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Multiple recycling routes: Canonical vs. non-canonical mitophagy in the heart☆

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ABSTRACT

The heart is composed of cardiomyocytes that require large amounts of energy to sustain contraction. Mitochondria are distinctive organelles of bacterial origin that generate most of the energy for the heart via oxidative phosphorylation. To ensure a healthy population of mitochondria that efficiently produce ATP, myocytes quickly eliminate any unhealthy or unwanted mitochondria via a process known as mitochondrial autophagy, or mitophagy. It is especially important to selectively remove damaged or aged mitochondria since they can become excessive producers of reactive oxygen species and release pro-death proteins. Because this is such a crucial cellular process, cells have several mechanisms in place to deal with potentially harmful mitochondria. Here, we review the various pathways identified to date and how they are regulated. We also discuss the importance of these canonical and non-canonical pathways in the heart and their link to cardiovascular health, disease and aging.

1. Introduction

The heart requires a lot of energy to meet the high metabolic demand of the contracting cardiomyocytes. The majority of the energy is produced by mitochondria via oxidative phosphorylation. Mitochondria are also important in other processes such as metabolite synthesis, calcium storage, and integration of cell death pathways [1]. Thus, maintaining a healthy and functional mitochondrial network is imperative to cardiac homeostasis. Cells have several mechanisms in place to deal with damaged or unwanted mitochondria. In general, the selective removal of a mitochondrion requires two distinct steps: 1) activation of a pathway responsible for labeling the mitochondrion for degradation and 2) initiation of the degradation machinery with the formation of a vesicle that will sequester the mitochondrion. The most well characterized pathway involved in degrading mitochondria is macroautophagy (herein called autophagy). Autophagy is an evolutionarily conserved mechanism used to facilitate the removal of protein aggregates and damaged or unwanted organelles. While originally thought to be a bulk process for removal, further studies revealed that this pathway has the capacity for selective degradation of organelles, such as mitochondria and ER [2]. Mitochondrial autophagy, or mitophagy, is required to eliminate fetal mitochondria during the metabolic transition in hearts after birth [3] and is an essential housekeeping process in adult cardiomyocytes [4]. Mitophagy facilitates the normal turnover of mitochondria as they age and become less effective in generating ATP. This process becomes even more important during exposure to stress that leads to mitochondrial damage [5]. When mitochondria become damaged, they are not only less efficient at generating ATP, but they can also become producers of excessive reactive oxygen species and release pro-death factors that result in activation of apoptotic and necrotic cell death pathways in cells. This is of particular concern in terminally differentiated cells, such as cardiomyocytes, as they do not possess regenerative potential. Impaired mitochondrial function and reduced mitophagy have also been implicated in the aging process and in the development and progression of heart failure [6–10], further emphasizing the need for elucidation of these pathways. In this review, we provide an overview of the pathways and proteins known to regulate the various steps involved in the selective elimination of mitochondria. Recent studies have identified additional non-canonical pathways that can also clear damaged mitochondria, including alternative autophagy [11], microautophagy [12] and the endosomal-lysosomal degradation pathway [13]. Here, we discuss our current understanding of the distinct pathways that regulate mitochondrial turnover and how they relate to one and other mechanistically.

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2. Canonical mitophagy pathway

Autophagy is an evolutionarily conserved degradation pathway that allows for the removal of various cellular components, including mitochondria. First described in yeast, a variety of autophagy-related ATG proteins exist to facilitate a series of discrete steps that ultimately compose the process of autophagy [2]. A defect in any of these sequential steps will affect the progress of this pathway and can potentially lead to disease. The phases of mitochondrial autophagy can be divided into three distinct stages: 1) the signals from mitochondria to initiate autophagy 2) the formation of autophagic vesicles and 3) the ultimate delivery of cargo to the lysosome, the compartment responsible for degradation.

2.1. Signals from mitochondria to initiate mitophagy

In order for the damaged or unwanted mitochondria to be cleared, they must first be labeled and then signal to the autophagic machinery to start forming vesicles. There are a variety of different signals and pathways that have been implicated in this activation process. Here, we list some of the mitochondrial triggers that have been reported to initiate selective mitophagy.

2.1.1. AMP-activated kinase (AMPK)

The mitochondrial membrane potential (ΔΨm) and ATP production are two parameters known to initiate mitochondrial clearance. The ΔΨm controls respiratory rate and ATP synthesis and a drop in the ΔΨm leads to reduced cellular ATP levels. The reduced energy levels are sensed by the serine/threonine kinase AMPK [14]. One of the main functions of AMPK is to maintain energy and metabolic homeostasis during energy-limiting conditions by activating catabolic processes such as autophagy. Activated AMPK initiates formation of autophagosomes through inhibition of mTORC1 [15], a negative regulator of autophagy (Fig. 1A). Additionally, AMPK phosphorylates ULK1 at serines 313 and 777 in order to directly activate autophagy [16] (Fig. 1A). Thus, under nutrient-rich conditions active mTORC1 phosphorylates ULK1, which prevents ULK1 from interacting with AMPK and keeps ULK1 inactive. However, once cellular energy is depleted, activated AMPK inhibits mTORC1, which reduces the inhibitory phosphorylation on ULK1 [17].

AMPK has also been linked to the removal of damaged mitochondria via direct activation of mitophagy. A link between AMPK and ULK1 in regulating mitophagy was initially discovered by Egan et al. who observed that cells lacking either AMPK or ULK1 accumulated dysfunctional mitochondria due to a defect in mitophagy [18]. A subsequent study reported that mitochondrial damage led to AMPK-mediated phosphorylation of ULK1 at Serine-555, which triggered translocation of ULK1 to the mitochondria and activation of mitophagy [19]. Mutational analyses confirmed that a phosphorylation resistant mutant, ULK1S555A failed to translocate to mitochondria during stress, while a phosphomimetic mutant, ULK1S555D induced mitophagy independently of AMPK [19]. The importance of this pathway was recently confirmed in vivo where AMPK-ULK1-mediated mitophagy was activated in skeletal muscle to eliminate mitochondria that had been damaged during intense exercise [20].

A number of studies have demonstrated that activation of AMPK and autophagy are cardioprotective [21–23]. However, the function of AMPK in mitophagy in the heart is less clear. It is important to note that although mice with cardiac specific deletion of LKB1, an upstream regulator of AMPK, developed cardiac hypertrophy and contractile dysfunction, mitochondrial function in these hearts was normal [24–25]. Conditional deletion of key AMPK subunits or overexpression of a dominant negative mutant of the AMPK α2 catalytic subunit in mouse hearts also had no effect on cardiac function or the high energy phosphate and adenine nucleotides contents under baseline conditions [26–27]. However, it was recently reported that AMPKα2 specifically activated mitophagy in cardiac myocytes in response to stress and that AMPKα2$^{-/-}$ mice exhibited exacerbated transverse aortic constriction-induced heart failure due to decreased mitophagy [22]. Aged mice with muscle-specific loss of AMPK also accumulated abnormal enlarged mitochondria with increased levels of mitochondrial DNA (mtDNA) deletions, indicating that mitophagy was impaired in the muscle cells [28]. Thus, this suggests that AMPK might play a limited role in the normal turnover of mitochondria under baseline conditions and that it is specifically involved in eliminating mitochondria in response to stress or during aging. Clearly, additional studies elucidating the molecular mechanism and determining under what conditions AMPK regulates mitophagy in various cells and tissues are needed.

