ANNUAL REVIEWS

*Annual Review of Cell and Developmental Biology*

# Mitochondrial Quality Control and Restraining Innate Immunity

Andrew T. Moehlman and Richard J. Youle

Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892, USA; email: youler@ninds.nih.gov

Annu. Rev. Cell Dev. Biol. 2020. 36:265–89

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

https://doi.org/10.1146/annurev-cellbio-021820-101354





## Keywords

mitochondria, immunity, inflammation, mitophagy, neurodegeneration

## Abstract

Maintaining mitochondrial health is essential for the survival and function of eukaryotic organisms. Misfunctioning mitochondria activate stress-responsive pathways to restore mitochondrial network homeostasis, remove damaged or toxic proteins, and eliminate damaged organelles via selective autophagy of mitochondria, a process termed mitophagy. Failure of these quality control pathways is implicated in the pathogenesis of Parkinson's disease and other neurodegenerative diseases. Impairment of mitochondrial quality control has been demonstrated to activate innate immune pathways, including inflammasome-mediated signaling and the antiviral cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING)–regulated interferon response. Immune system malfunction is a common hallmark in many neurodegenerative diseases; however, whether inflammation suppresses or exacerbates disease pathology is still unclear. The goal of this review is to provide a historical overview of the field, describe mechanisms of mitochondrial quality control, and highlight recent advances on the emerging role of mitochondria in innate immunity and inflammation.

## Contents



# 1. MITOCHONDRIAL QUALITY CONTROL OVERVIEW

## 1.1. Overview of Mitochondrial Surveillance Mechanisms

Mitochondria are highly specialized organelles that function as intracellular signaling hubs, generate cellular energy in the form of ATP, and regulate intrinsic apoptosis (Nunnari & Suomalainen 2012). The unique features of mitochondria and mitochondrial metabolism require eukaryotic cells to maintain mitochondrial health and to quickly repair or remove damaged mitochondria to prevent cell death or tissue damage. To this end, multiple mitochondrial quality control pathways have emerged to monitor mitochondrial health and activate appropriate responsive measures (Mishra & Chan 2016). This review first describes these mitochondrial surveillance mechanisms and the damage-induced signaling pathways responsible for clearing malfunctioning organelles,

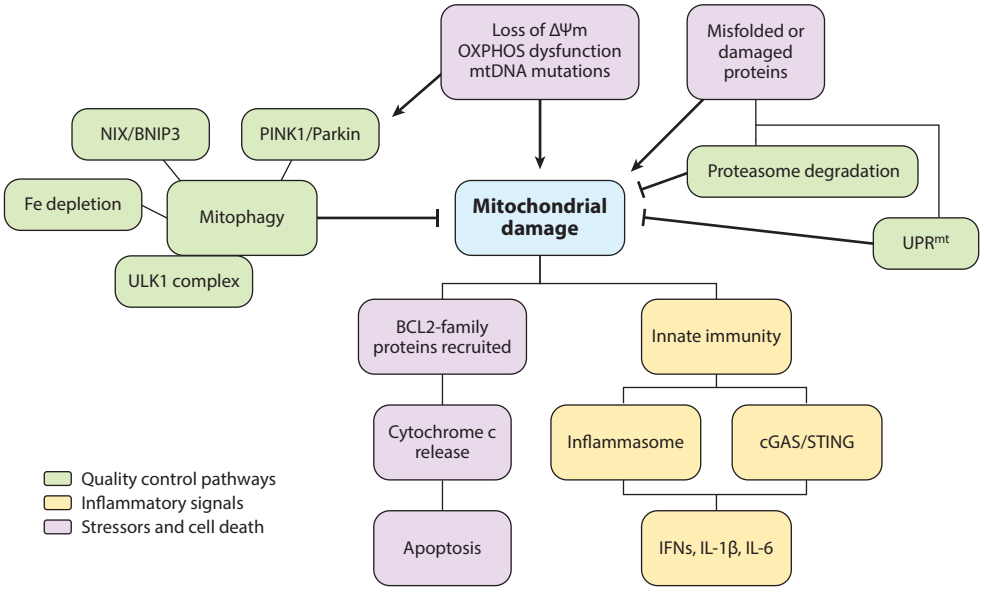


**Figure 1**

Mitochondrial stress invokes quality control pathways to resolve the stress or remove damaged organelles. Mitochondrial damage can occur through impaired electron transport chain function, aggregation of misfolded proteins, accumulation of mtDNA mutations, or chemical-induced dysfunction. Quality control pathways that are induced include (among others): mitophagy, the autophagosome-mediated degradation of mitochondria; $UPR^{mt}$, a translational and transcriptional response to misfolded proteins; and degradation of mitochondrial proteins through the proteasome. Damage signals released from mitochondria trigger inflammatory response pathways. Ultimately, if the mitochondrial and cellular stress remains unresolved, apoptosis can be induced via the activation of Bax and the opening of the outer mitochondrial membrane, leading to the release of caspase-inducing cytochrome c. Abbreviations: cGAS, cyclic GMP-AMP synthase; IFN, interferon; IL, interleukin; mtDNA, mitochondrial DNA; OXPHOS, oxidative phosphorylation; PINK1, PTEN-induced putative kinase protein 1; STING, stimulator of interferon genes; $UPR^{mt}$, mitochondrial unfolded protein response.

as outlined in **Figure 1**. Then we discuss the increasingly prominent topic of mitochondrial-damage-induced activation of the immune system and the research advances made in recent years to understand these pathways in vivo. Finally, we provide a brief overview of the emerging roles of mitochondrial quality control in neurodegenerative disorders, aging, and other diseases.

