JCB Journal of Cell Biology

REVIEW

Mitophagy pathways in health and disease

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Mitophagy is an evolutionarily conserved process involving the autophagic targeting and clearance of mitochondria destined for removal. Recent insights into the complex nature of the overlapping pathways regulating mitophagy illustrate mitophagy's essential role in maintaining the health of the mitochondrial network. In this review, we highlight recent studies that have changed the way mitophagy is understood, from initiation through lysosomal degradation. We outline the numerous mitophagic receptors and triggers, with a focus on basal and physiologically relevant cues, offering insight into why they lead to mitochondrial removal. We also explore how mitophagy maintains mitochondrial homeostasis at the organ and system levels and how a loss of mitophagy may play a role in a diverse group of diseases, including cardiovascular, metabolic, and neurodegenerative diseases. With disrupted mitophagy affecting such a wide array of physiological processes, a deeper understanding of how to modulate mitophagy could provide avenues for numerous therapies.

What is mitophagy?

Introduction

Macroautophagy, hereafter referred to as "autophagy," is an evolutionarily conserved pathway involving the engulfment of cytosolic contents by a lipid membrane for recycling of nutrients or removal of harmful aggregates, microbes, and organelles (He and Klionsky, 2009). Mitophagy is one form of macroautophagy that involves selectively targeting and engulfing mitochondria for removal through lysosomal degradation (Rodriguez-Enriquez et al., 2006). Activation of this pathway is a result of mitochondria being damaged beyond the capabilities of other quality control methods or in instances in which the cell needs to get rid of mitochondria for metabolic or developmental purposes (Palikaras et al., 2018). As is the case in other forms of selective autophagy, mitophagy involves some form of "eat me" signal on the surface of the mitochondria designated for clearance (Palikaras et al., 2018).

The efficient functioning of mitochondria is essential for their diverse roles in the cell, including but not limited to ATP synthesis, lipid and heme biosynthesis, calcium buffering, and innate immune surveillance (Friedman and Nunnari, 2014). During instances of mitochondrial damage, mitophagy removes malfunctioning mitochondria to maintain the population at an optimal state (Palikaras et al., 2018). Not only are damaged mitochondria deficient at making ATP and other biosynthetic products, but, as a result, they release greater levels of reactive oxygen species (ROS; Murphy, 2009). This turns into a feedback signal because mitochondria themselves are sensitive to the oxidizing damage of ROS to proteins and DNA in addition to downstream activation of the NLRP3 inflammasome (Heid et al., 2013). Accumulation of defective mitochondria also leads to cell death through the release of prodeath molecules and accumulation of mutations in the mitochondrial DNA (mtDNA; Youle, 2019). The removal of mitochondria is balanced through the regulated biogenesis of new mitochondria (Jornayvaz and Shulman, 2010). Uncontrolled mitophagy would disrupt homeostasis because there would not be enough remaining organelle, in addition to overwhelming lysosomes. Because the careful and timely removal of mitochondria appears crucial for cell survival, cells have evolved numerous and often overlapping pathways to ensure that mitophagy can occur in a balanced way in response to a wide array of stimuli and triggers (Palikaras et al., 2018).

Although this review focuses on mitophagy and its role in mitochondrial quality control, various mitochondrial quality control programs exist and function independently from mitophagy (Youle, 2019). The type of mitochondrial quality control activated depends on the category and level of stress or damage. The most basic of these involves regulation of mitochondrial dynamics through fission and fusion to locally sequester portions of damaged mitochondria (Ni et al., 2015). Before a segment of mitochondria can be fully engulfed in the growing mitophagophore, it must be asymmetrically divided from the remaining mitochondrial reticulum. This ensures that the damaged regions will be isolated in a way that minimizes the lost contents. The mitochondrial proteins Fis1 and MFF recruit

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https://doi.org/10.1083/jcb.202004029

and form complexes with DRP1, allowing it to encircle the region of mitochondria to be split off (Friedman and Nunnari, 2014). In addition, the mitochondrial unfolded protein response relieves the burden of misfolded and damaged proteins by increasing the expression and presence of chaperones such as heat shock protein 22 (Hsp22), Hsp60, and Hsp70 and the proteases Lon and ClpP within mitochondria (Moehle et al., 2019). Mitochondrial proteins can also be translocated to the cytosol for proteasomal clearance (Bragoszewski et al., 2015). Finally, other pathways related to mitophagy involve mitochondria-derived vesicles, piecemeal mitophagy, and mitochondria being taken up directly by lysosomes (microautophagy; Kiššová et al., 2007; Neuspiel et al., 2008; Soubannier et al., 2012; McLelland et al., 2014; Le Guerroué et al., 2017; Pickles et al., 2018). Recently, transmitophagy, the transfer of mitochondria from cell to cell for degradation, has been explored, especially in the central nervous system, reflecting the progress the field has made in understanding mitochondrial clearance from within a cell to a system of cells (Davis et al., 2014; Morales et al., 2020).

Autophagy proteins involved in mitophagy

We start with the question of whether mitophagy begins with the same machinery as the well-characterized autophagy initiation pathways (Fig. 1). There is coordinated colocalization of the ULK1 complex to ATG9 vesicles along the ER, and both are important for mitophagosome initiation (Itakura et al., 2012). The ULK1 complex transmits upstream signals of stress from mammalian target of rapamycin and AMP-activated protein kinase (AMPK) to promote autophagy initiation in times of need, and it is itself regulated by phosphorylation, whereas the ATG9 vesicles offer another source of lipid membrane that will be included in the new autophagosome (Kim et al., 2011; Yamamoto et al., 2012). These areas can then recruit VPS34, the class III phosphatidylinositol 3-kinase (PI3P) complex that produces PI3P (Burman and Ktistakis, 2010). Omegasomes are DFCP1-positive ER segments where PI3P is concentrated and are often regarded as focal points for phagophore synthesis and precursors to the isolation membranes that facilitate autophagy (Axe et al., 2008; Nanao et al., 2015). This PI3P also attracts WIPI1 and WIPI2, which recruit ATG16 and the associated ATG machinery needed to covalently modify ATG8s with phosphatidylethanolamine (Polson et al., 2010). The ATG8 family of proteins associate with phagophores through conjugation to phosphatidylethanolamine and are understood as the tether between phagophore and cargo (Kabeya et al., 2004; Pankiv et al., 2007). Once this machinery is in place, the mitophagophore continues to elongate and mature through the addition of lipids until it can be closed by the endosomal sorting complex required for transport machinery (Zhen et al., 2020).

The next question involves whether mitochondria are targeted to existing phagophores within the cytosol, like those created following starvation, or whether mitochondrial stress stimulates the de novo synthesis of the autophagosome around or even on the surface of the mitochondria that will be removed. Recent studies support the latter model, and from an energetic point of view, local synthesis would limit the size of the phagophore to one that is as small as possible (Lazarou et al., 2015; Vargas et al., 2019; Zachari et al., 2019). Depolarized mitochondria become systematically labeled with phosphorylated ubiquitin through the actions of PINK1 and Parkin, respectively (Vives-Bauza et al., 2010; Matsuda et al., 2010; Narendra et al., 2010). PINK1 is a mitochondrial-localized serine/threonine protein kinase that phosphorylates ubiquitin (Koyano et al., 2014). Parkin is an E3 ubiquitin ligase that is found in the cytosol until PINK1 phosphorylation directs its recruitment and activation on the surface of damaged mitochondria (Kazlauskaite et al., 2014). In this new location, Parkin ubiquitinates outer mitochondrial membrane (OMM) proteins, providing the substrate for additional PINK1-induced phosphorylation (Matsuda et al., 2010; Ordureau et al., 2014). Ubiquitin acts as a signal to cargo receptors such as OPTN and NDP52, which contain LC3/GABARAP-interacting region (LIR) motifs that allow the subsequent recruitment of ATG8 to the mitochondria (Lazarou et al., 2015). Phosphorylation of OPTN and NDP52 by the kinase TBK1 controls their localization to ubiquitinated mitochondrial proteins (Lazarou et al., 2015; Heo et al., 2015). Recent studies have shown that NDP52 can bring the ULK1 complex to the mitochondrial surface through direct binding to FIP200 to initiate mitophagy (Lazarou et al., 2015; Vargas et al., 2019). Additional cargo receptors such as p62, NBR1, and TAX1BP1 that are known for roles in other selective autophagy processes appear to play a more modest and dispensable role in mitophagy (Shi et al., 2015; Lazarou et al., 2015).

Mitophagosome synthesis has historically been perceived as the linear sequence described above. Using this understanding, the initiation of mitophagy would be LC3 independent because recruitment of NDP52 to mitochondria through ubiquitin binding is enough to initiate a phagophore at the surface of the mitochondria even in the absence of all ATG8s (Nguyen et al., 2016). However, mitophagosomes that form in the absence of LC3 are smaller and do not mature fully, suggesting that the LC3 family is important for elongation and maturation (Padman et al., 2019). This study later confirmed that there is a feedforward cycle whereby LC3 on the mitochondria can drive further recruitment of NDP52 and OPTN rather than strictly the opposite direction. Discovery of this feedback illustrates one of the major trends in mitophagy: that the pathway involves positive feedback signals and amplification loops instead of a linear sequence of effects (Heo et al., 2015). This also provides one explanation for why redundancy in certain steps of the mitophagy pathway may actually be a good thing; these are likely steps that benefit from multiple overlapping amplification loops so that when one protein is absent, the whole pathway can still operate. Interestingly, a recent study reported that OPTN and ATG13 interact with mitochondria in an oscillatory nature, supporting the cyclical amplification that mitochondrial targeting and initiation involves (Zachari et al., 2019).

The functional importance of LC3 localization remains a prominent focus of the field. Reports suggest that LC3 is still targeted to mitochondria in the absence of FIP200 or ATG9A, raising the possibility that LC3 may be brought to mitochondria without the mitophagosome and serve as a marker for damaged mitochondria such as ubiquitin (Itakura et al., 2012). Reports also suggest that LC3 and other ATG8s can facilitate recruitment

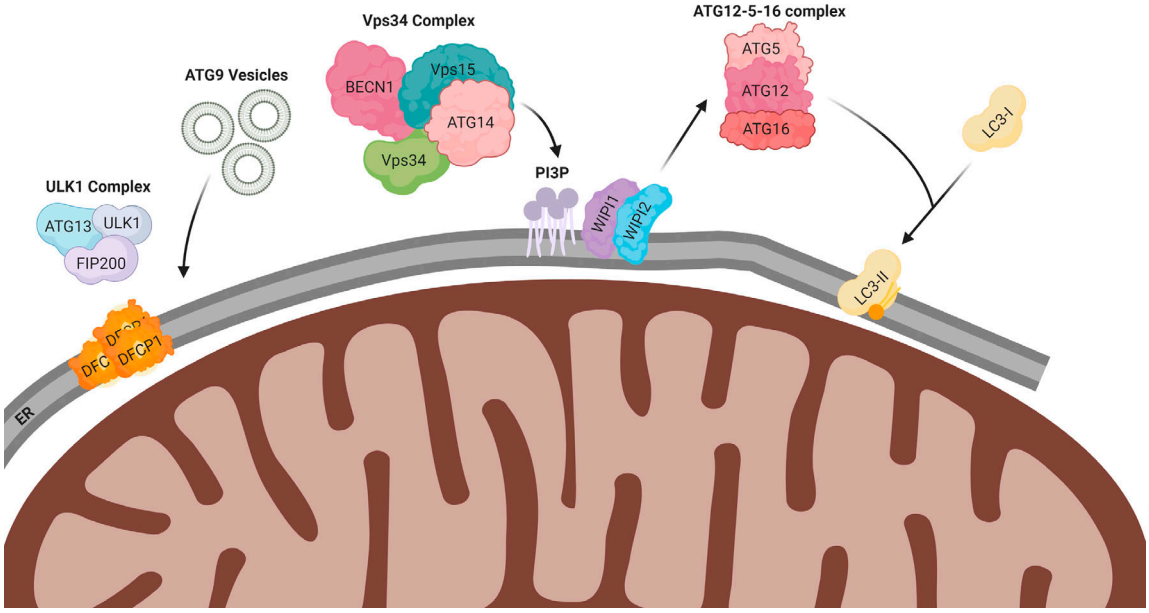

Figure 1. **Canonical autophagy machinery is involved in de novo mitophagy initiation on the surface of mitochondria.** ATG9 vesicles and the ULK1 complex are recruited to DFCP1-positive regions of the ER known as "omegasomes." Together, these bring the Vps34 complex, which amplifies the local PI3P signal that attracts WIPI1/2. Last, additional ATGs, including the ATG12–5–16 complex, facilitate LC3 lipidation and incorporation into the growing phagophore, ensuring proper maturation and elongation. Figure was created with BioRender.com.

of initiation machinery such as the ULK1 complex themselves by serving as a scaffold (Alemu et al., 2012). Interestingly, the need for NDP52 and LC3 can be completely bypassed by artificially targeting ULK1 to the mitochondria (Vargas et al., 2019). However, although components may be dispensable under certain artificial setups, extrapolating from these results is challenging at best.

Experiments involving the overexpression of Parkin in combination with a toxin that severely damages a large proportion of the mitochondria have led to a detailed understanding of mitophagy that may not be generalizable to mitophagy following unrelated treatments. In agreement with this, a study investigating the drug ivermectin found that mitophagy was ubiquitin dependent but Parkin independent (Zachari et al., 2019). Following ivermectin treatment, TBK1 acts independently of OPTN, and FIP200 acts independently of ULK1 (Zachari et al., 2019). By studying different experimental setups, groups can uncover notably different pathways for mediating mitophagy. In addition, receptor-mediated mitophagy pathways occur independently from mitochondrial surface ubiquitination, which raises questions involving how the autophagy initiation machinery would be targeted to the mitochondria and what components would be shared with the ubiquitin pathways (Schweers et al., 2007; Zhang et al., 2008; Liu et al., 2012; Murakawa et al., 2015; Bhujabal et al., 2017). NDP52 and OPTN, which are known to detect ubiquitin, may not be involved at all or until a later step, possibly being recruited to mitochondria by LC3 that is bound to the OMM mitophagy receptors. Redundancy and overlap are evolutionarily beneficial because mitochondrial quality control is essential, so the cell must have ways to fine-tune a path, depending on the current conditions.