2.1.2. Reactive oxygen species (ROS)

Reactive oxygen species (ROS) are by-products that arise as a result of oxidative phosphorylation. Due to their high mitochondrial content,

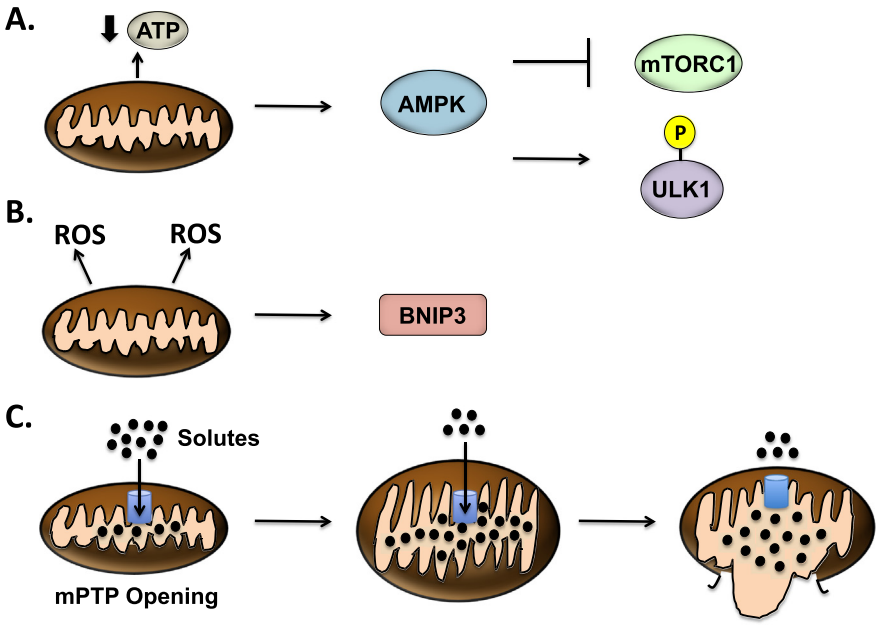

Fig. 1. Signals from the mitochondria that activate mitochondrial autophagy. **A**) Dysfunctional mitochondria produce less ATP which leads to activation of the energy sensor AMPK. Activated AMPK initiates autophagy by inhibiting mTORC1 and by activating ULK1. **B**) Damaged mitochondria produce more reactive oxygen species (ROS) which leads to activation of the mitophagy receptor BNIP3. **C**) Opening of the mitochondrial permeability transition pore (mPTP) results in an influx of solutes and water into the mitochondrial matrix. This leads to mitochondrial swelling and membrane depolarization, which serve as a signal to initiate mitophagy.

cardiomyocytes have a very strong antioxidant-dense system that quickly neutralizes ROS from mitochondrial respiration [29]. However, excess ROS production that exceeds the cell's capability to neutralize it can lead to damage of the mitochondrial DNA (mtDNA), mitochondrial dysfunction and a further increase in ROS generation. The DNA inside mitochondria is very susceptible the ROS to due to the lack of protective histones and close proximity to the electron transport chain [30]. Cardiac progenitor cells isolated from the POLG mutator mice that accumulate mtDNA mutations due to a mutation in the "proofreading" exonuclease domain of the DNA polymerase γ had elevated levels of mitophagy at baseline [31]. ROS can also trigger opening of the mitochondrial permeability transition pore (mPTP), which can disrupt the mitochondrial membrane potential and induce mitophagy (discussed below in more detail) [32,5,33–34]. The excess ROS from the mitochondria can also function as a direct signal to trigger mitophagy [35,29]. ROS can induce mitophagy by activating the mitophagy receptor BNIP3 [36] (Fig. 1B). Additionally, ROS can activate PINK1/PARKIN-mediated mitophagy and it has been shown that treatment of cells with ROS scavengers, *N*-acetyl-l-cysteine and catalase, substantially inhibited PINK1/PARKIN mediated mitophagy [37]. The specific functions of BNIP3 and PINK1/PARKIN in mitophagy will be discussed later.

It is now clear that while excessive ROS production is detrimental to cells, lower levels of ROS function as an important signaling molecule and are critical for cellular homeostasis. A study by Dorn's group demonstrated the importance of ROS as a signaling molecule and function in mitochondrial quality control. They found that transgenic mice overexpressing modest levels of a mitochondrial-targeted Catalase was cardioprotective. In contrast, the mouse line with much higher levels of mito-Catalase overexpression led to complete suppression of mitochondrial ROS and resulted in impaired mitophagy and development of cardiomyopathy [29]. However, determining the exact threshold required for ROS to trigger mitochondrial clearance, as well as the identification of additional cellular components in this pathway in the myocardium will be necessary moving forward.

2.1.3. Mitochondrial permeability transition pore (mPTP) opening

The mitochondrial permeability transition pore (mPTP) in the inner mitochondrial membrane is an important regulator of cell death [38]. Opening of the mPTP leads to an influx of solutes and water that induces swelling of the inner membrane, causing disruption of mitochondrial membrane potential and outer membrane rupture (Fig. 1C). This is an irreversible event and if a large number of the cell's mitochondria undergo mPTP opening, it will lead to cell death [38]. Opening of the mPTP can also induce mitophagy [33–34], where the loss of membrane potential will trigger PINK1/PARKIN-mediated mitophagy [32,5]. If only a few mitochondria in the cell have undergone outer membrane rupture, mitophagy then functions as a cleanup mechanism by eliminating these mitochondria. However, opening of the pore is not a pre-requisite for induction of mitophagy. Cyclophilin D (CypD) is an important regulator of the mPTP and cells lacking this protein were resistant to mPTP opening [38] but still had intact mitophagy. Overexpression of the mitophagy receptor BNIP3 led to similar induction of mitophagy in WT and CypD-deficient cells [39], confirming that initiation of mitophagy can occur independent of mPTP opening.

2.1.4. Metabolism

A number of recent studies have uncovered that mitophagy also plays a role in regulating cellular metabolism [40,3,41–42]. In general, this occurs through adjusting the mitochondrial content and by monitoring the mitochondrial phenotype (i.e. immature with low activity versus mature and highly respiring) in cells. For instance, embryonic and adult stem cells are metabolically distinct from mature differentiated cells. Stem cells are relatively quiescent and rely on glycolysis to generate ATP rather than on oxidative phosphorylation [43]. Uncommitted stem cells in a quiescent state exhibit low metabolic activity and contain low number of mitochondria with an immature phenotype. In these cells, mitophagy functions to maintain stem cell homeostasis and to prevent differentiation by continuously eliminating actively respiring mitochondria and suppressing oxidative metabolism [41]. Recent evidence has also demonstrated a pivotal role for mitophagy in regulating the metabolic shift to glycolysis in the reprogramming of somatic cells into inducible pluripotent stem cells (iPSCs). The reprogramming process requires a reduction in mitochondrial content, as well as inactivation of oxidative phosphorylation and a switch to glycolysis [44]. PINK1-dependent mitophagy was reported to play a key role in ensuring the decrease in mitochondrial mass during the cellular reprogramming [45]. Vazquez-Martin et al. found that reprogramming of PINK1-null mouse embryonic fibroblasts (MEFs) led to a mixed population of mature and immature mitochondria the iPSC cells and these cells also displayed an increased tendency to undergo spontaneous differentiation [45]. Similarily, BNIP3L/NIX-mediated mitophagy was reported to be responsible for mitochondrial elimination during reprogramming of MEFs [46].