## 1.2. Loss of Mitochondrial Membrane Potential Triggers Mitochondrial Quality Control

The complexes of the electron transport chain (ETC) generate ATP through oxidative phosphorylation (OXPHOS), a process that requires the presence of sufficient mitochondrial membrane potential ($\Delta\Psi m$) (Boyer et al. 1977, Hatefi 1985). Failure to maintain this voltage potential impairs ATP production and leads to the release of reactive oxygen species (ROS) (Reczek & Chandel 2015), mitochondrial damage-associated molecular patterns (DAMPs) (Zhang et al. 2010), and eventually cell death, described further in the sidebar titled Apoptosis: The Ultimate Mitochondrial Damage Response (Tait & Green 2013). To counter the potentially harmful and apoptosis-inducing effects of failed mitochondria, cells have adapted countermeasures to recognize decreases in $\Delta\Psi m$ and to resolve such stress (Lemasters 2005). These strategies employ cytosolic machinery

## APOPTOSIS: THE ULTIMATE MITOCHONDRIAL DAMAGE RESPONSE

A final method of mitochondrial damage response is the induction of regulated cell death, or apoptosis, through Bcl-2 family proteins mediating release of cytochrome c (Cyt c) from mitochondria, triggering downstream signaling events that have previously been reviewed extensively (Tait & Green 2013). When released from suppression by the antiapoptotic Bcl-2 proteins, proapoptotic Bax proteins are recruited to mitochondria and form pores in the outer mitochondrial membrane (OMM), causing the herniation and rupture of the inner membrane (IMM) (Hsu et al. 1997, Kluck et al. 1997, Yang et al. 1997). Damaged mitochondria will release Cyt c through these pores (Kluck et al. 1997, Yang et al. 1997). Cyt c in the cytosol activates the apoptosome, which in turn triggers a cascade of caspases, starting with caspase-9, that ultimately results in the controlled destruction of the cell (Tait & Green 2013). Recently, OMM permeabilization via Bak and Bax was reported to release mitochondrial DNA into the cytosol, activating intracellular immune pathways (Brokatzky et al. 2019, McArthur et al. 2018).

such as the ubiquitin–proteosome degradation pathway (Tanaka et al. 2010, Yoshii et al. 2011) (see Section 1.5), piecemeal degradation of mitochondrial regions via the autophagosome–lysosome pathway (Le Guerroué et al. 2017) (see Section 1.5), or wholesale autophagosome-mediated engulfment of damaged mitochondria, a process termed mitophagy (Lemasters 2005) (see Section 1.4). To study these pathways in vitro, mitochondrial uncoupling agents such as carbonyl cyanide m-chlorophenyl hydrazone (CCCP) and valinomycin, or a combination of respiratory complex inhibitors such as oligomycin and antimycin A, are commonly used to destabilize the mitochondrial membrane potential and induce mitochondrial damage (Gottlieb et al. 2003, Lemasters et al. 1998, Narendra et al. 2008, Whitworth & Pallanck 2017). These potent acute stressors have been useful in vitro to unravel the molecular mechanisms of mitochondrial damage pathways; however, how well these treatments represent mitochondrial damage in vivo remains controversial (Whitworth & Pallanck 2017).

### 1.3. Import of Mitochondrial Proteins

Mitochondrial dynamics and function depend on the proper trafficking and import of nuclear-encoded proteins destined for the outer mitochondrial membrane (OMM), the inner mitochondrial membrane (IMM), or the mitochondrial matrix (Dolezal et al. 2006, Neupert & Brunner 2002). The nascent peptides often contain a mitochondrial targeting signal that is recognized by cytoplasmic chaperones such as Hsp70, Hsp90, and the mitochondrial import-stimulating factor, which facilitates relocation of the translating peptide–ribosome complex to the OMM (reviewed thoroughly in Wiedemann & Pfanner 2017). This highly regulated transport process depends on many high-molecular-weight protein complexes, including the translocator of the outer membrane (TOM) and translocator of the inner membrane (TIM) complexes and mitochondrial chaperones. Failure to maintain this trafficking process or blockage of the import machinery results in mitochondrial decoupling and induction of the mitochondrial unfolded protein response ($UPR^{mt}$) and other mitochondrial quality control pathways such as mitophagy (Weidberg & Amon 2018, Wrobel et al. 2015). Mitophagy, in particular, requires the continued import of PTEN-induced putative kinase protein 1 (PINK1) into the IMM, where it is cleaved by the proteases MPP and PARL (Narendra et al. 2010a, Youle 2019). This cleavage leads to cytosolic relocation of the cleaved PINK1 and degradation by the proteasome following the N-end-rule pathway (Matsuda et al. 2010, Narendra et al. 2010a, Yamano & Youle 2013). When mitochondria are depolarized or when the import pathway is disrupted, PINK1 import is prevented, leading

to its autophosphorylation and activation (Okatsu et al. 2012); activated PINK1 phosphorylates ubiquitin, which in turn recruits and activates Parkin, as described in the next section.

### 1.4. PINK1/Parkin–Regulated Mitophagy Completely Removes Damaged Mitochondria via the Autophagy Pathway

When PINK1 is stabilized on damaged mitochondria, Parkin is recruited to phospho-Ser65 ubiquitin chains on mitochondria generated by PINK1 (Kane et al. 2014, Kazlauskaite et al. 2014, Koyano et al. 2014). Parkin ubiquitinates many proteins, including the OMM proteins mitofusin (Glauser et al. 2011, Poole et al. 2010, Tanaka et al. 2010, Ziviani et al. 2010), voltage-dependent anion channels (VDACs)/porin (Sun et al. 2012), and Miro (Wang et al. 2011), in addition to dozens of other mitochondrial proteins, autophagy receptors, and proteasome regulators, as discovered by an unbiased quantitative Lys-*ε*-Gly-Gly (diGLY) capture and proteomics method for identifying ubiquitination sites (Sarraf et al. 2013). The ubiquitin chains are recognized by autophagy adaptor proteins such as p62/sequestosome-1 (SQSTM1), NPD52, TAX1BP1, NBR1, and optineurin (OPTN) (Heo et al. 2015, Lazarou et al. 2015, Wong & Holzbaur 2014). Once the adaptors are recruited, the canonical autophagy machinery, including the Unc-51-like autophagy-activating kinase (ULK1) complex, the PI(3)P kinase complex, and the LC3-conjugation machinery, initiates formation of an isolation membrane (Johansen & Lamark 2011, Narendra et al. 2010b, Yoshii & Mizushima 2015). Next the ATG5/12/16 complex is recruited and elongates the autophagosome membrane (Yoshii & Mizushima 2015). Finally, the autophagosome fuses with the lysosome through a not fully characterized process involving syntaxin 17, LAMP2, and other factors (Hubert et al. 2016, Issa et al. 2018). The acidic autolysosome then degrades the targeted mitochondrial cargo.

Loss-of-function mutations in the core mitophagy-promoting factors such as PINK1/Parkin or the autophagy adaptor proteins lead to impaired mitophagy in some cell culture models; however, there are well-established PINK1/Parkin–independent mitophagy pathways (Allen et al. 2013, Lieber et al. 2019, Novak et al. 2010). For example, BNIP3, the related protein NIX (BNIP3L), and iron chelation have been shown to modulate mitophagy independently of PINK1/Parkin (Allen et al. 2013, Novak et al. 2010, Rikka et al. 2011).