Role of additional organelles in controlling mitophagy

Clearance of mitochondria is not a self-destruction program that mitochondria complete autonomously in isolation; rather, it depends on additional organelles with the ER as the key player (Böckler and Westermann, 2014; Zachari et al., 2019). The ER provides most lipids for the autophagosome, with notable contributions from the Golgi, plasma membrane, and the mitochondrion itself (Ravikumar et al., 2010; Tooze and Yoshimori, 2010; Böckler and Westermann, 2014). ER–mitochondria contacts are referred to as "mitochondria-associated membranes," and these sites are of significance for mitophagy for several reasons (Hamasaki et al., 2013). Upon stimulation of autophagy, DFCP1 translocates to mitochondria-associated membranes and is a marker for omegasomes (Axe et al., 2008; Itakura et al., 2012). Interestingly, PINK1-BECN1 localizes to omegasome sites as well, supporting the importance of the sites in mitophagy initiation (Gelmetti et al., 2017). Efficient calcium transfer and balance between the two organelles is needed for energy production and mitophagy initiation (MacVicar et al., 2015; Marchi et al., 2018). Last, crosstalk between mitochondrial and ER stresses has been proposed to induce Parkin expression, which can help trigger the PINK1/Parkin pathway for mitophagy, whereas certain mitochondrial stressors can also activate protein kinase R–like ER kinase (Bouman et al., 2011; Fessler et al., 2020; Guo et al., 2020).

ATG9A vesicles from the trans-Golgi network are targeted to the autophagosome formation site on damaged mitochondria independently from the ULK1 complex and are required for further recruitment of multiple ATG family members and subsequent expansion of the autophagophore (Itakura et al., 2012). Additional components that help facilitate mitophagy are endosomal Rab-GTPase family members (Yamano et al., 2014).

Through ubiquitin detection, RABGEF1 engages Rab5 and Rab7A, which in turn usher ATG9 vesicles to damaged mitochondria (Yamano et al., 2018). TBK1 phosphorylation of RAB7A is important for this role in bringing ATG9 vesicles to ubiquitin-labeled mitochondria (Heo et al., 2018). TBC1D15/17 is a Rab-GTPase–activating protein that controls autophagosome biogenesis by limiting phagophore growth around the cargo by releasing Rab7A from the mitochondrial surface, ensuring precise engulfment (Yamano et al., 2014). In addition to these roles of Rabs in canonical mitophagy, the endosomal signaling pathway has been described as a program of mitochondrial clearance that is dependent on multiple Rabs while being independent of major components of the autophagy machinery, such as the ULK1 complex (Hammerling et al., 2017). In this pathway, depolarized mitochondria are still ubiquitinated by Parkin, and BECN1 is recruited and activates Rab5, which, in combination with the endosomal sorting complex required for transport machinery, engulfs mitochondria in endosomes (Hammerling et al., 2017). These endosomes later mature into Rab7 endosomes toward the end of the pathway, before lysosomal degradation (Hammerling et al., 2017).

While canonical autophagy is induced through lipid conjugation of ATG8s thanks to the concerted actions of ATGs such as ATG5 and ATG7, noncanonical autophagy and mitophagy occur independently of ATG5, ATG7, and ATG8s (Codogno et al., 2012; Saito et al., 2019). Instead, following energetic stresses such as starvation or ischemia, AMPK, ULK1, BECN1, and the trans-Golgi network work in combination with Rab9 to bring late endosome membranes to the mitochondria (Saito et al., 2019). Notably, this method of clearance is Parkin and ubiquitin independent. It is apparent that the cell has multiple different ways of inducing both canonical and noncanonical mitochondrial clearance. This review focuses on the better-characterized pathways that employ canonical autophagy machinery.

At the final step of each of these mitophagy pathways, the mitophagosome fuses with the lysosome, where the acid hydrolase enzymes can recycle the building blocks of the cell for new purposes (Wong et al., 2019). Mitophagy efficiency can be impacted by lysosomal dysfunction as well, and if the lysosomes are not functioning properly, a buildup of toxic organelles and aggregates can accumulate, which would further affect mitochondrial health and clearance (Plotegher and Duchen, 2017). Similarly, mitochondrial health has been known to affect lysosomal function, so maintaining healthy crosstalk remains another important system in the cell (Wong et al., 2019; Deus et al., 2020).

How does mitophagy occur?

Triggers for mitophagy

Because it is advantageous for mitochondria to be removed in different circumstances, it is expected that multiple triggers can induce mitophagy. The most investigated stimulus for inducing mitochondrial clearance is depolarization, and several mitophagy inducers accomplish this by targeting various steps of the electron transport chain and oxidative phosphorylation (OXPHOS; Palikaras et al., 2018). Mitochondria that are depolarized are not able to efficiently generate ATP, so these are removed in order for new mitochondria to be synthesized (Narendra et al., 2008). Depolarized mitochondria have difficulty importing nuclear-encoded mitochondrial proteins, which PINK1 uses to relocalize to the surface of mitochondria as a signal for removal (Narendra et al., 2010). Some of the most common toxins are carbonyl cyanide 3-chlorophenylhydrazone and carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone, which both act as protonophores and disrupt the proton gradient across the inner mitochondrial membrane (IMM; Georgakopoulos et al., 2017). Aside from the protonophores, the combination of oligomycin and antimycin A is also commonly used. Oligomycin is an antibiotic that blocks the ATP synthase proton channel, and antimycin A is an inhibitor of the respiratory chain complex III (Georgakopoulos et al., 2017). Last, valinomycin is a potassium ionophore that also uncouples ATP generation by affecting the H^+ gradient (Georgakopoulos et al., 2017). Mitophagy following these treatments heavily relies on the PINK1–Parkin system (Georgakopoulos et al., 2017). Interestingly, depolarizing mitochondria alone may not be enough on the basis of a recent study arguing that some second signal, such as acidification of the cytosol, is needed, and these depolarizing treatments also increase acidity of the cytosol (Berezhnov et al., 2016). There are additional chemical triggers that are gaining interest in the field. As mentioned earlier, the antiparasitic drug ivermectin induces a ubiquitin-dependent, Parkin-independent mode of mitophagy, meaning other E3 ubiquitin ligases are involved (Zachari et al., 2019). The exact mechanistic details of this pathway remain to be resolved. Finally, both urolithin A and actinonin have been shown to induce mitophagy through novel mechanisms (Ryu et al., 2016; Fang et al., 2019b). Despite the focus on these triggers, recent work has begun questioning whether the severe mitochondrial damage these treatments induce is relevant to the quality and quantity of stimuli that may occur within the cell or organism physiologically (McWilliams et al., 2018b; Lee et al., 2018).

Hypoxia serves as a physiologically relevant trigger for mitophagy, because in low-oxygen conditions, the mitochondria cannot complete OXPHOS (Zhang et al., 2008). Hypoxic environments stabilize and activate the master regulator transcription factor hypoxia-inducible factor-1α (HIF1α), which drives the transcriptional upregulation of two mitophagy receptors, BNIP3 and BNIP3L (NIX), whereas a third mitophagy receptor, FUNDC1, becomes activated by dephosphorylation (Sowter et al., 2001; Zhang et al., 2008; Liu et al., 2012). Mitophagy following hypoxia is proposed to be independent of PINK1 stability (Wei et al., 2015). AMPK is a pivotal sensor of cellular metabolism and energetics that closely monitors the levels of ATP and ADP/AMP (Herzig and Shaw, 2018). Hypoxia also activates AMPK through the resulting oxidative stress (Mungai et al., 2011). Activated AMPK promotes mitophagy by activating ULK1 and the downstream initiation machinery (Kim et al., 2011; Laker et al., 2017). Another physiological stimulus closely related to hypoxia is iron chelation (Allen et al., 2013). Mitochondrial respiration and energy production rely on iron-sulfur clusters, and iron chelation also leads to HIF1α stabilization (Allen et al., 2013). Iron chelation by deferiprone drives the removal of mitochondria without depolarization and is independent from the PINK1/Parkin pathway (Allen et al., 2013).

One category of mitophagy that occurs during differentiation of RBCs and retinal ganglion cells is unique and distinct because it does not cause damage and is not a stressor (Schweers et al., 2007; Sandoval et al., 2008; Esteban-Martínez et al., 2017). In both types of cells, there is dramatic removal of mitochondria through NIX-dependent mitophagy, and deficiencies in mitophagy at these developmental milestones result in diseases such as anemia (Schweers et al., 2007; Esteban-Martínez et al., 2017). Another notably distinct trigger for mitophagy is *Listeria* infection through the toxin listeriolysin O (Zhang et al., 2019). Following infection, mitophagy promotes *Listeria* propagation through the reduction of ROS, which occurs through the Nod-like receptor NLRX1 independently from the PINK1/Parkin pathway, but many details of the mechanism remain to be explored further (Zhang et al., 2019). Another emerging mitophagy trigger is high glucose (HG) levels (Devi et al., 2017; Alcántar-Fernández et al., 2019). Although glucose is normally thought to prevent nonselective autophagy, levels at the high extreme can trigger removal of mitochondria. TBK1 inhibitor blocks this pathway, suggesting involvement of TBK1 substrates such as OPTN and NDP52 (Devi et al., 2019). TXNIP is involved in triggering mitophagy in HG conditions through increasing oxidative stress and AMPK activation rather than being involved in the targeting of mitochondria (Devi et al., 2017, 2019). Oxidative stress is a necessary signal because *N*-acetylcysteine can prevent HG-induced mitophagy (Devi et al., 2019). An alternative hypothesis is that HG supplementation shifts a cell's energy production away from OXPHOS to glycolytic metabolism, and therefore large mitochondrial content is not required (Doménech et al., 2015; Esteban-Martínez et al., 2017). Interestingly, mitochondrial dysfunction following most of the treatments listed above will invariably increase oxidative stress, suggesting that ROS could be the common stimulus for certain components of the pathway, while also amplifying mitochondrial dysfunction and the degradative signals (Zhang et al., 2008; Devi et al., 2019). Accumulation of misfolded proteins in the mitochondria due to the mitochondrial Hsp90 inhibitor Gamitrinib-triphenylphosphonium or overexpression of a mutant form of ornithine transcarbamylase also leads to mitophagy (Burman et al., 2017; Fiesel et al., 2017). Because these triggers induced PINK1-dependent mitophagy without membrane depolarization, it is likely that the accumulation of misfolded proteins alone was sufficient to prevent proper import of PINK1 and possibly other mitochondrial proteins (Burman et al., 2017; Fiesel et al., 2017).

The last mitophagy trigger we discuss is NAD^+. Recently, NAD^+ regulation has become a physiological target that is gaining interest in increasing mitochondrial function, biogenesis, and clearance (Jang et al., 2012; Fang et al., 2016). NAD^+ supplementation induces mitophagy mainly through Sirtuin-dependent pathways (Aman et al., 2020). Sirtuins are a class of signaling proteins that regulate many aspects of cellular metabolism, including mitochondrial biogenesis and mitophagy, and rely on NAD^+ levels to function (Aman et al., 2020). SIRT1 is a deacetylase that can modify multiple ATGs that are needed for LC3 lipidation while also affecting multiple targets downstream of AMPK, such as ULK1, PGC1α, FOXO1, and FOXO3a (Cantó et al., 2009; Fang et al., 2019a). Aside from SIRT1, SIRT2 can modify ATG5 acetylation, whereas SIRT3 affects the FUNDC1 mitophagy receptor pathway (Liu et al., 2017; Fang, 2019).

Aside from exogenous molecules or triggers to induce mitophagy, low levels of background mitophagy maintain the mitochondrial population at optimum efficiency (McWilliams et al., 2018b; Lee et al., 2018). This basal, physiological mitophagy likely happens with a local signal when only a small percentage of mitochondria are being turned over to maintain the health of the whole, rather than engaging in global depolarization. However, the cells must still receive some form of signal for clearance of these mitochondria, possibly a localized signature of oxidative stress or altered metabolite levels. Interestingly, although PINK1 and Parkin are important for basal turnover of mitochondrial proteins, PINK1- and Parkin-knockout flies and mice do not display any defect in basal mitophagy, which is especially high in metabolically active tissue such as the heart and muscle (Vincow et al., 2013; McWilliams et al., 2018b; Lee et al., 2018). On the contrary, the adenine nucleotide translocator (ANT) complex is involved in basal mitophagy, with genetic removal of ANT leading to accumulation of damaged mitochondria (Hoshino et al., 2019). This mechanism involves the ability of ANT to restrict mitochondrial protein import through TIM23 by directly interacting with TIM44 (Hoshino et al., 2019). Cells lacking ANT are unable to stabilize PINK1 following depolarization, but because PINK1 is not needed for basal mitophagy, this suggests that the retention of mitochondrial proteins in the cytosol may be a common mechanism multiple proteins use to induce mitophagy (Hoshino et al., 2019). Aside from this, other autophagy components are important for basal clearance, such as ATG5, as well as the mitophagy receptor BNIP3, whose deficiency leads to accumulation of defective mitochondria in mammary tumor cells and the liver (Tal et al., 2009; Chourasia et al., 2015b; Glick et al., 2012).