In addition, mitophagy is also rapidly activated in response to changes in bioenergetic needs in the cell, such as during differentiation [40,3,47]. For instance, Esteban-Martines et al. found that NIX-mediated programmed mitophagy was required for the glycolytic shift during retinal ganglion cell differentiation during the mouse retinal development [40]. The mitophagy preceded the glycolytic shift which is required for retinal ganglion cell differentiation and genetic disruption of autophagy or mitophagy led to impaired neuronal differentiation [40]. However, activation of mitophagy during metabolic reprogramming is not limited to stem cells. Gong et al. recently demonstrated the vital role of mitophagy in facilitating the metabolic transition in the heart after birth [3]. Cardiac metabolism changes dramatically during the transition from the fetal to postnatal environment. PARKIN-mediated mitophagy was found to be activated after birth to facilitate replacement of fetal mitochondria with mature mitochondria during the metabolic transition in the neonatal heart [3]. Interestingly, PARKIN-deficient myocytes retained their immature fetal mitochondria, which led to rapid cardiac dysfunction and death after birth. Although these studies clearly demonstrate the link between mitophagy and the metabolic state of cells, additional studies are needed to elucidate the molecular mechanisms underlying mitophagy-dependent metabolic reprogramming.

3. Transcriptional regulation of mitophagy

When a large number of aberrant mitochondria require elimination, the demand for the number of autophagosomes and lysosomes increases. Thus, activation of mitophagy is closely coordinated with increased transcription of genes involved in autophagosome generation and lysosomal biogenesis. The transcription factor EB (TFEB) is a basic helix-loop-helix leucine zipper transcription factor and a key regulator of autophagic flux. It activates transcription of a network of genes encoding proteins involved in regulating the complete autophagic-lysosomal degradation process [48–49]. Thus, overexpression of TFEB alone leads to increased autophagic flux in cells and tissues, including the heart [50]. In addition, TFEB was reported to be selectively activated in a PARKIN-dependent manner during mitophagy of depolarized mitochondria [51], confirming the close coordination between PARKIN and TFEB to ensure successful completion of the mitophagic elimination of mitochondria.

While TFEB activates transcription of a network of genes involved in the entire process, other transcription factors that activate a subset of genes involved in autophagy and mitophagy have also been described. For instance, the forkhead box O (FOXO) transcription factors functions as positive regulators of autophagy and mitophagy in cells by inducing transcription of select proteins involved in regulating these two processes. FOXO1 increases transcription of key autophagy genes such as

Atg3, *Atg5* and *Atg12* [52–53], while FOXO3 activates transcription of *Bnip3*, *lc3b* and *Gabarapl1* [54–55]. In addition, a key role for FOXO3 in regulating autophagy and mitophagy in the heart has been demonstrated by two independent groups using transgenic mice with cardiac specific overexpression of a constitutively active FOXO3 (CA-FOXO3). These mice had high levels of autophagy and mitophagy in their hearts due to elevated levels of regulators such as LC3 and BNIP3. This shift in the balance from protein synthesis to protein degradation by chronic expression of CA-FOXO3 led to reduced mitochondrial content and cardiac mass [56–57]. Interestingly, BNIP3 null mice with cardiac overexpression of CA-FOXO3 had diminished cardiac atrophy [57], confirming the specific role of FOXO3 in activating BNIP3-mediated autophagy and organelle degradation in myocytes.

In addition, transcriptional repressors of the autophagy degradation pathway have been identified. The zinc-finger family DNA-binding protein ZKSCAN3 was initially reported to function as a transcriptional repressor of a large number of genes involved in autophagosome formation and lysosomal biogenesis [58]. Chauhan et al. found that knockdown of ZKSCAN3 in cells led to enhanced autophagy and increased lysosome biogenesis in cell lines. This group also observed that ZKSCAN3 and TFEB were inversely regulated, where starvation led to repression of ZKSCAN3 and activation of TFEB. In contrast, Pan et al. recently reported that ZKSCAN3 is not a key regulator of autophagic flux in vivo [59]. The authors examined gene expression of autophagy and lysosomal genes previously reported to be targets of ZKSCAN3 in brain, heart and liver from WT and *Zkscan3* knockout mice but they found no differences in tissues lacking ZKSCAN3. Autophagic flux was also unaltered in the *Zkscan3* knockout mice [59]. The underlying reasons for the two opposing findings are currently unclear but could be due to differences in the regulation of autophagy between tissues and tumor cell lines. Another major difference between these two studies was the use of acute knockdown in cells versus chronic deletion of *Zkscan3* in mice. It is well known that chronic deletion of genes can lead to activation of compensatory pathways. Finally, a recent study identified the Bromodomain-containing protein 4 (BRD4) to function as a transcriptional repressor of several key autophagy and lysosomal genes by binding to their promoter regions under normal growth conditions [60]. The authors reported that BRD4 knockdown led to increased autophagic activity and lysosomal function in cells. They also found that BRD4 knockdown led to enhanced degradation of cytotoxic protein aggregates and provided resistance to starvation-mediated cell death. Unexpectedly, BRD4 knockdown did not enhance mitophagy even after mitochondrial depolarization, suggesting that regulation of autophagy/lysosomal activities by BRD4 might be specific for select environmental signals such as nutrient deprivation. Overall, these studies demonstrate the complex and multilevel regulation and coordination of autophagy and mitophagy in cells and tissues.

4. Mitophagy regulators

4.1. PINK1/PARKIN-mediated mitophagy

The most well characterized mitophagy mechanism to date involves the PINK1/PARKIN pathway. The serine/threonine kinase (PTEN)-induced kinase 1 (PINK1) is normally imported into healthy mitochondria by the translocase of the outer membrane (TOM) complex, where it then undergoes proteolysis and subsequent degradation [32]. However, following mitochondrial depolarization, PINK1 import is abrogated and it accumulates on the outer mitochondrial membrane [32]. This action leads to recruitment of the E3 ubiquitin ligase PARKIN from the cytosol to the depolarized mitochondria [5]. Once PARKIN has been recruited, it mediates K63-linked ubiquitination of proteins in the outer mitochondrial membrane, labeling the mitochondrion for autophagic degradation. Exactly how PINK1 recruits and activates PARKIN has been the focus of many investigations and several mechanisms have been identified to date. First, a recent study by Wang et al. reported that AMPKα2 phosphorylates PINK1 at Ser495, which enhances its activity [22]. Then, activated PINK1 phosphorylates Mitofusin 2 (MFN2) on the outer membrane, which functions as a mitochondrial receptor for PARKIN [61]. A key step in the induction of mitophagy is the PINK1-mediated phosphorylation of ubiquitin at Serine 65 [62–63]. PINK1 also phosphorylates the ubiquitin like (Ubl) domain in PARKIN at Serine 65 [64]. Most recently, Gladkova et al. reported that binding of the phospho-ubiquitin at the mitochondria was responsible for the recruitment of PARKIN and that subsequent PINK1 phosphorylation of PARKIN led to release of autoinhibition and activation of PARKIN [65].

It is well known that loss of mitochondrial membrane potential is a potent trigger of PINK1/PARKIN-mediated mitophagy in cells. Interestingly, it was recently reported that PARKIN was recruited to mitochondria upon activation of the mitochondrial unfolded protein response (UPR^{mt}) and that this occurred in the absence of mitochondrial depolarization [66]. In this scenario, accumulation of unfolded proteins in the matrix caused PINK1 accumulation on mitochondria that remained energetically healthy, which then led to PARKIN recruitment and induction of mitophagy [66]. Exactly how the UPR^{mt} regulates the mitochondrial import machinery to activate this pathway is still unknown and needs to be investigated.