Mitophagy can be assessed using mitochondria-targeted, pH-sensitive fluorophores. Two tools for this are the mitoQC sensor, a green fluorescent protein (GFP) fused to the mCherry red fluorescent protein targeted to the OMM (McWilliams et al. 2016), and the matrix-targeted mKeima, a fluorescent protein with an extreme pH-induced Stokes shift (Katayama et al. 2011). The mitoQC method is similar to the LC3-GFP-mCherry probe for measuring autophagy, which depends on the loss of GFP fluorescence in the acidic lysosome while mCherry remains stable. Therefore, the ratio of mCherry to GFP is a quantitative measure of autophagosome maturation; however, it was recently demonstrated that as an OMM localized protein mitoQC is sensitive to proteasomal degradation independently of mitophagy and therefore might overestimate true mitophagy events (Katayama et al. 2020). With the mKeima method, the matrix-localized Keima signal undergoes a pH-dependent change in fluorescence characteristics (Katayama et al. 2011). At neutral pH, Keima undergoes excitation at 440 nm and shows maximum emission at 620 nm; however, in an acidic environment the predominant excitation shifts to 586 nm. Therefore, the ratio of $_{em}620$ excited at $_{ex}586$ versus $_{ex}440$ can quantitatively measure mitophagy in live imaging or fluorescence-activated cell sorting (FACS) experiments (Wang 2020). Both of these mitophagy sensors have been engineered into rodents (McWilliams et al. 2016, Sun et al. 2015) and fruit flies (*Drosophila melanogaster*) (Cornelissen et al. 2018, Y. Kim et al. 2019, Lee et al. 2018), yet controversy exists about the prevalence of mitophagy in vivo (Cornelissen et al. 2018, Y. Kim et al.

2019, Lee et al. 2018, McWilliams et al. 2018, Sliter et al. 2018). During the preparation of this review, a novel mitophagy sensor named mito-SRAI was published that consists of two linked fluorophores, one of which is sensitive to low pH environments, that generate a quantitative Förster resonance energy transfer (FRET) signal (Katayama et al. 2020). Mito-SRAI also has the advantage over mKeima of being usable in fixed cells and tissues, so we predict this tool will greatly aid in studying mitophagy in neurons within animals (Katayama et al. 2020).

## 1.5. Removal of Damaged and Aggregated Proteins Maintains Mitochondrial Health

When mitochondrial proteins in the matrix, IMM, and OMM are damaged, malfunctioning, or otherwise destined for degradation, they must be cleaved or targeted for degradation. Some key proteins involved in the proteolysis of mitochondrial proteins include the ATPase proteases LonP and ClpP (in the inner matrix), Yme1 (IMM-anchored in the intermembrane space), and SPG7 (IMM-anchored in the matrix space) (Jin & Youle 2013, Koppen & Langer 2007). In addition, many OMM proteins are targeted to a proteasome by ubiquitin ligases (Karbowski & Youle 2011, Sarraf et al. 2013, Tanaka et al. 2010). These proteins are extracted from the OMM by a multistep process requiring valocin-containing protein (VCP)/p90 and are then transferred to the proteasome for destruction (Tanaka et al. 2010). Regulated ubiquitination of mitochondrial proteins is performed by E3 ligases, including MARCH5, MITOL, MUL1, and Parkin, depending on the protein target, cell or tissue type, and stress state (Karbowski & Youle 2011, Koyano et al. 2019, Yun et al. 2014).

In a related pathway, piecemeal degradation can selectively target mitochondrial domains to remove larger protein aggregates (Burman et al. 2017, Hughes et al. 2016, Le Guerroué et al. 2017, Vincow et al. 2013). Via mechanisms not completely understood, misfolded or damaged mitochondrial proteins are selected for degradation and sequestered within part of the organelle (Burman et al. 2017, Hughes et al. 2016). The mitochondrion is then fragmented via Drp1-mediated fission, and the damage-containing fragments are targeted to the autophagosome–lysosome pathway via the autophagy receptor p62 (Burman et al. 2017, Le Guerroué et al. 2017).

## 1.6. Mitochondrial Unfolded Protein Response–Signaling Pathways Upregulate Mitochondrial Chaperones to Clear Misfolded Peptides

Protein aggregates are toxic to cells, and multiple pathways sense and remove these misfolded proteins. Collectively known as the UPR, these pathways can be further distinguished based on the affected cellular compartment (such as $UPR^{mt}$ and $UPR^{ER}$), although cross talk exists between these pathways and cytosolic stress responses, collectively termed the integrated stress response (Walter & Ron 2011). In mitochondria, damaged membrane proteins or aggregated matrix protein can impair the mitochondrial OXPHOS process and hinder the import and export of mitochondrial proteins (Zhao et al. 2002). The $UPR^{mt}$ response leads to the transcriptional upregulation of mitochondrial chaperones and proteases including Hsp60 and LonP (Jin & Youle 2013, Zhao et al. 2002). In *Caenorhabditis elegans*, extensive work has determined the mechanism through which mitochondrial stress is transmitted to changes in nuclear transcription (Melber & Haynes 2018). Central to this is ATFS-1, a transcription factor with both a mitochondrial targeting sequence and a nuclear localization motif (Nargund et al. 2012). Similar to PINK1 import regulation, during proteotoxic mitochondrial stress and failure of the mitochondrial import machinery ATFS-1 instead enters the nucleus and activates its transcriptional targets (Nargund et al. 2012). In mammalian cells, similar roles have been proposed for the transcription factors ATF5 and ATF4; although the link from mitochondrial stress sensing to activation of

these signaling pathways remains unknown, it might require mTORC1 signaling (Fiorese et al. 2016, Khan et al. 2017, Quirós et al. 2017).