PINK1/Parkin pathway

PINK1/Parkin-driven mitophagy is the most characterized pathway, and the mitophagy field has been built on investigation of these two proteins (Park et al., 2006; Narendra et al., 2008, 2010). Recent reviews go into depth and detail on this path, so we will just touch on high-level points (Pickles et al., 2018; Montava-Garriga and Ganley, 2020). Under normal mitochondrial conditions, PINK1 is imported into the mitochondria, where it is exposed to and cleaved by mitochondrial proteases MPP and PARL (Whitworth et al., 2008; Deas et al., 2011a; Greene et al., 2012). Upon mitochondrial depolarization or accumulation of misfolded mitochondrial proteins, PINK1 import is prevented, and, as a result, PINK1 is stabilized on the surface of the mitochondria (Fig. 2; Narendra et al., 2010; Jin and Youle, 2013; Burman et al., 2017; Fiesel et al., 2017). Through its kinase activity, it phosphorylates the ubiquitin attached to several OMM proteins in addition to the cytosolic E3 ligase Parkin (Kane et al., 2014; Koyano et al., 2014; Shiba-Fukushima et al., 2014; Zhuang et al., 2016). Parkin is then recruited to these mitochondria and ubiquitinates multiple surface proteins as well, some of which will serve as a signal for autophagy receptors such as NDP52 and OPTN, whereas others will be targeted by the

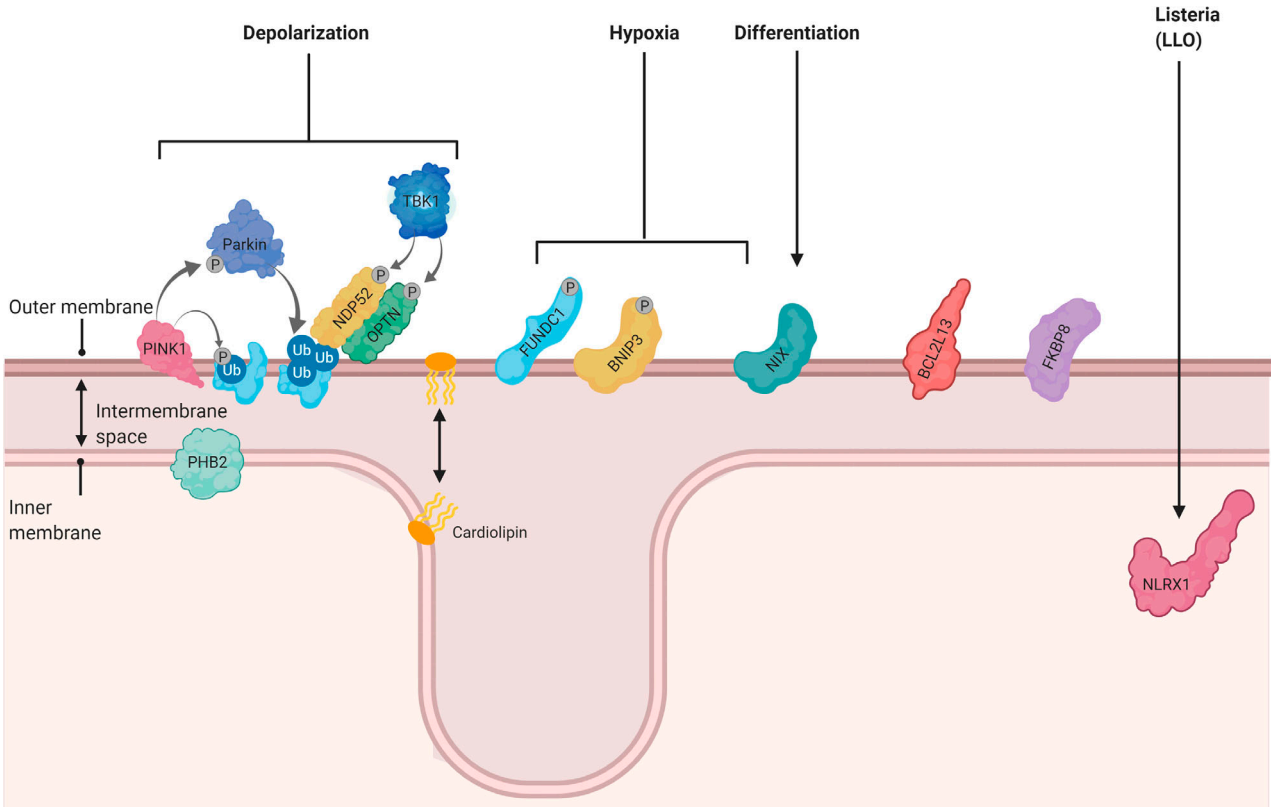

Figure 2. **Overview of the numerous receptors and pathways involved in mitophagy following different stressors.** Depolarization drives mitophagy through the PINK1/Parkin pathway, which involves the adapters NDP52 and OPTN, whose activity is modified by TBK1 phosphorylation. PHB2 is an IMM protein that is exposed during depolarization following proteolytic cleavage of the OMM. Similarly, cardiolipin is a lipid predominantly localized to the IMM, but it translocates to the OMM after stress. Additional mitophagy pathways involve the mitophagy receptors, mitochondrial proteins which possess LIR motifs that allow interaction and recruitment of ATG8 members directly following stressors such as hypoxia, differentiation, and infection. LLO, listeriolysin O; P, phosphate; Ub, ubiquitin. Figure was created with BioRender.com.

proteasome that degrades components of the OMM, which is essential for mitophagy (Vives-Bauza et al., 2010; Ziviani et al., 2010; Lazarou et al., 2015; Tanaka et al., 2010; Yoshii et al., 2011). This pathway contains an amplification loop whereby phosphorylation encourages Parkin recruitment and ubiquitination, resulting in more phosphorylation, sequentially building up the signal for degradation on the surface of mitochondria (Heo et al., 2015). As mentioned earlier, Parkin-mediated ubiquitination of mitochondria seems to be important for an endosomal Rab5-dependent path of clearance that operates independently of the canonical autophagy machinery (Hammerling et al., 2017).

Although the importance of this pathway is indisputable for in vitro assays that trigger mitophagy using depolarization, the role of PINK1 and Parkin in vivo has been more difficult to ascertain (McWilliams et al., 2018b; Lee et al., 2018). Mice lacking PINK1 or Parkin do not spontaneously develop a phenotype, and levels of basal mitophagy in metabolically active tissues such as the heart and the brain are not affected by the loss of either of these proteins (McWilliams et al., 2018b). Similarly, platelets from PINK1-deficient mice function normally and do not exhibit any defect in basal mitophagy (Walsh et al., 2018). These knockout animals require another hit such as the mtDNA mutator mouse background that induces mtDNA mutations due to deficiency in proofreading of the mtDNA polymerase POLG, exhaustive exercise, or aging before seeing a phenotype (Sliter et al., 2018; Noda et al., 2020). Because PINK1-Parkin mitophagy is triggered following severe mitochondrial stress, it is reasonable that some of the numerous other mitophagy pathways are important in maintaining basal levels of mitophagy when the stress is milder. Alternatively, due to the high levels of redundancy, perhaps loss of any one mitophagy pathway does not result in an overwhelming phenotype, where the others can compensate to accomplish the low levels of mitophagy needed to maintain balance. Deubiquitinating enzymes such as USP30 and USP35 are constitutively active and dampen the ubiquitin-dependent pathway, allowing the fine-tuning of the ubiquitin signal (Bingol et al., 2014; Wang et al., 2015; Marcassa et al., 2018). It makes sense, then, to have a ubiquitin-independent group of mitophagy receptors that can be activated and override this dampening through other means.

Mitophagy receptor pathways

Mitophagy receptors are typically mitochondrial proteins that contain an LIR motif that enables the recruitment of LC3 and the growing mitophagophore to the mitochondria designated for removal (Wang et al., 2019b). In addition to possessing this motif, these receptors are usually embedded in the OMM via a transmembrane domain, except for a few of the less characterized

receptors (Hamacher-Brady and Brady, 2016). As mentioned earlier, the mitophagy receptor NIX mediates mitophagy during RBC differentiation, as well as the hypoxia-driven glycolytic switch during metabolic transitions associated with retinal ganglion cell development (Schweers et al., 2007; Sandoval et al., 2008; Esteban-Martínez et al., 2017). NIX activity is enhanced through phosphorylation and dimerization and is transcriptionally regulated by HIF1α stabilization, supporting its role in hypoxia-mediated mitophagy (Sowter et al., 2001; Gustafsson, 2011). Closely related to NIX is BNIP3, which is similarly regulated transcriptionally by HIF1α, and also mediates mitophagy following hypoxia (Sowter et al., 2001; Zhang et al., 2008). The affinity that BNIP3 has for LC3 is regulated by phosphorylation within the LIR motif (Gustafsson, 2011). In addition to hypoxia, BNIP3 contributes to PINK1-Parkin mitophagy through multiple steps, including stabilizing PINK1 on the OMM, aiding in translocation of DRP1, and freeing BECN1 by preventing the interaction with BCL-2 (Zhang et al., 2008, 2016; Lee et al., 2011). The final mitophagy receptor induced by hypoxia is FUNDC1, which, instead of being transcriptionally regulated, is regulated by its own phosphorylation within the LIR motif (Wu et al., 2014). FUNDC1 has also been implicated in depolarization-induced mitophagy by maintaining the ER–mitochondria contact sites through interaction with IP3R2 and is regulated through direct phosphorylation by ULK1 (Wu et al., 2014, 2017). Recently, the critical function of FUNDC1 in exercise-induced mitophagy within skeletal muscle has been discovered and will be discussed in detail later (Fu et al., 2018).

Additional, less studied receptors have also been tied to mitophagy. Bcl2L13 is another OMM protein reported to regulate mitophagy by binding LC3 in addition to mediating mitochondrial fission (Murakawa et al., 2015). FKBP8 is another mitophagy receptor with a binding preference for LC3A that mediates mitophagy as well as fission, independently from Parkin (Bhujabal et al., 2017). Aside from the OMM proteins, some mitophagy receptors exist in other mitochondrial locations. Prohibitin 2 (PHB2) is an IMM protein that is unique not only because of its location but also because the proteasome-driven OMM rupture involved in PINK1-Parkin mitophagy is required for its exposure and activity (Wei et al., 2017). PHB2 regulates PINK1 stability on mitochondria in addition to binding LC3 upon OMM rupture and is required for mitophagy following depolarization (Wei et al., 2017; Yan et al., 2020). Another mitophagy receptor that resides within the mitochondria is NLRX1, a NOD-like receptor that is localized within the mitochondrial matrix and contains an LIR motif (Zhang et al., 2019). Recently, infection with *Listeria* was shown to promote a PINK1-independent mitophagy program through NLRX1 (Zhang et al., 2019). The direct mechanism remains to be determined, including an explanation of how a mitochondrial matrix protein could be interacting with LC3 from its location. The last mitophagy receptor we will mention is cardiolipin, a unique phospholipid that is present in the IMM (Chu et al., 2013). Upon mitochondrial damage, cardiolipin relocalizes to the OMM, where it can interact with LC3 and is potentially involved in PINK1-Parkin mitophagy (Chu et al., 2013).

When compared with the PINK1/Parkin pathway that has been studied for years, these mitophagy receptor pathways appear relatively new and underexplored. Apart from clarifying the location, the trigger for inducing mitophagy and the possession of an LIR motif, these pathways lack mechanistic exploration, and questions remain. Is this mitophagy independent of NDP52, OPTN, and TBK1, which are indispensable for ubiquitin-driven mitophagy, or is there some crosstalk and ability to recruit these components as well, possibly through the LC3-based targeting of NDP52 and OPTN because the ubiquitin label may not be present on the surface (Padman et al., 2019)? Ubiquitin has been reported to drive mitochondrial localization of ULK1 and LC3 (Lazarou et al., 2015; Padman et al., 2019). The state of being Parkin independent does not necessitate that ubiquitin is absent, because other E3 ligases have been reported, such as Gp78, ARIH1, CIAP1/2, and TRAF2 (Fu et al., 2013; Villa et al., 2017; Zachari et al., 2019). Mitophagy receptor pathways contain a ubiquitin-independent "eat me" signal in the form of a receptor that can bind LC3, but there is a possibility of ubiquitin-dependent recruitment of other components for autophagosome synthesis.

With the new paradigm of mitophagosomes forming on mitochondria, another question involves how ULK1 may be recruited without ubiquitin bringing NDP52 to mitochondria. AMPK phosphorylation and activation of ULK1 may lead to ULK1 localization on mitochondria independently of NDP52 following certain stimuli (Tian et al., 2015). Mitophagy receptors such as FUNDC1 and Bcl2L13 can bind and localize ULK1 to the mitochondria directly (Wu et al., 2014; Murakawa et al., 2019). In addition, the LC3 on the surface of mitochondria through interaction with the mitophagy receptors is the ideal replacement label for damaged mitochondria instead of ubiquitin. It can further drive NDP52/OPTN recruitment along with other ATG members (Padman et al., 2019). As mentioned earlier, the ATG8 family has also been proposed to serve as a scaffold for mobilizing the ULK1 complex, with many of the family members having the ability to bind ULK1, ULK2, FIP200, and ATG13 (Alemu et al., 2012). This would allow signal amplification and positive feedback because more LC3 labeling would allow more phagophore synthesis and additional LC3. These ideas are currently speculation, and there is a need for detailed mechanistic investigations into these mitophagy receptor pathways and triggers.

We end this discussion of how mitophagy is occurring by asking if these are truly separate and isolated pathways or if it is wrong to think of PINK1/Parkin-driven versus independent pathways. It is possible that many mitophagy receptors may be involved in the PINK1/Parkin pathway, because they are OMM proteins with the ability to bind LC3, so they could be involved in LC3 amplification steps due to their presence alone. Maybe PINK1-Parkin and depolarization-induced mitophagy uses these mitophagy receptors, whereas the sheer number and redundancy of them means that not all are necessary.

Impact of mitophagy in physiology and disease

In the beginning of the present review, we touched on why mitophagy is important for any cell that contains mitochondria. When moving from this idea of a nondescript eukaryotic cell to differentiated, specific cells that make up our body's organs,

certain tissues become focal points for discussion. These include muscle cells and neurons because of their specific functions, metabolism, and energy requirements (McWilliams et al., 2018b). These cells make up organs with high energy consumption and vulnerability, and slight perturbations in homeostasis can lead to pronounced effects (Baker et al., 2010; van der Kooij et al., 2018; Muchlinski et al., 2018). Although we focus on these two cell types, mitophagy is important in all organs where mitochondria play important roles. Multiple recent reviews explore the role of mitophagy in cardiovascular, liver, metabolic, immune, and inflammatory diseases and cancer (Chourasia et al., 2015a; Zhao et al., 2018; Ke, 2020; Liu et al., 2020; Morciano et al., 2020; Xu et al., 2020).

Cardiac and skeletal muscle

The heart is a highly energetic organ that generates ATP mainly through OXPHOS (Kolwicz et al., 2013). Cardiomyocytes are known to consume large amounts of fatty acids through β-oxidation to maintain contraction and blood circulation (Kolwicz et al., 2013). Interestingly, mitochondria within cardiomyocytes are more fragmented than in other cells, and turnover of mitochondrial proteins is less rapid than cytosolic proteins (Saito et al., 2019). There is a notable age-related reduction in mitophagy that is believed to contribute to the fibrotic nature of aged cardiac tissue through accumulated oxidative stress and misfolded proteins (Liang and Gustafsson, 2020). Following ischemia reperfusion in cardiomyocytes, both Parkin- and FUNDC1-mediated mitophagy are protective (Fig. 3; Kubli et al., 2013; Zhang et al., 2017). Aside from canonical mitophagy, the Rab9 alternative mitophagy pathway also plays a role in cardiomyocytes, an example of multiple redundant pathways functioning in parallel (Saito et al., 2019). An alternative trigger for mitophagy in the heart involves acute exercise, with LC3 lipidation increasing in cardiomyocytes after exercise (Ogura et al., 2011). Cardiac mitophagy under these conditions is likely beneficial for endurance capacity through optimized oxygen use during the stressful condition.