4.2. Adaptor proteins

Mitochondria that have been labeled for degradation via PARKIN-mediated protein ubiquitination must subsequently become physically linked to the forming autophagosome membrane to complete the process. This is accomplished by various autophagy adaptor proteins in the cell. These adaptor proteins contain motifs involved in binding ubiquitin (UBR) on the cargo and LC3 (LIR) on the autophagosome membrane [67]. Following adaptor-protein facilitated linkage of the mitochondria to the forming autophagosome membrane, the autophagosome can engulf the mitochondrion and deliver it to the lysosome for degradation to complete the process. Ultimately, adaptor proteins are degraded along with the cargo they labeled. Adaptor proteins that have been identified to play a role in selective mitophagy are: p62/sequestome 1 (p62), neighbor of BRCA1 gene 1 (NBR1), optineurin, NDP52, and Tax1-binding protein 1 (TAX1BP1) [68–70,67,71]. The first adaptor protein identified in mitophagy was p62 and it was observed that p62 was required for efficient PARKIN-mediated mitophagy in HeLa cells [69]. However, other studies have contested this finding and reported that while loss of p62 inhibited mitochondrial perinuclear clustering, it did not hinder mitochondrial degradation [72,73]. Although the underlying reasons for the opposing findings in these studies are still unclear, the existence of additional adaptor proteins suggests that the main function of p62 is not in mitophagy or other adaptors can compensate for a loss in p62. NBR1 is a functional homologue of p62 [67], and was still subjected to autophagic degradation in p62-deficient cells, suggesting that NBR1 functions independently of p62 [74]. However, deletion of NBR1 alone or in combination with p62 did not abrogate mitophagy of damaged mitochondria, suggesting that NBR1 is also dispensable for PARKIN-mediated mitophagy [75]. Most recently, Lazarou et al. knocked out all five aforementioned autophagy receptors and demonstrated that NDP52 and optineurin functioned as the primary adaptors for PINK1-and Parkin-mediated mitophagy [70]. Finally, another study confirmed that optineurin was specifically required for efficient mitophagy even though other receptors, such as NDP52 and TAX1BP1, were still recruited to damaged mitochondria with similar kinetics [71]. Clearly, there are a number of autophagy adaptor proteins in the cell that can facilitate autophagy of specific cargo. However, what dictates this specificity in cargo binding is still unclear. It is also likely that additional adaptor proteins will be identified in future studies.

4.3. Receptor-mediated mitophagy

There are proteins that are anchored in the outer or inner mitochondrial membranes that can also function as mitochondrial receptors for autophagosomes. In general, to be defined as a mitophagy receptor these proteins must contain a transmembrane domain to anchor them in the membrane and contain an LC3-Interacting Region (LIR) motif that allows them to bind directly to LC3 on the autophagosome. The fact that these proteins bind directly to LC3 suggests that this mechanism of mitophagy is independent of ubiquitin and adaptor proteins. Additionally, many of these proteins have alternative functions in the cell.

4.3.1. BNIP3/NIX

The BCL-2-related proteins BNIP3 and BNIP3L/NIX act as mitophagy receptors that can bind directly to LC3 to clear mitochondria, eliminating the need for adaptor proteins. While they were initially identified as pro-apoptotic BH3-only proteins, they are better known as regulators of mitophagy in a variety of cell types, including cardiomyocytes [76,77,10,78–80]. BNIP3 and NIX localize to the outer mitochondrial membrane and can directly recruit autophagosomes to mitochondria through the use of their LC3-binding motifs [81–82]. BNIP3 and NIX-deficient mice accumulate dysfunctional mitochondria with age, which correlates with cardiac dilation and contractile dysfunction [10]. Additionally, BNIP3 knockout mice accumulate dysfunctional mitochondria in their livers [10], indicating that these mitophagy receptors are involved in regulating mitochondrial clearance in tissues at baseline. NIX has also been reported to play a role in programmed mitophagy and is required for mitochondrial elimination in maturing reticulocytes [80,83]. Interestingly, BNIP3 and NIX can also directly activate autophagy in cells, presumably by disrupting the interaction between BCL-2 and BECLIN1 [84]. BNIP3 can promote mitophagy even when mitochondria retain their membrane potential [79]. Although BNIP3 and NIX can function as both mitophagy activators and pro-death proteins, the interplay between these two distinct functions and when one is activated over the other are still unclear.

4.3.2. FUNDC1

FUN14 domain-containing protein 1 (FUNDC1) is a highly conserved outer mitochondrial membrane protein that can bind to LC3 and act as a mitophagy receptor. Interestingly, ULK1 has been reported to regulate FUNDC1's pro-mitophagy activity at the mitochondria. In response to stress induced by hypoxia or mitochondrial uncouplers, FUNDC1 is activated by concurrent phosphorylation at Serine 17 by ULK1 and dephosphorylation at Serine 13 by the PGAM5 phosphatase [85–86]. The phosphorylation by ULK1 and dephosphorylation by PGAM5 enhance the ability of FUNDC1 to bind to LC3 through its LIR motif to facilitate mitophagy [85–86]. Recently, another layer of FUNDC1 regulation was uncovered where the mitochondrial resident E3 ubiquitin ligase MARCH5 acts as a negative regulator of FUNDC1-mediated mitophagy. MARCH5 ubiquitinates FUNDC1 at lysine 119, which leads to its proteasomal degradation and abrogation of mitophagy [87]. Although FUNDC1 is known to activate mitophagy during hypoxia [85], this study found that MARCH5 promoted ubiquitination and degradation of FUNDC1 during the hypoxic insult. The authors proposed that this degradation mechanism functions to “fine-tune” the mitophagic activity and prevents unnecessary degradation of undamaged mitochondria during hypoxia [87].

There is also evidence that FUNDC1 regulates mitochondrial dynamics and that this is linked to its mitophagy function. For instance, Chen et al. found that FUNDC1 interacted with the fission and fusion proteins, DRP1 and OPA1, to coordinate mitochondrial morphology and mitophagy [88]. Under baseline conditions, OPA1 interacted with FUNDC1, which prevented activation of mitochondrial fission and mitophagy. Mitochondrial stress led to dephosphorylation of FUNDC1 and dissociation from OPA1. It also led to increased association between FUNDC1 and DRP1 along with induction of mitophagy. Finally, FUNDC1 has also been reported to function at ER-mitochondria contact sites. These sites in the endoplasmic reticulum are called mitochondria-associated membranes (MAMs) and contain a unique set of proteins that interact with mitochondrial proteins. It has been reported that FUNDC1 localizes to MAMs in HeLa cells and cardiac myocytes [89–90]. It was reported that FUNDC1 accumulated at MAMs during acute hypoxia where it functioned as a DRP1 receptor to drive mitochondrial fission prior to mitophagy [89]. More recently, it was discovered that FUNDC1 was required for MAM formation in cardiomyocytes and that it interacted with inositol 1,4,5-trisphosphate type 2 receptor (IP3R2) at these sites to regulate calcium release from the ER into mitochondria and cytosol [90]. Although an increase in cytosolic calcium can activate DRP1 and fission [91], the authors found that DRP1 remained cytosolic during FUNDC1-mediated fission and that FIS1 was required for fission and mitophagy by FUNDC1. The underlying reason for these different results is unclear but might be due to the stimuli used in these studies (hypoxia versus FUNDC1 overexpression).

4.3.3. Other mitophagy receptors

The outer mitochondrial membrane protein BCL2-like protein 13 (BCL2-L-13), a mammalian homologue of the essential mitophagy yeast protein ATG32, can also function as a mitophagy receptor. Interestingly, it also has a role in regulating mitochondrial fission, which often precedes mitophagy [92]. Overexpression of BCL2-L-13 leads to mitochondrial fragmentation, which is facilitated by its conserved BH domains, and unexpectedly, is not dependent on the fission regulator DRP1 [92]. As a potential coupled function, BCL2-L-13 also binds to LC3 via a specific functional WXXI motif and facilitates mitophagy in a Parkin-independent manner [92]. These dual functions might serve to allow for increased coordination and efficiency of the removal of damaged mitochondria.