### 1.7. Mitochondrial Fission and Fusion Pathways Regulate the Number of Mitochondria and Contribute to Quality Control

Mitochondria are organelles that constantly undergo changes in size, morphology, and number depending on the health of the mitochondrial network and the energy demands of the cell (Mishra & Chan 2016). The number and morphology of mitochondria are controlled through tightly regulated fission and fusion events; these dynamics are necessary in both proliferative and postmitotic cells (Hoppins et al. 2007). Mitochondrial fission is facilitated by a cytosolic dynamin-like GTPase—Drp1 in mammals, flies, and worms and Dmn1 in yeast (Hoppins et al. 2007). This process is regulated by accessory components such as Mdv1 in yeast and Mff in mammals, which along with Fis1 recruit Drp1 to mitochondria (Otera et al. 2010, Tieu et al. 2002). In both yeast and mammalian cells, membrane contact sites between the endoplasmic reticulum (ER) and the mitochondrial network are proposed to regulate fission through the recruitment of Drp1 (Friedman et al. 2011, Murley et al. 2013) and generation of the biomechanical forces needed for separation of the membranes (Manor et al. 2015). The detailed function of ER–mitochondria contact sites in regulating mitochondrial fission and fusion remains a topic of ongoing investigation.

Mitochondrial fission responds to changes in cellular energy demands and is invoked during mitochondrial stress, when damaged mitochondrial subdomains are divided and degraded to maintain a healthy mitochondrial network (Burman et al. 2017, Yamashita et al. 2016). In contrast, mitochondrial fusion is performed by three mitochondrial membrane-localized, dynamin-like proteins: mitofusins (Mfn1 and Mfn2) on the OMM and Opa1 on the IMM (Mishra & Chan 2016). Damaged mitochondria harboring mitochondrial DNA (mtDNA) mutations can be sustained through fusion with healthy mitochondria containing compensating wild-type DNA (Yoneda et al. 1994). Fusion is normally inhibited upon mitochondrial damage, in part through the ubiquitination and degradation of Mfn1/2 by the E3 ligases Parkin (Deng et al. 2008, Poole et al. 2010, Tanaka et al. 2010, Ziviani et al. 2010) and Mul1 (Puri et al. 2019, Yun et al. 2014). In animals lacking the Pink1/Parkin pathway, Mfn proteins accumulate and mitochondria are primarily swollen; however, these defects can be rescued by the overexpression of fission-promoting factors such as Drp1 (Deng et al. 2008).

## 2. MITOCHONDRIAL DAMAGE ACTIVATES INNATE IMMUNITY

### 2.1. Mitochondrial Damage-Associated Molecular Patterns Trigger Antibacterial Signaling Pathways

Innate immune–signaling pathways have evolved in complex multicellular eukaryotes to recognize invasive pathogens, including bacteria, viruses, fungi, and protists. Perhaps the most critical function of these pathways is the ability to distinguish friend from foe. To prevent aberrant immune responses, the sentries involved in activation of the defense pathway must be able to accurately discriminate pathogens from the organism's own cells (see **Figure 2**). Therefore, the cell surface pattern-recognition receptors (PRRs) responsible for recognizing bacteria and triggering an immune response often respond to complex and unique pathogen-associated molecular patterns (PAMPs)—peptides, lipoproteins, and glycan moieties—on the bacterial cell wall (West & Shadel 2017). Peptidoglycans (PGNs), consisting of short peptides linked to glycan chains, are found in both gram-negative and gram-positive bacteria and constitute the major outer cell wall component of gram-positive bacteria (Raetz & Whitfield 2002, Takeuchi et al. 1999). PGNs are sensed by