The other tissue overtly activated during exercise-mediated stress is skeletal muscle (Fig. 3; Laker et al., 2017; Fu et al., 2018). FUNDC1-driven mitophagy in skeletal muscle greatly affected the endurance capacity of mice (Fu et al., 2018). This study further showed that skeletal muscle–localized mitophagy defects affect whole-body metabolism, with substantial effects on adipose tissue and fat metabolism (Fu et al., 2018). BNIP3 expression is enhanced in exercised muscle, suggesting that it has a role in mitophagy (Lira et al., 2013). In agreement with the importance of autophagy machinery in exercise, mice deficient in exercise-induced autophagy throughout the entire body, named BCL2 AAA mice, as well as whole-body BECN1 heterozygotes had a reduced endurance capacity (He et al., 2012). Exercise-induced mitophagy in skeletal muscle was shown to be ULK1 dependent, placing ULK1 activation downstream of AMPK (Laker et al., 2017). Interestingly, skeletal muscle–specific AMPK–dominant negative mice did not display defective endurance capacity, and mice with a ULK1 deficiency in skeletal muscle similarly did not have reduced endurance capacity (Laker et al., 2017). Similarly, skeletal muscle–specific knockout of ATG7 before exercise did not affect the endurance capacity of mice (Lo Verso et al., 2014). Taking the skeletal muscle–specific knockout data together with the whole-body data, the mice that consistently show a reduced endurance capacity lack mitophagy in more organs than just the skeletal muscle, which suggests that the cardiac muscle would be the most reasonable location of local mitophagy importance.

After three consecutive days of exhaustive exercise, PINK1/Parkin-dependent mitophagy is induced, preventing systemic inflammation and the resulting motor deficits seen in neurodegenerative disease (Sliter et al., 2018). Parkin was also reported to drive mitophagy following a much shorter, acute period of exercise; however, these data conflict with those of another study that did not see PINK1 stabilization following exercise (Chen et al., 2018; Drake et al., 2019). None of the studies noted any endurance capacity defect in animals lacking PINK1-Parkin. Because loss of FUNDC1-driven mitophagy in skeletal muscles was enough to decrease the endurance capacity of mice, perhaps this suggests the difference in experimental setup including an exercise regimen, or perhaps the method of quantifying endurance capacity is responsible for these differing results. A separate explanation is that the altered fatty acid metabolism seen in FUNDC1-deficient mice is independent of its mitophagy defect because it is not recapitulated when other proteins essential to mitophagy are absent in the muscle.

Neurodegenerative disease

The deep molecular understanding of mitophagy we have stems from the thorough investigation into PINK1 and Parkin, which are both major recessive risk factors for developing early onset Parkinson's disease (PD; Pickrell and Youle, 2015). Neurons are highly specialized cells built to generate and propagate action potentials and are dependent on mitochondria for several functions (Kann and Kovács, 2007). The unique structure of the neuron creates an environment where not only does the mitochondrial pool have to be healthy, but it also must be properly transported down the axon to quite distant sites where ATP production and calcium buffering are its two most important functions (Kann and Kovács, 2007). An aged nervous system coupled with a decline in mitophagy leads to accumulation of bad mitochondria and is a hallmark of neurodegeneration (Fivenson et al., 2017).

The second most common neurodegenerative disease, PD, is a motor neuron disease arising from the progressive loss of dopaminergic neurons in the substantia nigra pars compacta and characterized by bradykinesia, tremors, rigidity, and postural instability (Poewe et al., 2017). Genetic risk factors of PINK1, Parkin, DJ-1, and others led to a proposed disease mechanism of mitochondrial dysfunction (Poewe et al., 2017; Hsieh et al., 2016). Mitophagy is defective in PD tissue and in models that recapitulate the disease (Deas et al., 2011b; Hsieh et al., 2016; Gao et al., 2017). However, a question remains whether defective mitophagy is the cause of PD or if the cause is mitochondrial dysfunction more generally or even a completely other defective process that results in mitochondrial dysfunction (Chen et al., 2019). A recent study showed that the neurotoxin oxidopamine induces mitophagy in various neurons but not in dopaminergic

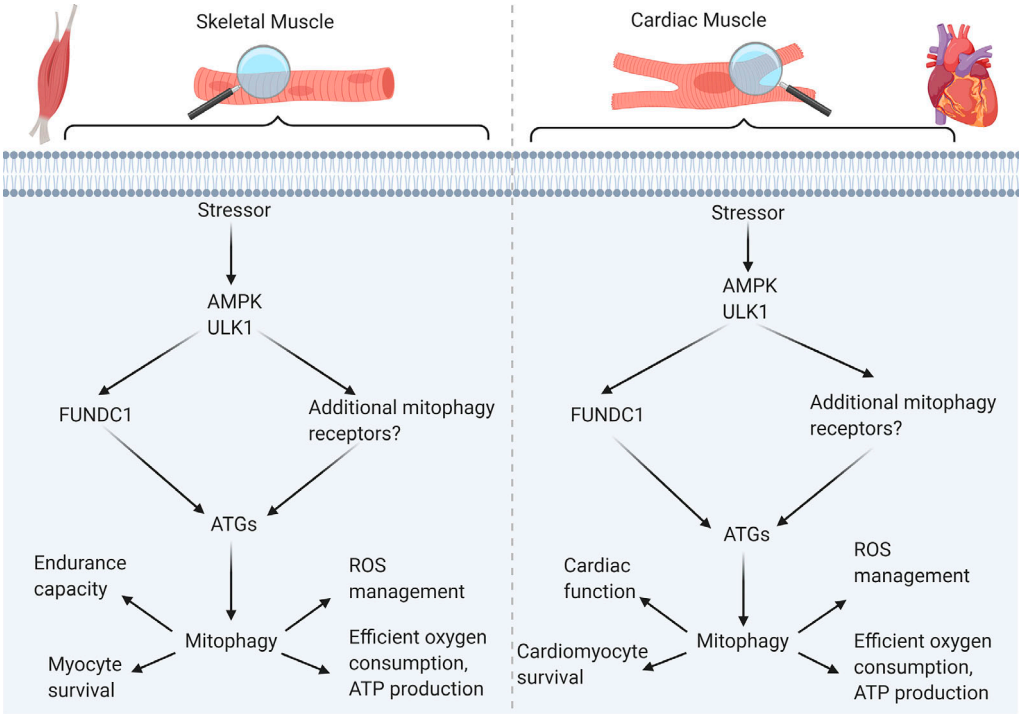

Figure 3. **Mitophagy pathways in skeletal and cardiac muscle following physiological stressors.** Mitophagy within skeletal muscle following acute exercise has been shown to involve AMPK and ULK1. FUNDC1 is involved downstream of ULK1, and other mitophagy receptors are likely at play as well. Mitophagy in these cells ensures effective ATP production and energy consumption while limiting ROS, and it manifests as an improvement in endurance capacity. Many similar pathways are likely at play in cardiac muscle after acute exercise as well as ischemia-reperfusion. Mitophagy in cardiac tissue ensures optimal cardiac function in addition to efficient oxygen consumption and ATP production. Figure was created with BioRender.com.

neurons, suggesting that dopaminergic neurons may not induce mitophagy as readily as others, which can explain why they are specifically vulnerable to underlying stress (Katayama et al., 2020). Inducing PINK1-Parkin mitophagy removes the damaged mitochondria, but this does not mean that defective clearance of the mitochondria was the cause of the disease; rather, it means that the buildup of toxic mitochondria leads to cell death (Chen et al., 2019). Interestingly, mice lacking PINK1 or Parkin do not develop spontaneous PD (Perez and Palmiter, 2005; McWilliams et al., 2018b). On the contrary, rats lacking PINK1 accumulate PD neurodegeneration and behavioral deficiencies, although rats lacking Parkin did not show any abnormalities, which supports a Parkin- and mitophagy-independent role of PINK1 driving the phenotype (Dave et al., 2014). In Parkin-knockin mice harboring a Ser65Ala (S65A) mutation, phosphorylation-based activation of Parkin by PINK1 is ablated. Although later developing motor deficiencies, these mice do not show neurodegeneration or defective mitophagy (McWilliams et al., 2018a). Last, the importance of Parkin was also demonstrated in mice aged 110 wk, in which Parkin-knockout mice showed motor deficiencies, neuron loss, and mitochondrial abnormalities, suggesting that the second hit of aging is needed before a PD phenotype is noticeable (Noda et al., 2020). Having learned more about the various functions of PINK1 and Parkin, we move from a model with defective mitophagy as the sole cause of PD to a more complex understanding in which multiple mitochondrial functions are disturbed in a neuronal cell type that is less efficient at clearing out mitochondria. However, disrupted mitochondrial function remains a commonality in PD, and various dangerous processes stem from it.

Mitochondrial dyshomeostasis can stem from multiple processes, including decreased mitochondrial biogenesis, misfolded protein stress, deficient OXPHOS, α-synuclein (α-syn) aggregation and toxicity, ROS accumulation, and iron dyshomeostasis (Chen et al., 2019). Although these have the potential to impact mitochondrial function, they are not caused exclusively by defective mitophagy. PINK1 and Parkin may affect these other processes because they have been implicated in the mitochondrial unfolded protein response (UPR^{mt}), clearance of the toxic protein PARIS whose buildup negatively regulates PGC1α levels, mitochondrial dynamics, transport, and biogenesis (Jin and Youle, 2013; Lee et al., 2017; Yu et al., 2011). Alternatively, deficiency in PINK1 leads to an exacerbated immune response to mitochondrial antigens and was recently shown to lead to PD symptoms following bacterial infection (Matheoud et al., 2016, 2019). This new direction suggests an underlying role for PINK1 and Parkin in modifying the immune system that may explain the genetic susceptibility seen in mutation-bearing patients and offers an interesting avenue for future research. Although Lewy bodies made up of aggregated α-syn remain the prominent pathology associated with PD, α-syn not only slows mitophagy but also impacts mitochondrial health in additional ways (Vicario et al., 2018; Shaltouki et al., 2018; Wang et al., 2019a). These parallel processes eventually overwhelm the quality control system and are seen as defective mitophagy (Chen et al., 2019). In summary, there is a circle of events underlying PD, and

mitochondrial dysfunction is part of that. Abrogated mitophagy causes mitochondrial dysfunction, making it a contributor to PD, but it is not the sole cause of PD; therefore, it may not be a sufficient treatment.

Until recently, surprisingly little evidence directly linked mitophagy to the most common neurodegenerative disease, Alzheimer's disease (AD; Kerr et al., 2017; Chakravorty et al., 2019; Fang et al., 2019b). AD is the major cause of dementia and involves loss of the cholinergic neurons in the brain, with a notable focus on the hippocampal region (Chen and Mobley, 2019). Historically, PD etiology was more focused on defective mitophagy, whereas investigation of AD has focused on accumulation of amyloid-β (Aβ) plaques and phospho-tau neurofibrillary tangles (Kerr et al., 2017). Recent studies have shown that mitophagy is, in fact, affected in AD and, more important, that inducing mitophagy could benefit the pathological and cognitive outcomes (Fang et al., 2019b). Tissues from postmortem patient samples and patient-derived induced pluripotent stem cells display defective mitophagy, contributing to the energetic stress and mitochondrial dysfunction that have been characterized in AD. Models of toxic Aβ and tau were shown to impair mitophagy, and increasing mitophagy helped to reduce the plaque and neurofibrillary tangle burden in *Caenorhabditis elegans* and mouse models (Fang et al., 2019b). Mitophagy was important not only in neurons but also in microglia that showed improved phagocytic function for clearing out Aβ plaques (Fang et al., 2019b).

AD might also be targeted through modification of NAD^+ levels, which were low in AD models (Martire et al., 2016; Hou et al., 2018). NAD^+ supplementation or addition of the precursor nicotinamide mononucleotide was able to induce mitophagy (Fang et al., 2019b). NAD^+ has also been linked to other pathways of axonal degeneration, which makes it an interesting treatment that could increase mitophagy and abrogate axonal degeneration–related cell death together (Figley and DiAntonio, 2020). Like in PD, AD pathogenesis is likely a cycle and feedback loop whereby Aβ and phosphorylated tau lead to mitochondrial dysfunction, which can further exacerbate the accumulation of protein aggregates, and modifying mitophagy is not the sole cause or solution. Once one part of the cycle is broken through targeted treatment, perhaps the other becomes more vulnerable as well, lending itself to be more amenable to additional therapy.

Targeting mitophagy as a therapeutic approach

As with most therapeutics that control a biological process, increasing levels of mitophagy must be carefully controlled because passing an upper limit would induce cell death, so careful modulation rather than constitutive activation would be ideal for this style of treatment. The diseases discussed earlier involve other contributors and steps in a disease amplification cycle which implies that controlling mitophagy may help reduce disease burden but may not be a cure for the underlying cause. When starting to discuss what compounds would be suitable for mitophagy-based treatment, it is obvious that compounds such as carbonyl cyanide 3-chlorophenylhydrazone are toxic and will not be usable due to their widespread off-target effects (Ashrafi et al., 2014). One strategy for a PD treatment involves upregulating the transcriptional coactivator PGC1α to induce mitochondrial biogenesis (Corona and Duchen, 2015). However, PGC1α modifies many different paths, so it will not be exclusively selective for mitochondrial control (Corona and Duchen, 2015). This setback is similar to other upstream components, such as AMPK or the ULK1 complex (Egan et al., 2015; Day et al., 2017).

A promising alternative involves investigating induced pluripotent stem cells from patients who have mutations commonly linked with diseases but are only carriers to see how they are compensating, potentially through other pathways that are being increased to maintain homeostasis (Chang et al., 2020; Penney et al., 2020). Similarly, in patients in whom PINK1/Parkin are mutated and deficient mitophagy is suspected to be a contributor to disease, treatment could involve inducing the other parallel mitophagy receptor pathways. This approach would be used when supplementing or inducing the PINK1/Parkin pathway would not be possible.

Last, by having a deeper understanding of basal mitophagy and its physiological trigger, we will gain insight into a readily tolerable treatment, one that does not irreparably damage the remaining mitochondrial population. Physiologically relevant stimulation through NAD^+ supplementation has been effective in mouse and *C. elegans* studies in AD (Fang et al., 2019b). By supplementing with a molecule that is already present in the body, the safety concerns are greatly reduced. Alternatively, by removing the brakes on the mitophagy system, such as the deubiquitinating enzymes, we would also increase levels of basal mitophagy (Bingol et al., 2014; Marcassa et al., 2018). Regardless of the treatment approach, the ideal therapy will be targeted to the dysfunctional organ because affecting the balance of mitophagy in off-target organs that do not have mitochondrial dysfunction will create additional problems.

Conclusions

Investigation of the molecular players involved in mitophagy is a rapidly advancing field, not only due to the layers of complexity and interest in the discovery but also because mitochondrial dysfunction is at the foundation of numerous diseases. Major advancements in any research field are usually associated with paradigm shifts in the way we understand a given pathway, and mitophagy is no different. By renewing our understanding of mitophagosome initiation, what localizes the initiation machinery to a designated mitochondrion, we gain insight into the triggers that the cell uses to remove the organelles. Through a new understanding of LC3 and its crucial role not only in linking cargo to the autophagosome but also in aiding the recruitment of the autophagy initiation machinery as well as ensuring phagosome maturation and elongation, the importance of LIR motifs and targeting LC3 is increased. By understanding the need for redundant mitophagy pathways, we start to question whether they truly operate in isolation or if they contribute to a cyclic amplification process whereby they are all important in their own way. Last, with a new appreciation for basal mitophagy that is likely independent of depolarization and the triggers usually associated with mitophagy, we begin to investigate how mitochondrial homeostasis is maintained at baseline, and we gain

new pathways and triggers that could be exploited for their therapeutic potential.