The most recent mitophagy receptor to be identified is Prohibitin 2 (PHB2). In contrast to other mitophagy receptors, PHB2 resides in the inner mitochondrial membrane and is normally not accessible to LC3 [93]. Under baseline conditions, PHB2 functions in a wide range of processes, including cell proliferation and cristae morphogenesis [94]. It has also been implicated in mitochondrial dynamics by regulating the processing of the fusion protein OPA1 [94]. However, PHB2 contains an LC3-binding domain that allows it to mediate the selective removal of mitophagy upon proteasome-dependent rupture of the outer mitochondrial membrane [93]. PHB2 is also required for Parkin-mediated mitophagy in mammalian cells, specifically in response to oligomycin/antimycin-induced stress, although whether this extends to other stressors has yet to be determined [93]. Importantly, PHB2 has been reported to play an essential role in the clearance of paternal mitochondria post-fertilization in *C. elegans*, suggesting that this is an evolutionarily conserved mechanism of mitochondrial clearance [93]. Of note, because opening of the mPTP leads to rupture of the outer mitochondrial membrane leading to exposure of the inner membrane where PHB2 is localized, it is possible that PHB2-mediated mitophagy plays a role in eliminating these mitochondria. However, the relationship mPTP opening and PHB2 still needs to be investigated.

Another atypical putative mitophagy receptor is cardiolipin. This unique phospholipid resides in the inner mitochondrial membrane, and numerous studies have solidified its role as a key mediator of mitochondrial metabolism [95]. However, in response to mitochondrial damage, it becomes a positive regulator of mitophagy. When mitochondria become damaged, cardiolipin is redistributed to the outer mitochondrial surface, where it binds to LC3II on the autophagosome [96]. Intriguingly, mitochondria were unable to be delivered to autophagosomes when the interaction between LC3 and cardiolipin was abrogated in neurons, confirming that this phospholipid can function as a mitophagy receptor to facilitate the clearance of damaged mitochondria [96]. However, additional studies extending these studies to in vivo models are still needed to clarify its physiological function as a

mitophagy receptor in tissues.

4.4. Mitochondrial dynamics

Mitochondrial dynamics play a key role in mitochondrial health and quality control. Mitochondrial fission involves the fragmentation of mitochondria into shorter sphere shaped organelles, whereas fusion merges individual mitochondria into tubular and elongated networks. Mitochondrial fission facilitates proper mitochondrial clearance [97–102], while fusion protects mitochondria against mitophagy [103–104]. Symmetrical fission occurs in dividing cells and results in two equal daughter mitochondria. Asymmetrical fission occurs upon mitochondrial damage and facilitates the segregation of damaged components from still healthy organelle parts. Thus, this form of fission allows for only the aberrant components of the mitochondria to be removed via mitophagy, while the functional components become part of the mitochondrial network by fusing with healthy and functional mitochondria [100]. Defects in asymmetrical fission have been reported to lead to excessive and detrimental mitophagy in the heart [101].

Mitochondrial fission is regulated by the dynamin-like GTPase dynamin-related protein 1 (DRP1). It translocates from the cytosol to the mitochondria, where it acts as a critical mediator of mitochondrial scission [105]. Cardiomyocyte-specific deletion of DRP1 leads to unopposed fusion resulting in mitochondrial elongation and aberrant mitophagy, ultimately contributing to cardiac dysfunction and death [97–98,106]. Similarly, a C452F mutation in DRP1 in *Python* mice, which prevents disassembly of DRP1 oligomers, also leads to defective mitophagy and heart failure [107]. Additional regulators of mitochondrial fission include MFF, FIS1, and MiD49/51, which have been found to function as receptors for DRP1 on the outer mitochondrial membrane [108] [109–110]. Mitofusin 1 and 2 (MFN1 and MFN2) on the outer mitochondrial membrane and optic atrophy 1 (OPA1) in the inner mitochondrial membrane function as regulators of mitochondrial fusion [111–112]. The importance of mitochondrial dynamics in mitochondrial turnover in vivo was recently confirmed by Song et al. who completely disrupted dynamics by generating cardiac-specific DRP1, MFN1, and MFN2 triple knockout mice [113]. Unexpectedly, these mice were viable and accumulated a mixture of fragmented and fused mitochondria in the heart [113]. However, these mice had a defect in mitochondrial turnover which led to a significant increase in mitochondrial mass, sarcomeric distortions and development of cardiac hypertrophy [113]. Overall, these studies demonstrate the importance of functional mitochondrial dynamics in ensuring proper mitochondrial quality control.

4.4.1. Initiation of autophagy and formation of autophagic vesicles

Once the mitochondria have sent signals to initiate autophagy, the actual autophagic machinery needs to be assembled to carry out the final steps in the process (Fig. 2). Initiation of the isolation membrane, or phagophore, is promoted by a complex composed of BECLIN1(Atg6)/ATG14L/VPS34/VPS15 [2]. This complex is activated by ULK1, which phosphorylates BECLIN1 at serine 14 [114–115]. The membrane for the autophagosome most commonly originates from the ER, but studies have reported that other sources can include Golgi, mitochondrial or plasma membranes [2,116]. Expansion of the nascent autophagosome is achieved through formation of phosphatidylinositol-3-phosphate (PI3P), which promotes translocation of multiple autophagy proteins including ATG18, ATG20, ATG21, and ATG24 to the phagophore assembly site [2]. The ATG conjugation system mediates the elongation, maturation and closure of the autophagosome. In this case, ATG7, ATG10, and ATG3 mediate conjugation of ATG12, a ubiquitin-like molecule, to ATG5 [2]. Next, the ATG12-ATG5 complex interacts with ATG16 to promote elongation of the maturing phagophore. ATG16 promotes lipidation of microtubule-associated protein 1 light chain 3 (LC3). The conjugated form of LC3, LC3II, is incorporated into the phagophore in order to mediate final maturation to an autophagosome [2]. Once this is complete, the mature LC3II remains intact on the autophagosome membrane, where it interacts with specific adaptor proteins or receptors to facilitate selective mitophagy [68]. The autophagosome then fully engulfs the mitochondrion, forming a double-membrane vesicle.

4.4.2. Autophagosome-lysosome fusion

After the mitochondrion is fully sequestered inside the autophagosome, the autophagosome delivers the cargo to the lysosome via fusion (Fig. 2). The fusion process occurs in an ATG14-dependent manner, along with the soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) proteins STX17, SNAP29, and VAMP8 [117–119]. Once fusion has occurred, acid hydrolase enzymes from the lysosome degrade the cellular content, allowing the resulting amino acids and fatty acids to be recycled for future use [119]. Defects in the autophagosome-lysosome fusion process are detrimental and have been implicated in disease. For example, loss-of-function mutations in the gene that encodes the lysosome-associated membrane protein 2 (LAMP2) cause Danon disease, which is an X-linked disorder that leads to cardiomyopathy [120]. Recently, it was demonstrated that a mutation in *LAMP2* can also cause a severe dilated cardiomyopathy distinct from the hypertrophic cardiomyopathy normally observed in Danon disease [121]. Molecular studies revealed that hiPSC-CMs derived from a patient diagnosed with Danon disease had reduced mitophagy and decreased mitochondrial function that could be rescued upon restoration of the most abundant LAMP-2 isoform in the heart, LAMP2B [122]. These findings were further confirmed in vivo, in which *Lamp2* knockout mice also showed impaired autophagic flux and mitochondrial defects, as well as early features of contractile dysfunction [122]. Taken together, these findings confirm that defects in mitochondrial clearance will have severe consequences for mitochondrial rich tissues such as the heart.