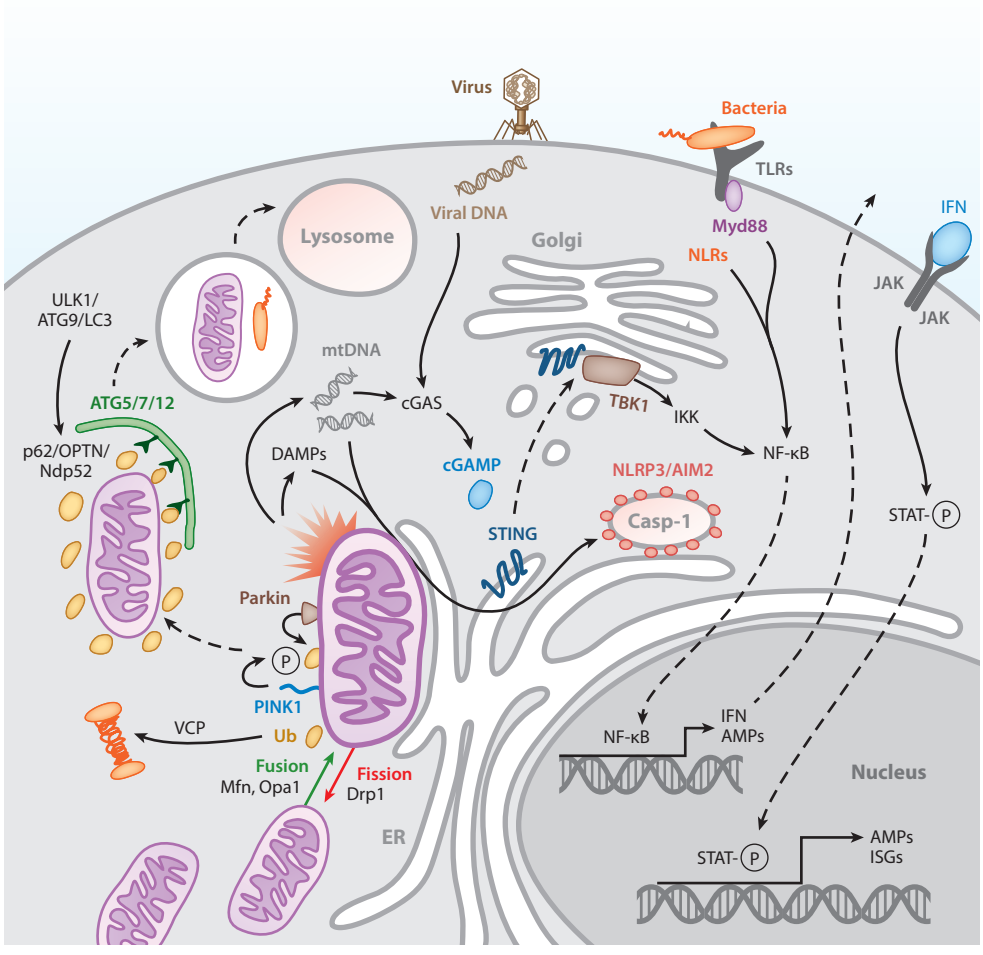


**Figure 2**

Mitochondrial quality control restrains innate immune pathways. Unmitigated mitochondrial damage may invoke innate immune responses due to the similarity of mitochondrial DAMPs to some PAMPs. These signals activate pathogen-response mechanisms, including the TLR- and NLR-mediated signaling pathways. Upon mitochondrial damage and permeabilization, mtDNA is released into the cytosol, triggering both the AIM2 and NLRP3 inflammasome pathways and cGAS, which generates cGAMP that then activates a STING-mediated type I interferon response. Abbreviations: AIM2, absent in melanoma 2; AMP, antimicrobial peptide; Casp-1, caspase-1; cGAMP, cyclic 2′3-GMP-AMP; cGAS, cyclic GMP-AMP synthase; DAMP, damage-associated molecular pattern; Drp1, dynamin-related protein 1 (also known as dynamin-1-like protein); IFN, interferon; IKK, inhibitor of nuclear factor kappa-B kinase; ISG, interferon-stimulated gene; JAK, Janus kinase; LC3, microtubule-associated protein 1A/1B–light chain 3; Mfn, mitofusin; mtDNA, mitochondrial DNA; Myd88, myeloid differentiation primary response 88; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NLR, NOD-like receptor; NLRP3, NOD-, LRR-, and pyrin domain–containing protein 3; Npd52, nuclear dot protein 52 kDa; Opa1, optic atrophy 1–mitochondrial dynamin-like GTPase; OPTN, optineurin; PAMP, pathogen-associated molecular pattern; PINK1, PTEN-induced putative kinase protein 1; STAT, signal transducer and activator of transcription; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; TLR, Toll-like receptor; Ub, ubiquitination; ULK1, Unc-51-like autophagy-activating kinase; VCP, valocin-containing protein.

specialized peptidoglycan-recognition proteins (PGRPs), including Toll-like receptor 2 (TLR2) in mammals (Takeuchi et al. 1999). Gram-negative bacteria possess an outermost lipopolysaccharide (LPS) layer that is predominantly sensed by TLR4 in mammals (Poltorak et al. 1998, Takeuchi et al. 1999).

If the invasive microbes evade the extracellular defenses and cell surface receptors, they can enter the host cell via regulated endocytosis or phagocytosis (Kanneganti et al. 2007). Inside the cell, the nucleotide-binding oligomerization domain–containing proteins (NODs) and the NOD-like receptors (NLRs) are attuned to sense cytosolic microbial signals (Carneiro et al. 2007). These patterns are the same ones that activate TLRs and PGRPs on the outer cell surface, including PGN, diaminopimelic acid (DAP), and LPS (Carneiro et al. 2007, Kanneganti et al. 2007). The downstream responses of these receptors include cleavage of procaspase-1 into the active caspase-1, activation of nuclear factor kappa light-chain-enhancer of activated B cells (NF-κB), generation of proinflammatory cytokines, induction of autophagy or xenophagy (to target and selectively remove the pathogen), and initiation of the cell-death pathway pyroptosis or apoptosis (Carneiro et al. 2007, Liston & Masters 2017).

Mitochondria present a special challenge to the cell's immune-recognition pathways due to their resemblance to ancient prokaryotes, according to the endosymbiotic origin theory (Dyall et al. 2004). Mitochondrial DAMPs are similar to some of the aforementioned patterns presented by infectious bacteria, including the start codon *N*-formylmethionine shared by bacterial and mitochondrial matrix translation machinery and the phospholipid cardiolipin (Carp 1982, Weinberg et al. 2015, Zhang et al. 2010). Sensing of these DAMPs by NOD-, LRR-, and pyrin domain–containing proteins (NLRPs) has been shown to activate inflammatory immune responses (Iyer et al. 2013, Zhang et al. 2010) (**Figure 2**). Additionally, released mtDNA itself has been shown to trigger DNA-sensing antiviral pathways (West et al. 2015), which we discuss in greater detail in Section 2.2. The failure of mitochondrial quality control pathways leads to the accumulation of these damage-associated signals. This failure can be due to the accumulation of misfolded mitochondrial proteins, oxidative stress, chemical- or pollutant-induced damage, or a decline in cellular health due to aging (Youle 2019). Innate immune activation has also been observed in disease mutations in mitophagy pathway genes such as *PRKN* (*PARK2*) and *PINK1* (*PARK6*) and key autophagy genes (Youle 2019). The inflammatory aspects of Parkinson's disease (PD) have been recognized clinically for some time (Dzamko et al. 2015, Frank-Cannon et al. 2008); however, the molecular mechanisms underlying these observations are still being investigated (Matheoud et al. 2019, Sliter et al. 2018, Zhong et al. 2016). The connections between the PINK1/Parkin pathway and bacterial infections were revealed recently in a study demonstrating that *PINK1*$^{-/-}$ mice had increased levels of gut infection and suggesting that alterations to the gut microbiome caused onset of Parkinsonian symptoms (Matheoud et al. 2019). This report proposes that mitochondrial quality control defects lead to induction of innate immunity and prime the adaptive immune system in a method termed mitochondrial antigen presentation (mitAP), leading to neurodegenerative symptoms (Matheoud et al. 2019).

## 2.2. Mitochondrial DNA Triggers Antiviral Defenses

Similar to the problems posed by mitochondria's resemblance to ancient prokaryotes, the presence of a second nonnuclear complement of DNA in the mitochondria posits a range of challenges to the host immune system (West & Shadel 2017). The release of mtDNA has been shown to occur due to continued failure of the mitochondrial clearance mechanisms (Oka et al. 2012). Exactly how mtDNA is released remains uncertain, although the prevailing theories include leakage through large-scale membrane rupture (Shimada et al. 2012), Bax/Bak–mediated membrane permeabilization during the initial steps of apoptosis (McArthur et al. 2018), and regulated

export such as via the VDAC complex (J. Kim et al. 2019). Regardless of the exact mechanism of mtDNA release, this free mtDNA can induce DNA-sensing antiviral mechanisms (West et al. 2015) (**Figure 2**). The antiviral immune system in vertebrates contains multiple sensors of foreign genetic material, including double-stranded DNA (dsDNA), double-stranded RNA (dsRNA), and single-stranded RNA. Cytosolic dsDNA from both pathogens and mitochondria activates multiple response pathways, including the TLR9 receptor pathway (Oka et al. 2012), the AIM2 and NLRP3 inflammasomes (Lugrin & Martinon 2018, Zhong et al. 2018), and the cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING) pathway (Ishikawa et al. 2009, Sun et al. 2013, West et al. 2015). These antiviral response pathways promote expression of proinflammatory cytokines and virus-targeting interferon (IFN) proteins (Ablasser & Chen 2019).