Acknowledgments

This work was supported by the Canadian Institutes of Health Research (operating grant 000303157).

The authors declare no competing financial interests.

Author contributions: S.A. Killackey, D.J. Philpott, and S.E. Girardin designed the manuscript's structure and organization. S.A. Killackey wrote the manuscript, which was edited by D.J. Philpott and S.E. Girardin.

Submitted: 5 June 2020
Revised: 12 August 2020
Accepted: 13 August 2020

References

Alcántar-Fernández, J., A. González-Maciel, R. Reynoso-Robles, M.E. Pérez Andrade, A.J. Hernández-Vázquez, A. Velázquez-Arellano, and J. Miranda-Ríos. 2019. High-glucose diets induce mitochondrial dysfunction in *Caenorhabditis elegans*. *PLoS One*. 14. e0226652. https://doi.org/10.1371/journal.pone.0226652

Alemu, E.A., T. Lamark, K.M. Torgersen, A.B. Birgisdottir, K.B. Larsen, A. Jain, H. Olsvik, A. Øvervatn, V. Kirkin, and T. Johansen. 2012. ATG8 family proteins act as scaffolds for assembly of the ULK complex: sequence requirements for LC3-interacting region (LIR) motifs. *J. Biol. Chem.* 287:39275–39290. https://doi.org/10.1074/jbc.M112.378109

Allen, G.F., R. Toth, J. James, and I.G. Ganley. 2013. Loss of iron triggers PINK1/Parkin-independent mitophagy. *EMBO Rep.* 14:1127–1135. https://doi.org/10.1038/embor.2013.168

Aman, Y., J. Frank, S.H. Lautrup, A. Matysek, Z. Niu, G. Yang, L. Shi, L.H. Bergersen, J. Storm-Mathisen, L.J. Rasmussen, et al. 2020. The NAD⁺-mitophagy axis in healthy longevity and in artificial intelligence-based clinical applications. *Mech. Ageing Dev.* 185. 111194. https://doi.org/10.1016/j.mad.2019.111194

Ashrafi, G., J.S. Schlehe, M.J. LaVoie, and T.L. Schwarz. 2014. Mitophagy of damaged mitochondria occurs locally in distal neuronal axons and requires PINK1 and Parkin. *J. Cell Biol.* 206:655–670. https://doi.org/10.1083/jcb.201401070

Axe, E.L., S.A. Walker, M. Manifava, P. Chandra, H.L. Roderick, A. Habermann, G. Griffiths, and N.T. Ktistakis. 2008. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *J. Cell Biol.* 182:685–701. https://doi.org/10.1083/jcb.200803137

Baker, J.S., M.C. McCormick, and R.A. Robergs. 2010. Interaction among skeletal muscle metabolic energy systems during intense exercise. *J. Nutr. Metab.* 2010. 905612. https://doi.org/10.1155/2010/905612

Berezhnov, A.V., M.P. Soutar, E.I. Fedotova, M.S. Frolova, H. Plun-Favreau, V.P. Zinchenko, and A.Y. Abramov. 2016. Intracellular pH modulates autophagy and mitophagy. *J. Biol. Chem.* 291:8701–8708. https://doi.org/10.1074/jbc.M115.691774

Bhujabal, Z., Å.B. Birgisdottir, E. Sjøttem, H.B. Brenne, A. Øvervatn, S. Habisov, V. Kirkin, T. Lamark, and T. Johansen. 2017. FKBP8 recruits LC3A to mediate Parkin-independent mitophagy. *EMBO Rep.* 18:947–961. https://doi.org/10.15252/embr.201643147

Bingol, B., J.S. Tea, L. Phu, M. Reichelt, C.E. Bakalarski, Q. Song, O. Foreman, D.S. Kirkpatrick, and M. Sheng. 2014. The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy. *Nature*. 510:370–375. https://doi.org/10.1038/nature13418

Böckler, S., and B. Westermann. 2014. Mitochondrial ER contacts are crucial for mitophagy in yeast. *Dev. Cell*. 28:450–458. https://doi.org/10.1016/j.devcel.2014.01.012

Bouman, L., A. Schlierf, A.K. Lutz, J. Shan, A. Deinlein, J. Kast, Z. Galehdar, V. Palmisano, N. Patenge, D. Berg, et al. 2011. Parkin is transcriptionally regulated by ATF4: evidence for an interconnection between mitochondrial stress and ER stress. *Cell Death Differ.* 18:769–782. https://doi.org/10.1038/cdd.2010.142

Bragoszewski, P., M. Wasilewski, P. Sakowska, A. Gornicka, L. Böttinger, J. Qiu, N. Wiedemann, and A. Chacinska. 2015. Retro-translocation of mitochondrial intermembrane space proteins. *Proc. Natl. Acad. Sci. USA*. 112:7713–7718. https://doi.org/10.1073/pnas.1504615112

Burman, C., and N.T. Ktistakis. 2010. Regulation of autophagy by phosphatidylinositol 3-phosphate. *FEBS Lett.* 584:1302–1312. https://doi.org/10.1016/j.febslet.2010.01.011

Burman, J.L., S. Pickles, C. Wang, S. Sekine, J.N.S. Vargas, Z. Zhang, A.M. Youle, C.L. Nezich, X. Wu, J.A. Hammer, et al. 2017. Mitochondrial fission facilitates the selective mitophagy of protein aggregates. *J. Cell Biol.* 216:3231–3247. https://doi.org/10.1083/jcb.201612106

Cantó, C., Z. Gerhart-Hines, J.N. Feige, M. Lagouge, L. Noriega, J.C. Milne, P.J. Elliott, P. Puigserver, and J. Auwerx. 2009. AMPK regulates energy expenditure by modulating NAD⁺ metabolism and SIRT1 activity. *Nature*. 458:1056–1060. https://doi.org/10.1038/nature07813

Chakravorty, A., C.T. Jetto, and R. Manjithaya. 2019. Dysfunctional mitochondria and mitophagy as drivers of Alzheimer's disease pathogenesis. *Front. Aging Neurosci.* 11:311. https://doi.org/10.3389/fnagi.2019.00311

Chang, C.Y., H.C. Ting, C.A. Liu, H.L. Su, T.W. Chiou, S.Z. Lin, H.J. Harn, and T.J. Ho. 2020. Induced pluripotent stem cell (iPSC)-based neurodegenerative disease models for phenotype recapitulation and drug screening. *Molecules*. 25:2000. https://doi.org/10.3390/molecules25082000

Chen, C.C.W., A.T. Erlich, M.J. Crilly, and D.A. Hood. 2018. Parkin is required for exercise-induced mitophagy in muscle: impact of aging. *Am. J. Physiol. Endocrinol. Metab.* 315:E404–E415. https://doi.org/10.1152/ajpendo.00391.2017

Chen, C., D.M. Turnbull, and A.K. Reeve. 2019. Mitochondrial dysfunction in Parkinson's disease—cause or consequence? *Biology (Basel)*. 8:38.

Chen, X.Q., and W.C. Mobley. 2019. Exploring the pathogenesis of Alzheimer disease in basal forebrain cholinergic neurons: converging insights from alternative hypotheses. *Front. Neurosci.* 13:446. https://doi.org/10.3389/fnins.2019.00446

Chourasia, A.H., M.L. Boland, and K.F. Macleod. 2015a. Mitophagy and cancer. *Cancer Metab.* 3:4. https://doi.org/10.1186/s40170-015-0130-8

Chourasia, A.H., K. Tracy, C. Frankenberger, M.L. Boland, M.N. Sharifi, L.E. Drake, J.R. Sachleben, J.M. Asara, J.W. Locasale, G.S. Karczmar, et al. 2015b. Mitophagy defects arising from BNip3 loss promote mammary tumor progression to metastasis. *EMBO Rep.* 16:1145–1163. https://doi.org/10.15252/embr.201540759

Chu, C.T., J. Ji, R.K. Dagda, J.F. Jiang, Y.Y. Tyurina, A.A. Kapralov, V.A. Tyurin, N. Yanamala, I.H. Shrivastava, D. Mohammadyani, et al. 2013. Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. *Nat. Cell Biol.* 15:1197–1205. https://doi.org/10.1038/ncb2837

Codogno, P., M. Mehrpour, and T. Proikas-Cezanne. 2012. Canonical and non-canonical autophagy: variations on a common theme of self-eating? *Nat. Rev. Mol. Cell Biol.* 13:7–12. https://doi.org/10.1038/nrm3249

Corona, J.C., and M.R. Duchen. 2015. PPARγ and PGC-1α as therapeutic targets in Parkinson's. *Neurochem. Res.* 40:308–316. https://doi.org/10.1007/s11064-014-1377-0

Dave, K.D., S. De Silva, N.P. Sheth, S. Ramboz, M.J. Beck, C. Quang, R.C. Switzer, III, S.O. Ahmad, S.M. Sunkin, D. Walker, et al. 2014. Phenotypic characterization of recessive gene knockout rat models of Parkinson's disease. *Neurobiol. Dis.* 70:190–203. https://doi.org/10.1016/j.nbd.2014.06.009

Davis, C.H., K.Y. Kim, E.A. Bushong, E.A. Mills, D. Boassa, T. Shih, M. Kinebuchi, S. Phan, Y. Zhou, N.A. Bihlmeyer, et al. 2014. Transcellular degradation of axonal mitochondria. *Proc. Natl. Acad. Sci. USA*. 111:9633–9638. https://doi.org/10.1073/pnas.1404651111

Day, E.A., R.J. Ford, and G.R. Steinberg. 2017. AMPK as a therapeutic target for treating metabolic diseases. *Trends Endocrinol. Metab.* 28:545–560. https://doi.org/10.1016/j.tem.2017.05.004

Deas, E., H. Plun-Favreau, S. Gandhi, H. Desmond, S. Kjaer, S.H. Loh, A.E. Renton, R.J. Harvey, A.J. Whitworth, L.M. Martins, et al. 2011a. PINK1 cleavage at position A103 by the mitochondrial protease PARL. *Hum. Mol. Genet.* 20:867–879. https://doi.org/10.1093/hmg/ddq526

Deas, E., N.W. Wood, and H. Plun-Favreau. 2011b. Mitophagy and Parkinson's disease: the PINK1-parkin link. *Biochim. Biophys. Acta.* 1813:623–633. https://doi.org/10.1016/j.bbamcr.2010.08.007

Deus, C.M., K.F. Yambire, P.J. Oliveira, and N. Raimundo. 2020. Mitochondria-lysosome crosstalk: from physiology to neurodegeneration. *Trends Mol. Med.* 26:71–88. https://doi.org/10.1016/j.molmed.2019.10.009

Devi, T.S., M. Somayajulu, R.A. Kowluru, and L.P. Singh. 2017. TXNIP regulates mitophagy in retinal Müller cells under high-glucose conditions:

implications for diabetic retinopathy. *Cell Death Dis.* 8. e2777. https://doi.org/10.1038/cddis.2017.190

Devi, T.S., T. Yumnamcha, F. Yao, M. Somayajulu, R.A. Kowluru, and L.P. Singh. 2019. TXNIP mediates high glucose-induced mitophagic flux and lysosome enlargement in human retinal pigment epithelial cells. *Biol. Open.* 8:bio038521. https://doi.org/10.1242/bio.038521

Doménech, E., C. Maestre, L. Esteban-Martínez, D. Partida, R. Pascual, G. Fernández-Miranda, E. Seco, R. Campos-Olivas, M. Pérez, D. Megias, et al. 2015. AMPK and PFKFB3 mediate glycolysis and survival in response to mitophagy during mitotic arrest. *Nat. Cell Biol.* 17:1304–1316. https://doi.org/10.1038/ncb3231

Drake, J.C., R.C. Laker, R.J. Wilson, M. Zhang, and Z. Yan. 2019. Exercise-induced mitophagy in skeletal muscle occurs in the absence of stabilization of Pink1 on mitochondria. *Cell Cycle.* 18:1–6. https://doi.org/10.1080/15384101.2018.1559556

Egan, D.F., M.G. Chun, M. Vamos, H. Zou, J. Rong, C.J. Miller, H.J. Lou, D. Raveendra-Panickar, C.C. Yang, D.J. Sheffler, et al. 2015. Small molecule inhibition of the autophagy kinase ULK1 and identification of ULK1 substrates. *Mol. Cell.* 59:285–297. https://doi.org/10.1016/j.molcel.2015.05.031

Esteban-Martínez, L., E. Sierra-Filardi, R.S. McGreal, M. Salazar-Roa, G. Mariño, E. Seco, S. Durand, D. Enot, O. Graña, M. Malumbres, et al. 2017. Programmed mitophagy is essential for the glycolytic switch during cell differentiation. *EMBO J.* 36:1688–1706. https://doi.org/10.15252/embj.201695916

Fang, E.F. 2019. Mitophagy and NAD$^+$ inhibit Alzheimer disease. *Autophagy.* 15:1112–1114. https://doi.org/10.1080/15548627.2019.1596497

Fang, E.F., H. Kassahun, D.L. Croteau, M. Scheibye-Knudsen, K. Marosi, H. Lu, R.A. Shamanna, S. Kalyanasundaram, R.C. Bollineni, M.A. Wilson, et al. 2016. NAD$^+$ replenishment improves lifespan and healthspan in ataxia telangiectasia models via mitophagy and DNA repair. *Cell Metab.* 24:566–581. https://doi.org/10.1016/j.cmet.2016.09.004

Fang, E.F., Y. Hou, S. Lautrup, M.B. Jensen, B. Yang, T. SenGupta, D. Caponio, R. Khezri, T.G. Demarest, Y. Aman, et al. 2019a. NAD$^+$ augmentation restores mitophagy and limits accelerated aging in Werner syndrome. *Nat. Commun.* 10:5284. https://doi.org/10.1038/s41467-019-13172-8

Fang, E.F., Y. Hou, K. Palikaras, B.A. Adriaanse, J.S. Kerr, B. Yang, S. Lautrup, M.M. Hasan-Olive, D. Caponio, X. Dan, et al. 2019b. Mitophagy inhibits amyloid-β and tau pathology and reverses cognitive deficits in models of Alzheimer's disease. *Nat. Neurosci.* 22:401–412. https://doi.org/10.1038/s41593-018-0332-9