5. Non-canonical mitochondrial quality control pathways

5.1. RAB9A/B-dependent alternative mitophagy

Traditional autophagy has been characterized by dependence on the proteins ATG5/7 and LC3, which are required for elongation of the phagophore and maturation of the autophagosome (Fig. 2A). However, a form of alternative autophagy that is independent of ATG5/ATG7 or LC3 has recently been identified. This ATG5/7 autonomous pathway is dependent on the small GTPase RAB9A/B and the membrane for the vesicle is derived from the trans-Golgi [11] (Fig. 2B). However, there is some overlap with the traditional autophagy pathway because ULK1 and BECLIN1 are both required for its activation [11,123] (Fig. 2). Additionally, another study confirmed these findings in HeLa cells, verifying the importance of RAB9A/B in alternative autophagy, and also revealing the requirement of the MAPKs MAPK1/ERK1 and MAPK14/p38 in this process of mitochondrial clearance [124]. Recently, RAB9-dependent alternative autophagy was also shown to be required for IGF-IIR-induced mitophagy in cardiomyocytes [125]. Intriguingly, PARKIN was also necessary for this process, thus raising the possibility that PARKIN recognizes the damaged mitochondria and labels them for degradation by RAB9-positive autophagosomes [125]. However, further studies are necessary to elucidate the exact nature of this relationship. It is also still unclear what specific conditions lead the cell to decide on activation of conventional versus alternative autophagy and whether they potentially have other overlapping factors that facilitate this decision. Furthermore, while RAB9A/B-dependent alternative autophagy was detected in several ATG5 deficient embryonic tissues in vivo [11], the functional role of this non-canonical pathway in the heart is still unclear.

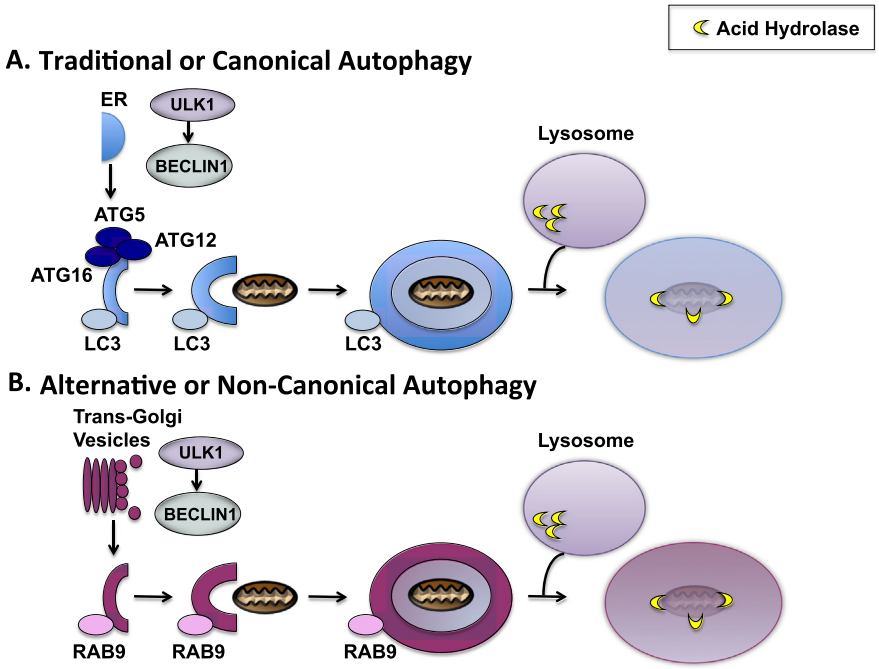

Fig. 2. Conventional versus alternative autophagy pathways. **A**) Canonical autophagy is initiated by ULK1, which then activates BECLIN1. The autophagosome membrane is derived from the endoplasmic reticulum (ER). ATG5/ATG12/ATG16 and LC3 facilitate elongation of the membrane. The autophagosome then sequesters the mitochondrion inside a double-membrane vesicle. The autophagosome fuses with a lysosome and the mitochondrion is degraded by the acid hydrolases. **B**) Alternative autophagy is activated by ULK1 and BECLIN1. The autophagosome membrane is derived from the trans-Golgi and elongation is dependent on RAB9. The autophagosome sequesters the mitochondrion and delivers the cargo to the lysosome for degradation.

5.2. Microautophagy

Microautophagy is another avenue for degradation of cellular components that is distinct from the conventional autophagy process. Microautophagy involves the direct internalization of proteins and organelles into lysosomes via invaginations in the lysosomal membrane [12] (Fig. 3A). Degradation can occur in a nonselective manner, in which randomly sequestered soluble intracellular material is broken down, or in a selective manner, involving the direct sequestration of organelles such as mitochondria, nucleus, and peroxisomes into lysosomes [12]. Recently, it was proposed that there are actually three distinct types of microautophagy based on membrane dynamics and molecular machineries: microautophagy with lysosomal protrusion, microautophagy with lysosomal invagination, and microautophagy with endosomal invagination [126]. Although very little is known about this process in mammalian cells, there is evidence that microautophagy can contribute to mitochondrial degradation. For instance, in response to ischemia/reperfusion (I/R) injury in the heart, mitochondria were directly targeted to lysosomes through a process that was dependent on GAPDH activity and negatively regulated by the protein kinase PKCδ [127]. Inhibition of this process led to accumulation of damaged mitochondria in the cytosol and initiation of cell death pathways [127]. The same group later confirmed that inactivated GAPDH associated with damaged mitochondria and served as a signaling molecule to induce direct engulfment of the mitochondria into lysosomes [128]. Still, very little is known about this process, such as its regulators in mammalian cells and its role in disease.

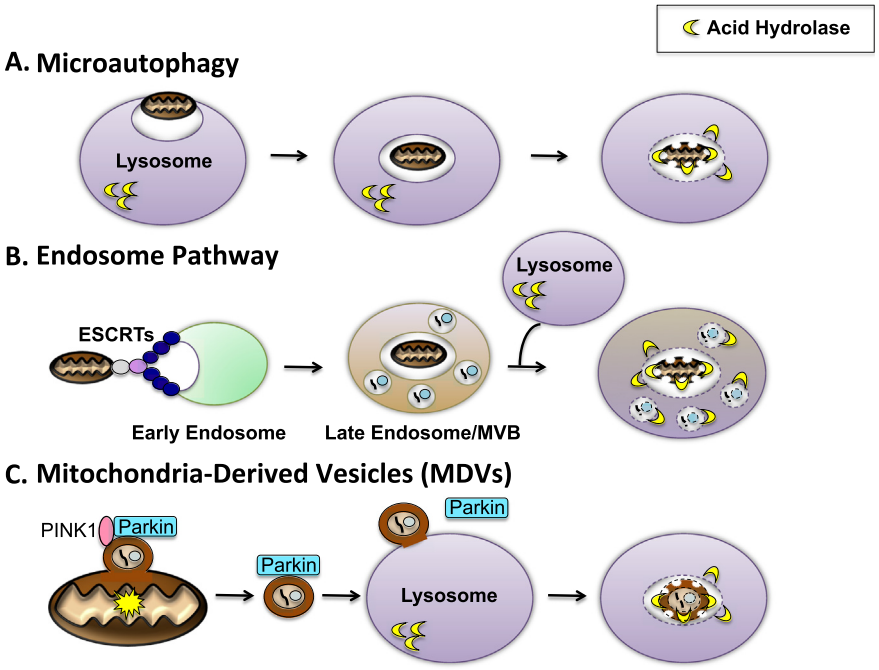

Fig. 3. Alternative pathways of mitochondrial clearance. **A**) In microautophagy, cargo is directly engulfed into lysosomes. The lysosome forms an invagination to envelop the cargo. The internalized vesicle and its content are then degraded by acid hydrolases. **B**) Clearance of mitochondria via the endosomal pathway. The endosomal sorting complexes required for transport (ESCORT) machinery allows mitochondria to be sequestered inside an early endosome. The early endosome then matures into a late endosome or multivesicular body (MVB), which fuses with a lysosome. **C**) Mitochondrial Derived Vesicles (MDVs) are generated in a PINK1/Parkin-dependent manner in response to enhanced oxidative stress from within the mitochondria. The MDVs bud off the mitochondria with their cargo and fuse with lysosomes to degrade the cargo.