Recently, this cGAS/STING–regulated inflammation has been found to contribute to the pathogenesis of inherited Parkinsonian phenotypes in mice (Sliter et al. 2018). Mice deficient for Parkin or PINK1, which normally lack any PD-like phenotypes, display elevated levels of serum cytokines following exhaustive exercise training. Similarly, expression of a proofreading-deficient polymerase gamma (PolG) mitochondrial polymerase in the *Parkin*$^{-/-}$ mutant background (*PRKN*$^{-/-}$; *PolG*$^{\text{mutator}}$) led to elevated cytokine production accompanied by PD-like age-dependent neurodegeneration of dopaminergic neurons in the substantia nigra as well as motor defects (Sliter et al. 2018). Critically, loss of STING prevented the inflammation observed in both models and the neurodegeneration phenotype of the *PRKN*$^{-/-}$; *PolG*$^{\text{mutator}}$ mice, supporting the hypothesis that PINK1/Parkin–dependent mitophagy restrains innate immunity (Sliter et al. 2018, Zhong et al. 2016). The cell-type specificity of STING signaling in the context of neurodegenerative disorders remains unknown, as this question is further complicated due to the possibility of the intercellular transfer of both cytokines and cyclic nucleotides (Ablasser et al. 2013, Luteijn et al. 2019, Ritchie et al. 2019).

## 2.3. Inflammasome Signaling and Mitochondria

In addition to the cGAS/STING DNA-sensing pathway, mtDNA also has the potential to stimulate inflammasome signaling in macrophages, leading to the activation of caspase-1 and the release of interleukin (IL)-1β (Shimada et al. 2012) (**Figure 2**). Inflammasomes share the same core components, the cysteine protease caspase-1 and ASC/PYCARD, but can contain one of four different receptors: NLRP3, AIM2, NLRC4, or NRLP1 (Liston & Masters 2017). The first three of these inflammasome complexes can be stimulated by mtDNA or oxidized mtDNA during varying stress conditions in bone marrow–derived macrophages (BMDMs) (He et al. 2016, Shimada et al. 2012). The AIM2 inflammasome is a canonical sensor of cytosolic DNA from invasive pathogens or damaged mitochondria (Lugrin & Martinon 2018). NLR-family inflammasome signaling is activated by multiple additional stimuli including disruption of the cellular $K^+$ gradient, oxidative stress, urea crystals, and ATP levels, among others (Nomura et al. 2015, Yaron et al. 2015). These inflammasome-inducing compounds can trigger mitochondrial damage, leading to Parkin-dependent ubiquitination and mitophagy via a p62 adaptor protein; in *PRKN* or *p62* mutant macrophages, secretion of IL-1β is enhanced, suggesting that the elimination of damaged mitochondria counteracts inflammasome activation (Zhong et al. 2016). Following Toll-pathway signaling in macrophages, increased synthesis of mtDNA contributes to the priming of the inflammasome response upon mitochondrial damage by increasing the level of oxidized mtDNA, which activates NLRP3 upon release to the cytosol (Zhong et al. 2018). ROS and specific lipids such as cardiolipin released from damaged mitochondria also activate the NLRP3 inflammasome (Iyer et al. 2013, Yaron et al. 2015). NLRP3 directly binds cardiolipin, and physical association of the NLRP3 inflammasome with the mitochondria may be necessary for activation (Iyer et al. 2013).

### 2.4. Mitochondria Coordinate Antiviral Signaling

Cytosolic mitochondrial RNA (mtRNA) or viral-derived dsRNA is sensed by MDA5 and retinoic acid–inducible gene 1 (RIG-1), both members of the RIG-1-like receptor (RLR) family (Dhir et al. 2018, Liu et al. 2017). The binding of RNAs triggers RIG-1 to localize onto mitochondria and interact with mitochondrial antiviral signaling (MAVS) protein, ultimately leading to the expression of IFNs and other antiviral factors (Castanier et al. 2010, Sánchez-Aparicio et al. 2017). The MAVS complex must be localized to the OMM via a C-terminal tail to function (Seth et al. 2005). Interestingly, impaired or altered mitochondrial dynamics is linked to defects in RIG-1 signaling (Castanier et al. 2010, Sánchez-Aparicio et al. 2017). Thus, mitochondria have emerged as a hub of antiviral innate immune pathways, and mitochondrial quality control is critical to maintaining these functions and to avoiding the aberrant activation of the cytokine response.

## 3. CONSERVED ROLES OF MITOCHONDRIAL QUALITY CONTROL AND IMMUNITY

### 3.1. *Drosophila melanogaster* Models of Mitochondrial Dysfunction

Clinical genetic studies have identified mutations in the genes encoding Parkin, Pink1, and DJ-1 as hereditary causes of some PD (Bonifati et al. 2003, Kitada et al. 1998, Valente et al. 2004). However, mouse models for the PD-linked loss-of-function *PRKN* and *PINK1* mutants fail to display the classic PD characteristics (Bobela et al. 2014, Kitada et al. 2009). The first identified function for the Pink1/Parkin pathway in mitochondrial biology came from studies of *parkin* and *pink1* mutant alleles in *D. melanogaster* (Clark et al. 2006, Greene et al. 2003, Park et al. 2006). Flies that are *parkin* and *pink1* null harbor multiple mitochondria-related defects, including thorax structure defects, flight muscle damage, locomotion defects, and dopaminergic neuron degeneration (Clark et al. 2006, Greene et al. 2003, Park et al. 2006, Whitworth et al. 2005). Underlying these muscle defects are severely enlarged and fused mitochondria, which are indicative of defects in mitochondrial quality control, mitochondrial fission, or both (Deng et al. 2008). These mutants also have male fertility defects, attributed to spermatid mitochondrial morphology defects in the organization of the Nebekurn structure, similar to mutants in the testis-specific mitochondrial fusion gene *fzo* (Riparbelli & Callaini 2007). The overexpression of Parkin could rescue the *pink1* mutant phenotypes, but the overexpression of Pink1 had no effect on the *parkin* mutant phenotypes (Clark et al. 2006, Park et al. 2006). These epistasis results strongly support the idea that Pink1 functions upstream of Parkin.