Fessler, E., E.M. Eckl, S. Schmitt, I.A. Mancilla, M.F. Meyer-Bender, M. Hanf, J. Philippou-Massier, S. Krebs, H. Zischka, and L.T. Jae. 2020. A pathway coordinated by DELE1 relays mitochondrial stress to the cytosol. *Nature.* 579:433–437. https://doi.org/10.1038/s41586-020-2076-4

Fiesel, F.C., E.D. James, R. Hudec, and W. Springer. 2017. Mitochondrial targeted HSP90 inhibitor Gamitrinib-TPP (G-TPP) induces PINK1/Parkin-dependent mitophagy. *Oncotarget.* 8:106233–106248. https://doi.org/10.18632/oncotarget.22287

Figley, M.D., and A. DiAntonio. 2020. The SARM1 axon degeneration pathway: control of the NAD$^+$ metabolome regulates axon survival in health and disease. *Curr. Opin. Neurobiol.* 63:59–66. https://doi.org/10.1016/j.conb.2020.02.012

Fivenson, E.M., S. Lautrup, N. Sun, M. Scheibye-Knudsen, T. Stevnsner, H. Nilsen, V.A. Bohr, and E.F. Fang. 2017. Mitophagy in neurodegeneration and aging. *Neurochem. Int.* 109:202–209. https://doi.org/10.1016/j.neuint.2017.02.007

Friedman, J.R., and J. Nunnari. 2014. Mitochondrial form and function. *Nature.* 505:335–343. https://doi.org/10.1038/nature12985

Fu, M., P. St-Pierre, J. Shankar, P.T. Wang, B. Joshi, and I.R. Nabi. 2013. Regulation of mitophagy by the Gp78 E3 ubiquitin ligase. *Mol. Biol. Cell.* 24:1153–1162. https://doi.org/10.1091/mbc.e12-08-0607

Fu, T., Z. Xu, L. Liu, Q. Guo, H. Wu, X. Liang, D. Zhou, L. Xiao, L. Liu, Y. Liu, et al. 2018. Mitophagy directs muscle-adipose crosstalk to alleviate dietary obesity. *Cell Rep.* 23:1357–1372. https://doi.org/10.1016/j.celrep.2018.03.127

Gao, F., J. Yang, D. Wang, C. Li, Y. Fu, H. Wang, W. He, and J. Zhang. 2017. Mitophagy in Parkinson's disease: pathogenic and therapeutic implications. *Front. Neurol.* 8:527. https://doi.org/10.3389/fneur.2017.00527

Gelmetti, V., P. De Rosa, L. Torosantucci, E.S. Marini, A. Romagnoli, M. Di Rienzo, G. Arena, D. Vignone, G.M. Fimia, and E.M. Valente. 2017. PINK1 and BECN1 relocalize at mitochondria-associated membranes during mitophagy and promote ER-mitochondria tethering and autophagosome formation. *Autophagy.* 13:654–669. https://doi.org/10.1080/15548627.2016.1277309

Georgakopoulos, N.D., G. Wells, and M. Campanella. 2017. The pharmacological regulation of cellular mitophagy. *Nat. Chem. Biol.* 13:136–146. https://doi.org/10.1038/nchembio.2287

Glick, D., W. Zhang, M. Beaton, G. Marsboom, M. Gruber, M.C. Simon, J. Hart, G.W. Dorn, II, M.J. Brady, and K.F. Macleod. 2012. BNip3 regulates mitochondrial function and lipid metabolism in the liver. *Mol. Cell. Biol.* 32:2570–2584. https://doi.org/10.1128/MCB.00167-12

Greene, A.W., K. Grenier, M.A. Aguileta, S. Muise, R. Farazifard, M.E. Haque, H.M. McBride, D.S. Park, and E.A. Fon. 2012. Mitochondrial processing peptidase regulates PINK1 processing, import and Parkin recruitment. *EMBO Rep.* 13:378–385. https://doi.org/10.1038/embor.2012.14

Guo, X., G. Aviles, Y. Liu, R. Tian, B.A. Unger, Y.T. Lin, A.P. Wiita, K. Xu, M.A. Correia, and M. Kampmann. 2020. Mitochondrial stress is relayed to the cytosol by an OMA1-DELE1-HRI pathway. *Nature.* 579:427–432. https://doi.org/10.1038/s41586-020-2078-2

Gustafsson, A.B. 2011. Bnip3 as a dual regulator of mitochondrial turnover and cell death in the myocardium. *Pediatr. Cardiol.* 32:267–274. https://doi.org/10.1007/s00246-010-9876-5

Hamacher-Brady, A., and N.R. Brady. 2016. Mitophagy programs: mechanisms and physiological implications of mitochondrial targeting by autophagy. *Cell. Mol. Life Sci.* 73:775–795. https://doi.org/10.1007/s00018-015-2087-8

Hamasaki, M., N. Furuta, A. Matsuda, A. Nezu, A. Yamamoto, N. Fujita, H. Oomori, T. Noda, T. Haraguchi, Y. Hiraoka, et al. 2013. Autophagosomes form at ER-mitochondria contact sites. *Nature.* 495:389–393. https://doi.org/10.1038/nature11910

Hammerling, B.C., R.H. Najor, M.Q. Cortez, S.E. Shires, L.J. Leon, E.R. Gonzalez, D. Boassa, S. Phan, A. Thor, R.E. Jimenez, et al. 2017. A Rab5 endosomal pathway mediates Parkin-dependent mitochondrial clearance. *Nat. Commun.* 8:14050. https://doi.org/10.1038/ncomms14050

He, C., and D.J. Klionsky. 2009. Regulation mechanisms and signaling pathways of autophagy. *Annu. Rev. Genet.* 43:67–93. https://doi.org/10.1146/annurev-genet-102808-114910

He, C., M.C. Bassik, V. Moresi, K. Sun, Y. Wei, Z. Zou, Z. An, J. Loh, J. Fisher, Q. Sun, et al. 2012. Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. *Nature.* 481:511–515. https://doi.org/10.1038/nature10758

Heid, M.E., P.A. Keyel, C. Kamga, S. Shiva, S.C. Watkins, and R.D. Salter. 2013. Mitochondrial reactive oxygen species induces NLRP3-dependent lysosomal damage and inflammasome activation. *J. Immunol.* 191:5230–5238. https://doi.org/10.4049/jimmunol.1301490

Heo, J.M., A. Ordureau, J.A. Paulo, J. Rinehart, and J.W. Harper. 2015. The PINK1-PARKIN mitochondrial ubiquitylation pathway drives a program of OPTN/NDP52 recruitment and TBK1 activation to promote mitophagy. *Mol. Cell.* 60:7–20. https://doi.org/10.1016/j.molcel.2015.08.016

Heo, J.-M., A. Ordureau, S. Swarup, J.A. Paulo, K. Shen, D.M. Sabatini, and J.W. Harper. 2018. RAB7A phosphorylation by TBK1 promotes mitophagy via the PINK-PARKIN pathway. *Sci. Adv.* 4:eaav0443.

Herzig, S., and R.J. Shaw. 2018. AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat. Rev. Mol. Cell Biol.* 19:121–135. https://doi.org/10.1038/nrm.2017.95

Hoshino, A., W.J. Wang, S. Wada, C. McDermott-Roe, C.S. Evans, B. Gosis, M.P. Morley, K.S. Rathi, J. Li, K. Li, et al. 2019. The ADP/ATP translocase drives mitophagy independent of nucleotide exchange. *Nature.* 575:375–379.

Hou, Y., S. Lautrup, S. Cordonnier, Y. Wang, D.L. Croteau, E. Zavala, Y. Zhang, K. Moritoh, J.F. O'Connell, B.A. Baptiste, et al. 2018. NAD$^+$ supplementation normalizes key Alzheimer's features and DNA damage responses in a new AD mouse model with introduced DNA repair deficiency. *Proc. Natl. Acad. Sci. USA.* 115:E1876–E1885. https://doi.org/10.1073/pnas.1718819115

Hsieh, C.H., A. Shaltouki, A.E. Gonzalez, A. Bettencourt da Cruz, L.F. Burbulla, E. St Lawrence, B. Schüle, D. Krainc, T.D. Palmer, and X. Wang. 2016. Functional impairment in Miro degradation and mitophagy is a shared feature in familial and sporadic Parkinson's disease. *Cell Stem Cell.* 19:709–724. https://doi.org/10.1016/j.stem.2016.08.002

Itakura, E., C. Kishi-Itakura, I. Koyama-Honda, and N. Mizushima. 2012. Structures containing Atg9A and the ULK1 complex independently target depolarized mitochondria at initial stages of Parkin-mediated mitophagy. *J. Cell Sci.* 125:1488–1499. https://doi.org/10.1242/jcs.094110

Jang, S.Y., H.T. Kang, and E.S. Hwang. 2012. Nicotinamide-induced mitophagy: event mediated by high NAD$^+$/NADH ratio and SIRT1 protein activation. *J. Biol. Chem.* 287:19304–19314. https://doi.org/10.1074/jbc.M112.363747

Jin, S.M., and R.J. Youle. 2013. The accumulation of misfolded proteins in the mitochondrial matrix is sensed by PINK1 to induce PARK2/Parkin-mediated

mitophagy of polarized mitochondria. *Autophagy*. 9:1750–1757. https://doi.org/10.4161/auto.26122

Jornayvaz, F.R., and G.I. Shulman. 2010. Regulation of mitochondrial biogenesis. *Essays Biochem*. 47:69–84. https://doi.org/10.1042/bse0470069

Kabeya, Y., N. Mizushima, A. Yamamoto, S. Oshitani-Okamoto, Y. Ohsumi, and T. Yoshimori. 2004. LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation. *J. Cell Sci*. 117: 2805–2812. https://doi.org/10.1242/jcs.01131

Kane, L.A., M. Lazarou, A.I. Fogel, Y. Li, K. Yamano, S.A. Sarraf, S. Banerjee, and R.J. Youle. 2014. PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J. Cell Biol*. 205:143–153. https://doi.org/10.1083/jcb.201402104

Kann, O., and R. Kovács. 2007. Mitochondria and neuronal activity. *Am. J. Physiol. Cell Physiol*. 292:C641–C657. https://doi.org/10.1152/ajpcell.00222.2006

Katayama, H., H. Hama, K. Nagasawa, H. Kurokawa, M. Sugiyama, R. Ando, M. Funata, N. Yoshida, M. Homma, T. Nishimura, et al. 2020. Visualizing and modulating mitophagy for therapeutic studies of neurodegeneration. *Cell*. 181:1176–1187.e16. https://doi.org/10.1016/j.cell.2020.04.025

Kazlauskaite, A., C. Kondapalli, R. Gourlay, D.G. Campbell, M.S. Ritorto, K. Hofmann, D.R. Alessi, A. Knebel, M. Trost, and M.M. Muqit. 2014. Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. *Biochem. J*. 460:127–141. https://doi.org/10.1042/BJ20140334

Ke, P.Y. 2020. Mitophagy in the pathogenesis of liver diseases. *Cells*. 9:831. https://doi.org/10.3390/cells9040831

Kerr, J.S., B.A. Adriaanse, N.H. Greig, M.P. Mattson, M.Z. Cader, V.A. Bohr, and E.F. Fang. 2017. Mitophagy and Alzheimer's disease: cellular and molecular mechanisms. *Trends Neurosci*. 40:151–166. https://doi.org/10.1016/j.tins.2017.01.002

Kim, J., M. Kundu, B. Viollet, and K.L. Guan. 2011. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol*. 13: 132–141. https://doi.org/10.1038/ncb2152

Kiššová, I., B. Salin, J. Schaeffer, S. Bhatia, S. Manon, and N. Camougrand. 2007. Selective and non-selective autophagic degradation of mitochondria in yeast. *Autophagy*. 3:329–336. https://doi.org/10.4161/auto.4034

Kolwicz, S.C., Jr., S. Purohit, and R. Tian. 2013. Cardiac metabolism and its interactions with contraction, growth, and survival of cardiomyocytes. *Circ. Res*. 113:603–616. https://doi.org/10.1161/CIRCRESAHA.113.302095

Koyano, F., K. Okatsu, H. Kosako, Y. Tamura, E. Go, M. Kimura, Y. Kimura, H. Tsuchiya, H. Yoshihara, T. Hirokawa, et al. 2014. Ubiquitin is phosphorylated by PINK1 to activate parkin. *Nature*. 510:162–166. https://doi.org/10.1038/nature13392

Kubli, D.A., X. Zhang, Y. Lee, R.A. Hanna, M.N. Quinsay, C.K. Nguyen, R. Jimenez, S. Petrosyan, A.N. Murphy, and A.B. Gustafsson. 2013. Parkin protein deficiency exacerbates cardiac injury and reduces survival following myocardial infarction. *J. Biol. Chem*. 288:915–926. https://doi.org/10.1074/jbc.M112.411363

Laker, R.C., J.C. Drake, R.J. Wilson, V.A. Lira, B.M. Lewellen, K.A. Ryall, C.C. Fisher, M. Zhang, J.J. Saucerman, L.J. Goodyear, et al. 2017. Ampk phosphorylation of Ulk1 is required for targeting of mitochondria to lysosomes in exercise-induced mitophagy. *Nat. Commun*. 8:548. https://doi.org/10.1038/s41467-017-00520-9

Lazarou, M., D.A. Sliter, L.A. Kane, S.A. Sarraf, C. Wang, J.L. Burman, D.P. Sideris, A.I. Fogel, and R.J. Youle. 2015. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature*. 524:309–314. https://doi.org/10.1038/nature14893

Lee, Y., H.Y. Lee, R.A. Hanna, and Å.B. Gustafsson. 2011. Mitochondrial autophagy by Bnip3 involves Drp1-mediated mitochondrial fission and recruitment of Parkin in cardiac myocytes. *Am. J. Physiol. Heart Circ. Physiol*. 301:H1924–H1931. https://doi.org/10.1152/ajpheart.00368.2011

Lee, Y., D.A. Stevens, S.U. Kang, H. Jiang, Y.I. Lee, H.S. Ko, L.A. Scarffe, G.E. Umanah, H. Kang, S. Ham, et al. 2017. PINK1 primes Parkin-mediated ubiquitination of PARIS in dopaminergic neuronal survival. *Cell Rep*. 18: 918–932. https://doi.org/10.1016/j.celrep.2016.12.090

Lee, J.J., A. Sanchez-Martinez, A.M. Zarate, C. Benincá, U. Mayor, M.J. Clague, and A.J. Whitworth. 2018. Basal mitophagy is widespread in *Drosophila* but minimally affected by loss of Pink1 or parkin. *J. Cell Biol*. 217: 1613–1622. https://doi.org/10.1083/jcb.201801044

Le Guerroué, F., F. Eck, J. Jung, T. Starzetz, M. Mittelbronn, M. Kaulich, and C. Behrends. 2017. Autophagosomal content profiling reveals an LC3C-dependent piecemeal mitophagy pathway. *Mol. Cell*. 68:786–796.e6. https://doi.org/10.1016/j.molcel.2017.10.029

