson, A.R. Tor, et al., Mitochondrial reactive oxygen species in lipotoxic hearts induce post-translational modifications of AKAP121, DRP1, and OPA1 that promote mitochondrial fission, Circ. Res. 122 (2018) 58–73, https://doi.org/10.1161/CIRCRESAHA.117.311307.
[179] Q. Liang, S. Kobayashi, Journal of molecular and cellular cardiology, J. Mol. Cell. Cardiol. (2016) 1–13, https://doi.org/10.1016/j.yjmcc.2015.12.025.
[180] M.N. Quinsay, R.L. Thomas, Y. Lee, A.B. Gustafsson, Bnip3-mediated mitochondrial autophagy is independent of the mitochondrial permeability transition pore, Autophagy 6 (2010) 855–862, https://doi.org/10.4161/auto.6.7.13005.
[181] A. Hamacher-Brady, N.R. Brady, S.E. Logue, M.R. Sayen, M. Jinno, L.A. Kirshenbaum, et al., Response to myocardial ischemia/reperfusion injury involves Bnip3 and autophagy, Cell Death Differ. 14 (2006) 146–157, https://doi.org/10.1038/sj.cdd.4401936.
[182] M.N. Quinsay, Y. Lee, S. Rikka, M.R. Sayen, J.D. Molkentin, R.A. Gottlieb, et al., Bnip3 mediates permeabilization of mitochondria and release of cytochrome c via a novel mechanism, J. Mol. Cell. Cardiol. 48 (2010) 1146–1156, https://doi.org/10.1016/j.yjmcc.2009.12.004.
[183] W. Zhang, H. Ren, C. Xu, C. Zhu, H. Wu, D. Liu, et al., Hypoxic mitophagy regulates mitochondrial quality and platelet activation and determines severity of I/R heart injury, elife 5 (2016) 71, https://doi.org/10.7554/eLife.21407.
[184] Z. Shen, C. Ye, K. McCain, M.L. Greenberg, Review article the role of cardiolipin in cardiovascular health, Biomed. Res. Int. (2015) 1–12, https://doi.org/10.1155/2015/891707.
[185] Y. Liu, B. Levine, Autosis and Autophagic Cell Death: the Dark Side of Autophagy, vol. 22, (2014), pp. 367–376, https://doi.org/10.1038/cdd.2014.143.
[186] H. Zhu, P. Tannous, J.L. Johnstone, Y. Kong, J.M. Shelton, J.A. Richardson, et al., Cardiac autophagy is a maladaptive response to hemodynamic stress, J. Clin. Investig. 117 (2007) 1782–1793, https://doi.org/10.1172/JCI27523.
[187] Y. Matsui, H. Takagi, X. Qu, M. Abdellatif, H. Sakoda, T. Asano, et al., Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy, Circ. Res. 100 (2007) 914–922, https://doi.org/10.1161/01.RES.0000261924.76669.36.
[188] Naresh B.V. Sepuri, R. Angireddy, S. Srinivasan, M. Guha, J. Spear, Bin Lu, et al., Mitochondrial LON protease-dependent degradation of cytochrome c oxidase subunits under hypoxia and myocardial ischemia, Biochim. Biophys. Acta Bioenerg. 1858 (2017) 519–528, https://doi.org/10.1016/j.bbabio.2017.04.003.
[189] B. Guillon, A.-L. Bulteau, M. Wattenhofer-Donzé, S. Schmucker, B. Friguet, H. Puccio, et al., Frataxin deficiency causes upregulation of mitochondrial Lon and ClpP proteases and severe loss of mitochondrial Fe-S proteins, FEBS J. 276 (2009) 1036–1047, https://doi.org/10.1111/j.1742-4658.2008.06847.x.
[190] A. Kasahara, S. Cipolat, Y. Chen, G.W. Dorn, L. Scorrano, Mitochondrial fusion directs cardiomyocyte differentiation via calcineurin and Notch signaling, Science 342 (2013) 734–737, https://doi.org/10.1126/science.1241359.
[191] P.M. Quirós, A.J. Ramsay, D. Sala, E. Fernández-Vizarra, F. Rodríguez, J.R. Peinado, et al., Loss of mitochondrial protease OMA1 alters processing of the GTPase OPA1 and causes obesity and defective thermogenesis in mice, EMBO J. 31 (2012) 2117–2133, https://doi.org/10.1038/emboj.2012.70.