Both *parkin*- and *pink1*-null flies exhibit abnormal mitochondrial fusion, which can be prevented either by overexpression of the fission-promoting GTPase Drp1 or by genetic ablation of the fusion factors Opa1 and/or Marf (dMfn) (Deng et al. 2008). However, the mechanisms through which defective mitochondrial quality control results in the observed in vivo fly phenotypes remain controversial: Some researchers argue that these impaired mitochondrial fission and fusion dynamics in the *pink1* and *parkin* fly mutants lead to defective and fused mitochondria (Deng et al. 2008, Ma et al. 2018, Pogson et al. 2014), while others support the lack of Parkin-dependent mitophagy as the causative factor, although the amount of in vivo mitophagy is still debated (Cornelissen et al. 2018, Y. Kim et al. 2019, Lee et al. 2018, Vincow et al. 2013, Whitworth & Pallanck 2017). These two explanations are not mutually exclusive; however, it is difficult to separate mitophagy from mitochondrial dynamics, as extensive cross talk occurs between the two intertwined processes. Regardless, both hypotheses incorporate the accumulation of mitochondria-derived damage, the induction of cellular stress-response pathways, and, ultimately, apoptosis.

Fly *pink1* mutants also feature lower ATP production and impaired ETC complex I activity (Morais et al. 2009). Interestingly, this appears to be a Parkin-independent effect, as the overexpression of a complex I subunit rescued ATP production and other visible phenotypes in *pink1* mutants but had no effect in *parkin* mutants (Pogson et al. 2014). Pink1 has been reported to phosphorylate a subunit of complex I (Morais et al. 2014, Pogson et al. 2014). This phosphorylation is thought to stabilize the complex in the matrix, but the localization of the phosphorylation event remains unclear, as Pink1 appears to be quickly removed from the membranes of healthy mitochondria and then degraded. Regardless, the role of Pink1 kinase targets other than Parkin and ubiquitin in mitochondrial health remains an interesting open question (Voigt et al. 2016).

### 3.2. Innate Immune Pathways in Flies

*Drosophila* mutants defective in mitochondrial quality control demonstrate a higher expression of antimicrobial peptides (AMPs). Early microarray analysis of the *parkin* mutant transcriptome revealed an enrichment of genes involved in both the oxidative stress response and innate immune signaling (Greene et al. 2005). Similar transcriptional changes are observed in very long-lived flies, suggesting that mitochondrial failure might be a root cause (Koehler et al. 2017). Flies with *pink1* or *parkin* loss-of-function mutations are hypersensitive to bacterial infections, implying that either innate immune signaling and defense against infection are impaired or excessive activation of the immune pathways is leading to the induction of cell death genes (Cho et al. 2015). Although invertebrates lack adaptive immunity, many of the core innate immune pathways are conserved, including the central NF-κB and inhibitor of nuclear factor kappa-B kinase (IKK) signaling hubs, along with the antimicrobial TLR and PGRPs (Buchon et al. 2014). The major pathways are the Toll pathway, involved in the sensing of gram-positive bacteria and fungi; the immune deficiency (IMD) pathway, for recognition of gram-negative bacteria; and the small interfering RNA (siRNA) pathway, which targets viral genetic material (Buchon et al. 2014). The ultimate output of these pathways is the activation of cell stress-response pathways including Janus kinase (JAK)/signal transducer and activator of transcription (STAT) and c-Jun N-terminal kinase

#### THE ROLES OF THE JAK/STAT AND JNK PATHWAYS DURING INFECTION AND MITOCHONDRIAL DAMAGE

Both infection and mitochondrial damage in flies can elicit the activation of general stress pathways including the c-Jun N-terminal kinase (JNK) and Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathways. Fly *parkin* mutants have elevated levels of activated phospho-JNK in muscle cells and dopaminergic neurons (Cha et al. 2005). The overexpression of Parkin prevented defects in a dominant-negative JNK mutant, suggesting that Parkin suppresses JNK signaling (Cha et al. 2005). JNK activation, potentiated by NF-κB and tumor necrosis factor (TNF) signaling, is one pathway through which apoptosis is initiated during infection in flies (Moreno et al. 2002). In mammals, stimulation of innate immunity leads to the generation of cytokines, including type I interferon IFNβ, which activates the JAK1/2-mediated phosphorylation of STAT (Schindler et al. 2007). STAT-mediated transcription in macrophages and T cells potentiates innate and adaptive immunity (Schindler et al. 2007). In flies, a similar pathway is induced during stress and infection through the release of the cytokine-like extracellular ligands Upd1/2/3, which are recognized by the receptor Domeless, triggering the activation of JAK and the upregulation of defense genes in fly immune tissues (Agaisse & Perrimon 2004). The consequences of this pathway on mitochondrial damage remain unclear; however, the involvement of STING signaling in genetic models of PD (Sliter et al. 2018) suggests that cytokine signaling and JAK/STAT activation are important downstream from mitochondrial damage.

(JNK)/Basket (described further in the sidebar titled The Roles of the JAK/STAT and JNK Pathways During Infection and Mitochondrial Damage), the stimulation of autophagy, and the upregulation of AMPs (Buchon et al. 2014, McPhee & Baehrecke 2009). Similar to inflammatory cytokines in mammals, innate immunity–regulated AMPs are hallmarks of aging and neurodegenerative disease models. In *Drosophila* neurons, the neuropeptides appear harmful (Shukla et al. 2019). Forced overexpression of AMPs causes the degeneration of dopaminergic neurons and locomotion defects that are enhanced in autophagy-deficient flies (Shukla et al. 2019).

Although invertebrates lack IFN signaling, the cyclic DNA sensor STING is conserved in flies, although it lacks the TBK1-interacting C-terminal domain (Martin et al. 2018). However, loss of *sting* results in immune-compromised flies with defects in the IMD/Relish–induced activation of AMPs (Goto et al. 2018, Liu et al. 2018, Martin et al. 2018). The upstream regulators of STING in invertebrates remain unknown, as the closest homolog to cGAS lacks the DNA-binding domain (Martin et al. 2018). Fly STING can directly bind to cyclic 2′3-GMP-AMP (cGAMP), so perhaps there is a novel source of cGAMP in invertebrates or, alternatively, STING is responding to cyclic dinucleotides (cGAMP or cyclic di-GMP) derived from bacteria (Goto et al. 2018, Martin et al. 2018). The importance of the STING signaling pathway in disease and stress responses remains unclear in flies, and much remains to be uncovered about the molecular inputs upstream of STING, the conservation of its ER–Golgi trafficking-based activating mechanism, and the details of its downstream signaling outputs. In addition to its role in innate immunity, STING also regulates autophagy in a manner that depends on ATG5 and WIPI2 but is independent of the ULK1 and VPS34 complexes (Gui et al. 2019). STING's regulatory role in autophagy is conserved in sea anemones (Gui et al. 2019) and potentially in *D. melanogaster*, where infection with Zika virus resulted in increased levels of neuronal autophagy, a process that was impaired in *STING*-null mutants (Liu et al. 2018).