Liang, W.J., and Å.B. Gustafsson. 2020. The aging heart: mitophagy at the center of rejuvenation. *Front. Cardiovasc. Med*. 7:18. https://doi.org/10.3389/fcvm.2020.00018

Lira, V.A., M. Okutsu, M. Zhang, N.P. Greene, R.C. Laker, D.S. Breen, K.L. Hoehn, and Z. Yan. 2013. Autophagy is required for exercise training-induced skeletal muscle adaptation and improvement of physical performance. *FASEB J*. 27:4184–4193. https://doi.org/10.1096/fj.13-228486

Liu, L., D. Feng, G. Chen, M. Chen, Q. Zheng, P. Song, Q. Ma, C. Zhu, R. Wang, W. Qi, et al. 2012. Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. *Nat. Cell Biol*. 14:177–185. https://doi.org/10.1038/ncb2422

Liu, G., S.H. Park, M. Imbesi, W.J. Nathan, X. Zou, Y. Zhu, H. Jiang, L. Parisiadou, and D. Gius. 2017. Loss of NAD-dependent protein deacetylase sirtuin-2 alters mitochondrial protein acetylation and dysregulates mitophagy. *Antioxid. Redox Signal*. 26:849–863. https://doi.org/10.1089/ars.2016.6662

Liu, L., X. Liao, H. Wu, Y. Li, Y. Zhu, and Q. Chen. 2020. Mitophagy and its contribution to metabolic and aging-associated disorders. *Antioxid. Redox Signal*. 32:906–927. https://doi.org/10.1089/ars.2019.8013

Lo Verso, F., S. Carnio, A. Vainshtein, and M. Sandri. 2014. Autophagy is not required to sustain exercise and PRKAA1/AMPK activity but is important to prevent mitochondrial damage during physical activity. *Autophagy*. 10:1883–1894. https://doi.org/10.4161/auto.32154

MacVicar, T.D., L.V. Mannack, R.M. Lees, and J.D. Lane. 2015. Targeted siRNA screens identify ER-to-mitochondrial calcium exchange in autophagy and mitophagy responses in RPE1 cells. *Int. J. Mol. Sci*. 16:13356–13380. https://doi.org/10.3390/ijms160613356

Marcassa, E., A. Kallinos, J. Jardine, E.V. Rusilowicz-Jones, A. Martinez, S. Kuehl, M. Islinger, M.J. Clague, and S. Urbé. 2018. Dual role of USP30 in controlling basal pexophagy and mitophagy. *EMBO Rep*. 19:e45595. https://doi.org/10.15252/embr.201745595

Marchi, S., S. Patergnani, S. Missiroli, G. Morciano, A. Rimessi, M.R. Wieckowski, C. Giorgi, and P. Pinton. 2018. Mitochondrial and endoplasmic reticulum calcium homeostasis and cell death. *Cell Calcium*. 69: 62–72. https://doi.org/10.1016/j.ceca.2017.05.003

Martire, S., A. Fuso, L. Mosca, E. Forte, V. Correani, M. Fontana, S. Scarpa, B. Maras, and M. d'Erme. 2016. Bioenergetic impairment in animal and cellular models of Alzheimer's disease: PARP-1 inhibition rescues metabolic dysfunctions. *J. Alzheimers Dis*. 54:307–324. https://doi.org/10.3233/JAD-151040

Matheoud, D., A. Sugiura, A. Bellemare-Pelletier, A. Laplante, C. Rondeau, M. Chemali, A. Fazel, J.J. Bergeron, L.E. Trudeau, Y. Burelle, et al. 2016. Parkinson's disease-related proteins PINK1 and Parkin repress mitochondrial antigen presentation. *Cell*. 166:314–327. https://doi.org/10.1016/j.cell.2016.05.039

Matheoud, D., T. Cannon, A. Voisin, A.M. Penttinen, L. Ramet, A.M. Fahmy, C. Ducrot, A. Laplante, M.J. Bourque, L. Zhu, et al. 2019. Intestinal infection triggers Parkinson's disease-like symptoms in Pink1$^{-/-}$ mice. *Nature*. 571:565–569. https://doi.org/10.1038/s41586-019-1405-y

Matsuda, N., S. Sato, K. Shiba, K. Okatsu, K. Saisho, C.A. Gautier, Y.S. Sou, S. Saiki, S. Kawajiri, F. Sato, et al. 2010. PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. *J. Cell Biol*. 189:211–221. https://doi.org/10.1083/jcb.200910140

McLelland, G.L., V. Soubannier, C.X. Chen, H.M. McBride, and E.A. Fon. 2014. Parkin and PINK1 function in a vesicular trafficking pathway regulating mitochondrial quality control. *EMBO J*. 33:282–295.

McWilliams, T.G., E. Barini, R. Pohjolan-Pirhonen, S.P. Brooks, F. Singh, S. Burel, K. Balk, A. Kumar, L. Montava-Garriga, A.R. Prescott, et al. 2018a. Phosphorylation of Parkin at serine 65 is essential for its activation *in vivo*. *Open Biol*. 8:180108. https://doi.org/10.1098/rsob.180108

McWilliams, T.G., A.R. Prescott, L. Montava-Garriga, G. Ball, F. Singh, E. Barini, M.M.K. Muqit, S.P. Brooks, and I.G. Ganley. 2018b. Basal mitophagy occurs independently of PINK1 in mouse tissues of high metabolic demand. *Cell Metab*. 27:439–449.e5. https://doi.org/10.1016/j.cmet.2017.12.008

Moehle, E.A., K. Shen, and A. Dillin. 2019. Mitochondrial proteostasis in the context of cellular and organismal health and aging. *J. Biol. Chem*. 294: 5396–5407. https://doi.org/10.1074/jbc.TM117.000893

Montava-Garriga, L., and I.G. Ganley. 2020. Outstanding questions in mitophagy: what we do and do not know. *J. Mol. Biol*. 432:206–230. https://doi.org/10.1016/j.jmb.2019.06.032

Morales, I., A. Sanchez, R. Puertas-Avendaño, C. Rodriguez-Sabate, A. Perez-Barreto, and M. Rodriguez. 2020. Neuroglial transmitophagy and Parkinson's disease. *Glia*. https://doi.org/10.1002/glia.23839

Morciano, G., S. Patergnani, M. Bonora, G. Pedriali, A. Tarocco, E. Bouhamida, S. Marchi, G. Ancora, G. Anania, M.R. Wieckowski, et al. 2020. Mitophagy in

cardiovascular diseases. *J. Clin. Med.* 9:892. https://doi.org/10.3390/jcm9030892

Muchlinski, M.N., H.W. Hemingway, J. Pastor, K.M. Omstead, and A.M. Burrows. 2018. How the brain may have shaped muscle anatomy and physiology: a preliminary study. *Anat. Rec. (Hoboken)*. 301:528–537. https://doi.org/10.1002/ar.23746

Mungai, P.T., G.B. Waypa, A. Jairaman, M. Prakriya, D. Dokic, M.K. Ball, and P.T. Schumacker. 2011. Hypoxia triggers AMPK activation through reactive oxygen species-mediated activation of calcium release-activated calcium channels. *Mol. Cell. Biol.* 31:3531–3545. https://doi.org/10.1128/MCB.05124-11

Murakawa, T., O. Yamaguchi, A. Hashimoto, S. Hikoso, T. Takeda, T. Oka, H. Yasui, H. Ueda, Y. Akazawa, H. Nakayama, et al. 2015. Bcl-2-like protein 13 is a mammalian Atg32 homologue that mediates mitophagy and mitochondrial fragmentation. *Nat. Commun.* 6:7527. https://doi.org/10.1038/ncomms8527

Murakawa, T., K. Okamoto, S. Omiya, M. Taneike, O. Yamaguchi, and K. Otsu. 2019. A mammalian mitophagy receptor, Bcl2-L-13, recruits the ULK1 complex to induce mitophagy. *Cell Rep.* 26:338–345.e6. https://doi.org/10.1016/j.celrep.2018.12.050

Murphy, M.P. 2009. How mitochondria produce reactive oxygen species. *Biochem. J.* 417:1–13. https://doi.org/10.1042/BJ20081386

Nanao, T., M. Koike, J. Yamaguchi, M. Sasaki, and Y. Uchiyama. 2015. Cellular localization and tissue distribution of endogenous DFCP1 protein. *Biomed. Res.* 36:121–133. https://doi.org/10.2220/biomedres.36.121

Narendra, D., A. Tanaka, D.F. Suen, and R.J. Youle. 2008. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J. Cell Biol.* 183:795–803. https://doi.org/10.1083/jcb.200809125

Narendra, D.P., S.M. Jin, A. Tanaka, D.F. Suen, C.A. Gautier, J. Shen, M.R. Cookson, and R.J. Youle. 2010. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol.* 8. e1000298. https://doi.org/10.1371/journal.pbio.1000298

Neuspiel, M., A.C. Schauss, E. Braschi, R. Zunino, P. Rippstein, R.A. Rachubinski, M.A. Andrade-Navarro, and H.M. McBride. 2008. Cargo-selected transport from the mitochondria to peroxisomes is mediated by vesicular carriers. *Curr. Biol.* 18:102–108. https://doi.org/10.1016/j.cub.2007.12.038

Nguyen, T.N., B.S. Padman, J. Usher, V. Oorschot, G. Ramm, and M. Lazarou. 2016. Atg8 family LC3/GABARAP proteins are crucial for autophagosome-lysosome fusion but not autophagosome formation during PINK1/Parkin mitophagy and starvation. *J. Cell Biol.* 215:857–874. https://doi.org/10.1083/jcb.201607039

Ni, H.M., J.A. Williams, and W.X. Ding. 2015. Mitochondrial dynamics and mitochondrial quality control. *Redox Biol.* 4:6–13. https://doi.org/10.1016/j.redox.2014.11.006

Noda, S., S. Sato, T. Fukuda, N. Tada, Y. Uchiyama, K. Tanaka, and N. Hattori. 2020. Loss of Parkin contributes to mitochondrial turnover and dopaminergic neuronal loss in aged mice. *Neurobiol. Dis.* 136. 104717. https://doi.org/10.1016/j.nbd.2019.104717

Ogura, Y., M. Iemitsu, H. Naito, R. Kakigi, C. Kakehashi, S. Maeda, and T. Akema. 2011. Single bout of running exercise changes LC3-II expression in rat cardiac muscle. *Biochem. Biophys. Res. Commun.* 414:756–760. https://doi.org/10.1016/j.bbrc.2011.09.152

Ordureau, A., S.A. Sarraf, D.M. Duda, J.M. Heo, M.P. Jedrychowski, V.O. Sviderskiy, J.L. Olszewski, J.T. Koerber, T. Xie, S.A. Beausoleil, et al. 2014. Quantitative proteomics reveal a feedforward mechanism for mitochondrial PARKIN translocation and ubiquitin chain synthesis. *Mol. Cell.* 56:360–375. https://doi.org/10.1016/j.molcel.2014.09.007

Padman, B.S., T.N. Nguyen, L. Uoselis, M. Skulsuppaisarn, L.K. Nguyen, and M. Lazarou. 2019. LC3/GABARAPs drive ubiquitin-independent recruitment of optineurin and NDP52 to amplify mitophagy. *Nat. Commun.* 10:408. https://doi.org/10.1038/s41467-019-08335-6

Palikaras, K., E. Lionaki, and N. Tavernarakis. 2018. Mechanisms of mitophagy in cellular homeostasis, physiology and pathology. *Nat. Cell Biol.* 20:1013–1022. https://doi.org/10.1038/s41556-018-0176-2

Pankiv, S., T.H. Clausen, T. Lamark, A. Brech, J.A. Bruun, H. Outzen, A. Øvervatn, G. Bjørkøy, and T. Johansen. 2007. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J. Biol. Chem.* 282:24131–24145. https://doi.org/10.1074/jbc.M702824200

Park, J., S.B. Lee, S. Lee, Y. Kim, S. Song, S. Kim, E. Bae, J. Kim, M. Shong, J.M. Kim, et al. 2006. Mitochondrial dysfunction in *Drosophila* PINK1 mutants is complemented by parkin. *Nature.* 441:1157–1161. https://doi.org/10.1038/nature04788

Penney, J., W.T. Ralvenius, and L.H. Tsai. 2020. Modeling Alzheimer's disease with iPSC-derived brain cells. *Mol. Psychiatry.* 25:148–167. https://doi.org/10.1038/s41380-019-0468-3

Perez, F.A., and R.D. Palmiter. 2005. Parkin-deficient mice are not a robust model of parkinsonism. *Proc. Natl. Acad. Sci. USA.* 102:2174–2179. https://doi.org/10.1073/pnas.0409598102

Pickles, S., P. Vigié, and R.J. Youle. 2018. Mitophagy and quality control mechanisms in mitochondrial maintenance. *Curr. Biol.* 28:R170–R185. https://doi.org/10.1016/j.cub.2018.01.004

Pickrell, A.M., and R.J. Youle. 2015. The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. *Neuron.* 85:257–273. https://doi.org/10.1016/j.neuron.2014.12.007

Plotegher, N., and M.R. Duchen. 2017. Crosstalk between lysosomes and mitochondria in Parkinson's disease. *Front. Cell Dev. Biol.* 5:110. https://doi.org/10.3389/fcell.2017.00110

Poewe, W., K. Seppi, C.M. Tanner, G.M. Halliday, P. Brundin, J. Volkmann, A.E. Schrag, and A.E. Lang. 2017. Parkinson disease. *Nat. Rev. Dis. Primers.* 3:17013. https://doi.org/10.1038/nrdp.2017.13

Polson, H.E., J. de Lartigue, D.J. Rigden, M. Reedijk, S. Urbé, M.J. Clague, and S.A. Tooze. 2010. Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. *Autophagy.* 6:506–522. https://doi.org/10.4161/auto.6.4.11863

Ravikumar, B., K. Moreau, L. Jahreiss, C. Puri, and D.C. Rubinsztein. 2010. Plasma membrane contributes to the formation of pre-autophagosomal structures. *Nat. Cell Biol.* 12:747–757. https://doi.org/10.1038/ncb2078

Rodriguez-Enriquez, S., I. Kim, R.T. Currin, and J.J. Lemasters. 2006. Tracker dyes to probe mitochondrial autophagy (mitophagy) in rat hepatocytes. *Autophagy.* 2:39–46. https://doi.org/10.4161/auto.2229

Ryu, D., L. Mouchiroud, P.A. Andreux, E. Katsyuba, N. Moullan, A.A. Nicolet-Dit-Félix, E.G. Williams, P. Jha, G. Lo Sasso, D. Huzard, et al. 2016. Urolithin A induces mitophagy and prolongs lifespan in *C. elegans* and increases muscle function in rodents. *Nat. Med.* 22:879–888. https://doi.org/10.1038/nm.4132