5.3. Endosome pathway

New studies have also uncovered a role for the endosomal-lysosomal degradation pathway in mitochondrial quality control. Although the endosomes are known for delivering plasma membrane proteins to the lysosomes for degradation, the endosomes can also sequester damaged mitochondria (Fig. 3B). First, it was reported that a novel RAB5-dependent endosomal pathway could clear depolarized mitochondria that had been labeled for degradation by PARKIN [13]. Moreover, the RAB5-positive endosomes internalized mitochondria through the endosomal sorting complexes required for transport (ESCRT) machinery and then delivered them to lysosomes for degradation (Fig. 3B). Although induced by the same stressors as traditional mitophagy, this mechanism was activated prior to the onset of autophagy, indicating that it is temporally distinct. There is, however, suggested cross talk between the two pathways, as the autophagy protein BECLIN1 was required for the formation of RAB5 positive vesicles in response to stress, implicating it as a potential master regulator for multiple mitochondrial quality control pathways. Interestingly, the endosomal-mediated clearance of mitochondria is not always dependent on PARKIN and can also be utilized by the mitophagy receptor BNIP3 to clear mitochondria in cells [129]. These findings are indicative of a redundancy in downstream degradation pathways for the various mitophagy pathways, ensuring efficient clearance of potentially harmful mitochondria. Further evidence suggesting overlap between regulators of the various mitochondrial quality control pathways comes from the findings that many of the RAB GTPases regulate both the endosomal and traditional autophagy pathways. For instance, TBC1D15 and TBC1D17, two mitochondrial RAB GTPase-activating proteins (GAPs) function downstream of PARKIN and were shown to regulate RAB7 activity at the interface between mitochondria and isolation membranes to mediate proper autophagosomal biogenesis during mitophagy [130]. Another recent study by the same group further demonstrated that the upstream factor of the endosomal RAB GTPase cascade, RABGEF1, facilitated recruitment of RAB5 and RAB7A to damaged mitochondria, where RAB7A was shown to be necessary for ATG9A vesicle assembly and enclosure of the mitochondria in autophagic membranes, [131]. Clearly, there is a lot of cross talk and overlap between the various degradation pathway and more functions for the RAB proteins in regulating autophagy are likely to be identified in future studies.

5.4. Mitochondria-derived vesicles (MDVs)

Mitochondria-derived vesicles (MDVs) also deviate from conventional autophagy and function to selectively eliminate smaller portions of a damaged mitochondrion. MDVs are generated as an early response to oxidative stress and do not require mitochondrial depolarization or the autophagy proteins ATG5 and LC3 [132] (Fig. 3C). Ultrastructural analysis confirmed the presence of mitochondrial proteins and lipids within these vesicles, indicating that MDVs function an alternative quality control mechanism [132]. Interestingly, biogenesis of MDVs was shown to occur in a PINK1/PARKIN-dependent manner in response to enhanced oxidative stress from within the mitochondria [133] (Fig. 3C). These MDVs containing damaged cargo then bud off the mitochondria for delivery to the lysosome where they are degraded [132–133] (Fig. 3C). Formation of MDVs has been identified in the heart, thus extending these findings to a more physiologically relevant system. MDV formation was confirmed both under baseline conditions, as well as in response to stress, in H9c2 cardiac myoblasts [134]. Interestingly, in vivo, MDV formation was more prevalent than mitophagy in mouse hearts under baseline conditions, whereas, doxorubicin-induced stress and mitochondrial dysfunction triggered both MDV formation and mitophagy [134]. Taken together, these findings suggest that MDV formation likely acts as a homeostatic mechanism and first line of defense against mitochondrial damage in the heart. Similar to the endosomal degradation pathway, the MDV pathway appears to be temporally distinct from autophagy and could have its own niche to maintain mitochondrial homeostasis at baseline and as a first response to mild or moderate stress that does not require removal of the entire mitochondrion.

6. Mitophagy in cardiovascular disease and aging

It is clear that functional mitochondrial quality control in the heart is critical for cardiac homeostasis and to prevent accelerated aging and development of disease. Many studies have reported that disruption of key autophagy proteins have severe consequences for the heart, both under baseline conditions and in response to stress [4,120,135,9–10,8,7]. Also, a number of studies have demonstrated the importance of mitophagy in protecting the heart against stress. For instance, PARKIN-mediated mitophagy is cardioprotective after a myocardial infarction (MI) [2]. PARKIN-deficient mice were more sensitive to MI and accumulated dysfunctional mitochondria in hearts which correlated with reduced survival [7]. Mitophagy was also shown to be protective in response to pressure overload-induced heart failure [97]. Specifically, mitophagy was transiently activated in response to TAC, but its subsequent downregulation contributed to accumulation of dysfunctional mitochondria and development of heart failure [97]. These defects could be rescued by Tat-BECLIN1, a potent autophagy inducer, in a DRP1–dependent manner, indicating that mitophagy acts as a crucial response in combating the stress induced by pressure overload [97]. Another recent study showed that cardiac-specific overexpression of BECLIN1 promoted mitophagy via the PINK1/PARKIN pathway and protected the heart during sepsis [136]. Additionally, inhibition of PARKIN-dependent mitophagy has been reported to play a role in the development of diabetic cardiomyopathy [137].

Other mitophagy pathways also function to maintain mitochondrial health and cardiac homeostasis. In fact, studies suggest that FUNDC1 is a key regulator of mitochondrial health in the heart. Cardiac specific deletion of FUNDC1 led to increased mitochondrial mass and reduced mitochondrial respiration [90], an indication that mitochondrial degradation is reduced in these hearts. In addition, FUNDC1-mediated mitophagy also protected against stress such as I/R injury [138–141]. Interestingly, Casein kinase 2α (CK2α), a negative regulator of FUNDC1, was found to be upregulated in the heart in response to acute I/R injury which led to suppression of FUNDC1-mediated mitophagy, accumulation of damaged mitochondria, and loss of myocytes [139]. Cardiac-specific CK2α null mice had reduced mitochondrial damage and were protected against IR injury. Similar to PARKIN knockout mice [7], loss of FUNDC1 in the heart led to increased susceptibility to MI and reduced survival after the infarct compared to wild type mice [90]. However, because FUNDC1 also regulates mitochondrial dynamics and MAM formation, it is possible that the cardiac phenotype observed in the *Fundc1*-deficient hearts is due to disruption of the multiple processes regulated by FUNDC1. Also, it is unclear whether PARKIN-mediated mitophagy is altered in the FUNDC1 knockout hearts. Similar concerns apply to the PARKIN-deficient mice and whether FUNDC1 can compensate for the lack of Parkin. Several recent studies have identified non-mitophagy roles for PARKIN [142].