Saito, T., J. Nah, S.I. Oka, R. Mukai, Y. Monden, Y. Maejima, Y. Ikeda, S. Sciarretta, T. Liu, H. Li, et al. 2019. An alternative mitophagy pathway mediated by Rab9 protects the heart against ischemia. *J. Clin. Invest.* 129:802–819. https://doi.org/10.1172/JCI122035

Sandoval, H., P. Thiagarajan, S.K. Dasgupta, A. Schumacher, J.T. Prchal, M. Chen, and J. Wang. 2008. Essential role for Nix in autophagic maturation of erythroid cells. *Nature.* 454:232–235. https://doi.org/10.1038/nature07006

Schweers, R.L., J. Zhang, M.S. Randall, M.R. Loyd, W. Li, F.C. Dorsey, M. Kundu, J.T. Opferman, J.L. Cleveland, J.L. Miller, et al. 2007. NIX is required for programmed mitochondrial clearance during reticulocyte maturation. *Proc. Natl. Acad. Sci. USA.* 104:19500–19505. https://doi.org/10.1073/pnas.0708818104

Shaltouki, A., C.H. Hsieh, M.J. Kim, and X. Wang. 2018. Alpha-synuclein delays mitophagy and targeting Miro rescues neuron loss in Parkinson's models. *Acta Neuropathol.* 136:607–620. https://doi.org/10.1007/s00401-018-1873-4

Shi, J., G. Fung, H. Deng, J. Zhang, F.C. Fiesel, W. Springer, X. Li, and H. Luo. 2015. NBR1 is dispensable for PARK2-mediated mitophagy regardless of the presence or absence of SQSTM1. *Cell Death Dis.* 6. e1943. https://doi.org/10.1038/cddis.2015.278

Shiba-Fukushima, K., T. Inoshita, N. Hattori, and Y. Imai. 2014. PINK1-mediated phosphorylation of Parkin boosts Parkin activity in *Drosophila*. *PLoS Genet.* 10. e1004391. https://doi.org/10.1371/journal.pgen.1004391

Sliter, D.A., J. Martinez, L. Hao, X. Chen, N. Sun, T.D. Fischer, J.L. Burman, Y. Li, Z. Zhang, D.P. Narendra, et al. 2018. Parkin and PINK1 mitigate STING-induced inflammation. *Nature.* 561:258–262. https://doi.org/10.1038/s41586-018-0448-9

Soubannier, V., G.L. McLelland, R. Zunino, E. Braschi, P. Rippstein, E.A. Fon, and H.M. McBride. 2012. A vesicular transport pathway shuttles cargo from mitochondria to lysosomes. *Curr. Biol.* 22:135–141. https://doi.org/10.1016/j.cub.2011.11.057

Sowter, H.M., P.J. Ratcliffe, P. Watson, A.H. Greenberg, and A.L. Harris. 2001. HIF-1-dependent regulation of hypoxic induction of the cell death factors BNIP3 and NIX in human tumors. *Cancer Res.* 61:6669–6673.

Tal, M.C., M. Sasai, H.K. Lee, B. Yordy, G.S. Shadel, and A. Iwasaki. 2009. Absence of autophagy results in reactive oxygen species-dependent amplification of RLR signaling. *Proc. Natl. Acad. Sci. USA.* 106:2770–2775. https://doi.org/10.1073/pnas.0807694106

Tanaka, A., M.M. Cleland, S. Xu, D.P. Narendra, D.F. Suen, M. Karbowski, and R.J. Youle. 2010. Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. *J. Cell Biol.* 191:1367–1380. https://doi.org/10.1083/jcb.201007013

Tian, W., W. Li, Y. Chen, Z. Yan, X. Huang, H. Zhuang, W. Zhong, Y. Chen, W. Wu, C. Lin, et al. 2015. Phosphorylation of ULK1 by AMPK regulates translocation of ULK1 to mitochondria and mitophagy. *FEBS Lett.* 589: 1847–1854. https://doi.org/10.1016/j.febslet.2015.05.020

Tooze, S.A., and T. Yoshimori. 2010. The origin of the autophagosomal membrane. *Nat. Cell Biol.* 12:831–835. https://doi.org/10.1038/ncb0910-831

van der Kooij, M.A., T. Jene, G. Treccani, I. Miederer, A. Hasch, N. Voelxen, S. Walenta, and M.B. Müller. 2018. Chronic social stress-induced hyperglycemia in mice couples individual stress susceptibility to impaired spatial memory. *Proc. Natl. Acad. Sci. USA.* 115:E10187–E10196. https://doi.org/10.1073/pnas.1804412115

Vargas, J.N.S., C. Wang, E. Bunker, L. Hao, D. Maric, G. Schiavo, F. Randow, and R.J. Youle. 2019. Spatiotemporal control of ULK1 activation by NDP52 and TBK1 during selective autophagy. *Mol. Cell.* 74:347–362.e6. https://doi.org/10.1016/j.molcel.2019.02.010

Vicario, M., D. Cieri, M. Brini, and T. Calì. 2018. The close encounter between alpha-synuclein and mitochondria. *Front. Neurosci.* 12:388. https://doi.org/10.3389/fnins.2018.00388

Villa, E., E. Proïcs, C. Rubio-Patiño, S. Obba, B. Zunino, J.P. Bossowski, R.M. Rozier, J. Chiche, L. Mondragón, J.S. Riley, et al. 2017. Parkin-independent mitophagy controls chemotherapeutic response in cancer cells. *Cell Rep.* 20:2846–2859. https://doi.org/10.1016/j.celrep.2017.08.087

Vincow, E.S., G. Merrihew, R.E. Thomas, N.J. Shulman, R.P. Beyer, M.J. MacCoss, and L.J. Pallanck. 2013. The PINK1-Parkin pathway promotes both mitophagy and selective respiratory chain turnover in vivo. *Proc. Natl. Acad. Sci. USA.* 110:6400–6405. https://doi.org/10.1073/pnas.1221132110

Vives-Bauza, C., C. Zhou, Y. Huang, M. Cui, R.L. de Vries, J. Kim, J. May, M.A. Tocilescu, W. Liu, H.S. Ko, et al. 2010. PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proc. Natl. Acad. Sci. USA.* 107: 378–383. https://doi.org/10.1073/pnas.0911187107

Walsh, T.G., M.T.J. van den Bosch, K.E. Lewis, C.M. Williams, and A.W. Poole. 2018. Loss of the mitochondrial kinase PINK1 does not alter platelet function. *Sci. Rep.* 8:14377. https://doi.org/10.1038/s41598-018-32716-4

Wang, Y., M. Serricchio, M. Jauregui, R. Shanbhag, T. Stoltz, C.T. Di Paolo, P.K. Kim, and G.A. McQuibban. 2015. Deubiquitinating enzymes regulate PARK2-mediated mitophagy. *Autophagy.* 11:595–606. https://doi.org/10.1080/15548627.2015.1034408

Wang, X., K. Becker, N. Levine, M. Zhang, A.P. Lieberman, D.J. Moore, and J. Ma. 2019a. Pathogenic alpha-synuclein aggregates preferentially bind to mitochondria and affect cellular respiration. *Acta Neuropathol. Commun.* 7:41. https://doi.org/10.1186/s40478-019-0696-4

Wang, Y., N. Liu, and B. Lu. 2019b. Mechanisms and roles of mitophagy in neurodegenerative diseases. *CNS Neurosci. Ther.* 25:859–875.

Wei, H., L. Liu, and Q. Chen. 2015. Selective removal of mitochondria via mitophagy: distinct pathways for different mitochondrial stresses. *Biochim. Biophys. Acta.* 1853:2784–2790. https://doi.org/10.1016/j.bbamcr.2015.03.013

Wei, Y., W.C. Chiang, R. Sumpter, Jr., P. Mishra, and B. Levine. 2017. Prohibitin 2 is an inner mitochondrial membrane mitophagy receptor. *Cell.* 168:224–238.e10. https://doi.org/10.1016/j.cell.2016.11.042

Whitworth, A.J., J.R. Lee, V.M. Ho, R. Flick, R. Chowdhury, and G.A. McQuibban. 2008. Rhomboid-7 and HtrA2/Omi act in a common pathway with the Parkinson's disease factors Pink1 and Parkin. *Dis. Model. Mech.* 1:168–174. https://doi.org/10.1242/dmm.000109

Wong, Y.C., S. Kim, W. Peng, and D. Krainc. 2019. Regulation and function of mitochondria-lysosome membrane contact sites in cellular homeostasis. *Trends Cell Biol.* 29:500–513. https://doi.org/10.1016/j.tcb.2019.02.004

Wu, W., W. Tian, Z. Hu, G. Chen, L. Huang, W. Li, X. Zhang, P. Xue, C. Zhou, L. Liu, et al. 2014. ULK1 translocates to mitochondria and phosphorylates FUNDC1 to regulate mitophagy. *EMBO Rep.* 15:566–575. https://doi.org/10.1002/embr.201438501

Wu, S., Q. Lu, Q. Wang, Y. Ding, Z. Ma, X. Mao, K. Huang, Z. Xie, and M.H. Zou. 2017. Binding of FUN14 domain containing 1 with inositol 1,4,5-trisphosphate receptor in mitochondria-associated endoplasmic reticulum membranes maintains mitochondrial dynamics and function in hearts in vivo. *Circulation.* 136:2248–2266. https://doi.org/10.1161/CIRCULATIONAHA.117.030235

Xu, Y., J. Shen, and Z. Ran. 2020. Emerging views of mitophagy in immunity and autoimmune diseases. *Autophagy.* 16:3–17. https://doi.org/10.1080/15548627.2019.1603547

Yamamoto, H., S. Kakuta, T.M. Watanabe, A. Kitamura, T. Sekito, C. Kondo-Kakuta, R. Ichikawa, M. Kinjo, and Y. Ohsumi. 2012. Atg9 vesicles are an important membrane source during early steps of autophagosome formation. *J. Cell Biol.* 198:219–233. https://doi.org/10.1083/jcb.201202061

Yamano, K., A.I. Fogel, C. Wang, A.M. van der Bliek, and R.J. Youle. 2014. Mitochondrial Rab GAPs govern autophagosome biogenesis during mitophagy. *eLife.* 3. e01612. https://doi.org/10.7554/eLife.01612

Yamano, K., C. Wang, S.A. Sarraf, C. Münch, R. Kikuchi, N.N. Noda, Y. Hizukuri, M.T. Kanemaki, W. Harper, K. Tanaka, et al. 2018. Endosomal Rab cycles regulate Parkin-mediated mitophagy. *eLife.* 7:e31326. https://doi.org/10.7554/eLife.31326

Yan, C., L. Gong, L. Chen, M. Xu, H. Abou-Hamdan, M. Tang, L. Désaubry, and Z. Song. 2020. PHB2 (prohibitin 2) promotes PINK1-PRKN/Parkin-dependent mitophagy by the PARL-PGAM5-PINK1 axis. *Autophagy.* 16: 419–434. https://doi.org/10.1080/15548627.2019.1628520

Yoshii, S.R., C. Kishi, N. Ishihara, and N. Mizushima. 2011. Parkin mediates proteasome-dependent protein degradation and rupture of the outer mitochondrial membrane. *J. Biol. Chem.* 286:19630–19640. https://doi.org/10.1074/jbc.M110.209338

Youle, R.J. 2019. Mitochondria—striking a balance between host and endosymbiont. *Science.* 365:eaaw9855. https://doi.org/10.1126/science.aaw9855

Yu, W., Y. Sun, S. Guo, and B. Lu. 2011. The PINK1/Parkin pathway regulates mitochondrial dynamics and function in mammalian hippocampal and dopaminergic neurons. *Hum. Mol. Genet.* 20:3227–3240. https://doi.org/10.1093/hmg/ddr235

Zachari, M., S.R. Gudmundsson, Z. Li, M. Manifava, R. Shah, M. Smith, J. Stronge, E. Karanasios, C. Piunti, C. Kishi-Itakura, et al. 2019. Selective autophagy of mitochondria on a ubiquitin-endoplasmic-reticulum platform. *Dev. Cell.* 50:627–643.e5. https://doi.org/10.1016/j.devcel.2019.06.016

Zhang, H., M. Bosch-Marce, L.A. Shimoda, Y.S. Tan, J.H. Baek, J.B. Wesley, F.J. Gonzalez, and G.L. Semenza. 2008. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. *J. Biol. Chem.* 283: 10892–10903. https://doi.org/10.1074/jbc.M800102200

Zhang, T., L. Xue, L. Li, C. Tang, Z. Wan, R. Wang, J. Tan, Y. Tan, H. Han, R. Tian, et al. 2016. BNIP3 protein suppresses PINK1 kinase proteolytic cleavage to promote mitophagy. *J. Biol. Chem.* 291:21616–21629. https://doi.org/10.1074/jbc.M116.733410

Zhang, W., S. Siraj, R. Zhang, and Q. Chen. 2017. Mitophagy receptor FUNDC1 regulates mitochondrial homeostasis and protects the heart from I/R injury. *Autophagy.* 13:1080–1081. https://doi.org/10.1080/15548627.2017.1300224

Zhang, Y., Y. Yao, X. Qiu, G. Wang, Z. Hu, S. Chen, Z. Wu, N. Yuan, H. Gao, J. Wang, et al. 2019. Listeria hijacks host mitophagy through a novel mitophagy receptor to evade killing. *Nat. Immunol.* 20:433–446. https://doi.org/10.1038/s41590-019-0324-2

Zhao, Y., S. Huang, J. Liu, X. Wu, S. Zhou, K. Dai, and Y. Kou. 2018. Mitophagy contributes to the pathogenesis of inflammatory diseases. *Inflammation.* 41:1590–1600. https://doi.org/10.1007/s10753-018-0835-2

Zhen, Y., H. Spangenberg, M.J. Munson, A. Brech, K.O. Schink, K.W. Tan, V. Sørensen, E.M. Wenzel, M. Radulovic, N. Engedal, et al. 2020. ESCRT-mediated phagophore sealing during mitophagy. *Autophagy.* 16:826–841. https://doi.org/10.1080/15548627.2019.1639301

Zhuang, N., L. Li, S. Chen, and T. Wang. 2016. PINK1-dependent phosphorylation of PINK1 and Parkin is essential for mitochondrial quality control. *Cell Death Dis.* 7. e2501. https://doi.org/10.1038/cddis.2016.396

Ziviani, E., R.N. Tao, and A.J. Whitworth. 2010. *Drosophila* parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. *Proc. Natl. Acad. Sci. USA.* 107:5018–5023. https://doi.org/10.1073/pnas.0913485107