Aging is generally associated with reduced autophagy in the heart, leading to accumulation of dysfunctional mitochondria and increased susceptibility to stress [143]. Many of the necessary autophagy components are reduced with age, contributing to the reduced autophagy [144–146]. For instance, cardiac-specific ATG5 knockout mice exhibit an accelerated cardiac aging phenotype with development of cardiomyopathy and reduced lifespan [4]. In contrast, ATG5 overexpression was found to be protective by activating autophagy and extending lifespan [147]. Additionally, *Parkin* knockout mice showed accelerated accumulation of abnormal mitochondria, mtDNA deletion mutations, and oxidative damage in the heart with age [6,9], while PARKIN

Table 1
Mouse models used to study mitophagy in the heart.

Mouse model	Effect on mitophagy	Cardiac phenotype	References
Pink1 knockout mice	Disruption of PINK1- mediated mitophagy	Development of cardiac hypertrophy. Failure to activate exercise-induced mitophagy in cardiac tissue	[8,156]
Park2 knockout mice	Disruption of PARKIN mediated mitophagy	Increased susceptibility to myocardial infarction and aging due to accumulation of dysfunctional mitochondria.	[6,7,9]
Cardiac specific *Park2* knockout mice	Disruption of /PARKIN mediated mitophagy in myocytes	Leads to failure to undergo metabolic transition after birth. Loss of Parkin is lethal.	[3]
Bnip3/Bnip3l double knockout mice	Disruption of BNIP3 and NIX-mediated mitophagy	Accelerated accumulation of dysfunctional mitochondria with age	[10]
Cardiac specific *Fundc1* knockout mice	Disruption of FUNDC1-mediated mitophagy in myocytes	Increased mitochondrial mass and reduced mitochondrial function. Increased susceptibility to myocardial infarction.	[90]
Cardiac specific *AMPKα2* knockout mice	Reduced activation of PINK1-mediated mitophagy	Exacerbated transverse aortic constriction-induced heart failure due to decreased mitophagy.	[22]
Cardiac specific *Drp1* knockout mice	Impaired DRP1-mediated fission. Leads to altered mitophagy	Development of pathological hypertrophy due to altered mitophagy. Studies have reported both increased and decreased mitophagy.	[97–98,106]
Cardiac specific *Drp1Mfn1/Mfn2* triple knockout mice	Abrogation in both mitochondrial fission and fusion. Leads to impaired mitophagy.	Dramatic accumulation of mitochondria in myocytes due to impaired degradation. Leads to cardiac hypertrophy and death.	[113]
Cardiac specific *Atg5* knockout mice	Abrogation in traditional ATG%-mediated autophagy.	Accumulation of dysfunctional mitochondria in the heart. Development of cardiac hypertrophy and accelerated aging.	[4]

overexpression mitigated the effects of aging in the heart [9]. Recent findings indicate that a thiol-dependent process can negatively regulate autophagy through direct oxidation of the key autophagy-related proteins ATG3 and ATG7, leading to prevention of LC3 lipidation in vitro and in mouse aorta [148]. While the mechanisms need to be further clarified, this has potential implications for impaired mitophagy during aging. Additionally, cardiac-specific deletion of SIRT3, which contributes to the prevention of redox stress and cell aging, impaired PARKIN-mediated mitophagy by increasing P53-PARKIN binding and blocking its translocation to the mitochondria [149]. AKT2 ablation was also shown to reverse age-induced reductions in the mitophagy proteins PINK1, PARKIN, and BNIP3, and resulted in improved myocardial contractile function and prolonged lifespan [150]. Of note, this study found that the beneficial effects of rapamycin and the SIRT1 activator SRT1720 on age-induced contractile dysfunction and loss of mitophagy in cardiomyocytes occurred in a PARKIN-dependent manner [150]. Taken together, these findings support a cardioprotective role for autophagy and mitophagy in response to aging, but further studies are required before utilizing these pathways as direct therapeutic targets.

7. Mitophagy and exercise

Exercise is well known to induce autophagy in mitochondrial-rich tissues such as skeletal muscle and the heart [151–153]. Recent studies have also highlighted that mitophagy is activated in response to exercise and during the recovery period [154–155,20,156]. During intense exercise, mitochondria increase their ATP generation to meet the increased energy need of the muscle tissue. However, the increased respiratory activity also leads to enhanced generation of ROS which can cause damage to the mitochondria in skeletal muscle cells [157]. Activation of mitophagy ensures the removal of these damaged mitochondria before they can cause harm to the cell. Laker et al. found that mitophagy was activated via the AMPK-ULK1 signaling pathway during the recovery period immediately after the exercise to eliminate the mitochondria that had been damaged in cells during the exercise [20]. Another study found that PARKIN-deficient mice failed to enhance mitophagy in skeletal muscle in response to acute exercise, implicating the PINK1/PARKIN pathway in exercise-induced mitochondrial degradation [158]. The importance of this process is supported by the observation that abrogating autophagy in skeletal muscle led to accumulation of damaged mitochondria during exercise [159]. However, whether AMPK/ULK1 coordinate with PINK1/PARKIN to facilitate mitophagy in response to exercise have yet to be investigated.

Although autophagy is activated in the heart during exercise, much less is known about activation of mitophagy in the heart after exercise. Lee et al. found that autophagic flux and BNIP3 protein levels were significantly increased in mouse hearts after treadmill running [153] while Sliter et al. reported that exhaustive treadmill running induced PINK1-mediated mitophagy mouse hearts [156]. Interestingly, Zhao et al. recently showed that short-duration swimming exercise attenuated cardiac dysfunction following MI in aged mice, in part, by regulating mitophagy [160]. Thus, whether one of the health benefits of exercise-induced mitophagy is due to mitochondrial rejuvenation and prevention of cellular aging in post-mitotic tissues remains to be investigated in more detail.

8. Conclusion and future directions

Our current understanding of the pathways that regulate mitophagy in the heart is still fairly limited. Most of our knowledge to date comes from studies performed in vitro utilizing immortalized cell lines. However, these studies need to be expanded into more relevant in vivo models. The current mitophagy mouse models available and their cardiac phenotypes are listed in Table 1. There are also many unanswered questions regarding the crosstalk between the conventional and non-canonical mitochondrial clearance pathways. For instance, is targeting one of these pathways a therapeutic option to treat or prevent development of heart disease? Also, if one pathway is impaired, is it possible to target one of the alternative pathways to enhance mitochondrial clearance and prevent unwanted cardiac impairment? The key to uncovering this will be to continue to find the specific regulators of these pathways in order to illuminate their distinct functional mechanisms. Already, some overlap in the regulation between these pathways has been established and additional studies on these potential master regulators, such as ULK1 and BECLIN1, are needed. While developing drugs/agents that are capable of inducing mitophagy in the heart is of significant interest, much more research is necessary to determine the viability of this approach. Although many studies suggest that general autophagy elicits cardioprotective benefits, finding safe and efficient targets remains challenging. A recent study reported that the natural polyamine spermidine, which is a dietary compound, enhanced autophagy and mitophagy and led to cardioprotection and increased lifespan in mice and rats [161]. Additionally, this study included epidemiological data indicating that spermidine may also play a cardioprotective role in humans, although these results are just correlative and further clarification is necessary [161]. Interestingly, the protective effects of spermidine were abrogated in mice that lacked the autophagy protein ATG5 in cardiomyocytes [161]. However, since mitophagy-specific agents are lacking, inducing general autophagy may not

selectively remove damaged mitochondria. Additionally, even if mitophagy-specific targets are pursued, there may be other adverse effects from their overexpression, as many mitophagy proteins are known to have additional functions. Thus, effectively targeting mitophagy in the heart currently remains elusive.

Transparency document

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

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Conflicts of interest

None.

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