### 3.3. Mitochondrial DNA Mutations Trigger Mitochondrial Defects and Dopaminergic Neuron Degeneration

Unlike the mouse mtDNA PolG-mutator model, attempts to study the effects of a proofreading-deficient mtDNA PolG in flies have been controversial. Genetic engineering–mediated replacement of the endogenous fly mtDNA PolG (encoded by the gene *tamas*) with a proofreading-deficient or polymerase-dead mutant failed to rescue the adult viability of the PolG mutants (Bratic et al. 2015). Flies that had one wild-type PolG allele and one mutator allele showed no defects in aging or health and had little to no mtDNA mutational burden (Bratic et al. 2015, Kauppila et al. 2018). Transheterozygous animals with one copy of the proofreading-deficient allele and one copy of a polymerase-dead PolG allele were viable and had increased levels of mtDNA mutation; however, these mtDNA-mutation-prone flies had no outward health defects, such as a reduction in life span or motor defects, leading the research group to conclude that the flies are resistant to mtDNA mutation accumulation (Kauppila et al. 2018). A competing study used an endogenously regulated transgenic insertion of the *PolG*$^{\text{mutator}}$ (*tamas*$^{\text{D263A}}$) allele along with a heterozygous deletion of the genomic region containing the *tamas* and the subunit *DNApol-γ35* genes (Samstag et al. 2018). This results in the dose-dependent accumulation of mtDNA mutations and age-related climbing defects, similar to the murine *PolG*$^{\text{mutator}}$ models (Samstag et al. 2018). The differences in the observed effects of *PolG*$^{\text{mutator}}$ models on neurodegeneration and aging led to the generation of a third mtDNA mutator system using a mitochondria-targeted cytidine deaminase called APOBEC1 (Andreazza et al. 2019). This system leads to an accumulation of C to T mutations and causes decreased animal fitness, including shorter life spans and climbing defects (Andreazza et al. 2019).

In addition to the prevention of harmful mtDNA mutations in postmitotic cells, sensing of and selection against mtDNA mutations in the fly germ line is critical for the correct transfer of mitochondria from mother to offspring (Hill et al. 2014, Ma et al. 2014). This process, termed purifying selection, is critical for promoting the survival of offspring as well as the prevention of cross-species hybridization. Mitochondrial fitness is assessed through mitochondrial fragmentation in the developing oocyte, and energetically impaired mitochondria are selected for degradation by autophagy in a process that requires Atg1 (ULK1) and BNIP3 (Lieber et al. 2019). Another recent study described a novel role for Pink1 in this quality control pathway in which Pink1 mediates the phosphorylation of the local mitochondrial translational factor Larp (Zhang et al. 2019); however, this report claims that Pink1 was functioning independently of its role in mitophagy in the germ line.

## 3.4. Mitophagy in Worms Promotes Longevity and Immunity

In the model organism *C. elegans*, multiple longevity studies have revealed the importance of maintaining a healthy mitochondrial network during aging. Loss of the NIX/BNIP homolog *dct-1* in worms results in the accumulation of mitochondria and the disruption of mitochondrial morphology (Palikaras et al. 2015). DCT-1 recruits damaged mitochondria to the autophagy machinery in a process that also requires PINK1 (*pink-1*) and the Parkin worm homolog (*pdr-1*), which ubiquitinates DCT-1 (Palikaras et al. 2015). Mitophagy via a Parkin and DCT-1 pathway is also induced in *C. elegans* by knockdown of the iron–sulfur cluster–generating factor *frataxin* in a genetic model for Friedreich's ataxia, a devastating neurodegenerative disease (Schiavi et al. 2015). Intriguingly, chronic mild induction of mitophagy via iron depletion resulted in life span extension, demonstrating the importance of mitochondrial quality control both in dealing with acute mitochondrial respiration inhibition and during aging (Schiavi et al. 2015). PINK1/Parkin–dependent mitophagy is triggered during *Pseudomonas aeruginosa* infection in *C. elegans*, which triggers iron depletion and mitochondrial damage through the virulence factor pyoverdine (Kirienko et al. 2015). In these infection experiments, loss of *pink-1* or *pdr-1*, or the autophagy factor *bec-1* (BECN1) or *lgg-1* (LC3), led to increased lethality, demonstrating a link between mitophagy and innate immune protection in worms (Kirienko et al. 2015).

## 3.5. The Mitochondrial Unfolded Protein Response Engages Antimicrobial Peptides and Innate Immunity

Another major contributor to innate immunity in worms is the UPR$^{mt}$ pathway; recent studies have shown that *C. elegans* mutants defective in UPR$^{mt}$ components, such as the core regulator ATFS-1, are more likely to die during bacterial infections. Included in ATFS-1's repertoire of transcriptional targets are multiple innate immunity–related genes and AMPs (Pellegrino et al. 2014). These genes are induced during both mitochondrial dysfunction and bacterial infection (Pellegrino et al. 2014). Mutants in ATFS-1 were hypersensitive to *P. aeruginosa* infection, and worms expressing a constitutively activated mutant ATFS-1 showed resistance to infection (Pellegrino et al. 2014). Recently, *P. aeruginosa* was discovered to hijack an endogenous regulatory transcription factor called ZIP-3 that leads to repression of the UPR$^{mt}$ pathway (Deng et al. 2019). Worms mutant for *zip-3* were resistant to infection by wild-type *P. aeruginosa*, which was found to utilize phenazine-based toxins to disrupt the worms' mitochondrial health, signifying the existence of an evolutionary arms race between the pathogen and the worms' UPR$^{mt}$-signaling pathways (Deng et al. 2019). A novel layer of regulation was uncovered through studies on the SUMOylation of ATFS-1 and its partner DVE-1 (Gao et al. 2019). During low-stress states, the presence of this posttranslational modification restrains DVE-1 in the cytosol and ATFS-1 degradation;

however, upon UPR$^{mt}$ activation, the small ubiquitin-like modifier (SUMO) tags are removed through the activity of ULP-4, a SUMO protease, and ATFS-1 and DVE-1 are fully activated (Gao et al. 2019). The loss of ULP-4 impairs the UPR$^{mt}$ pathway and also makes the worms susceptible to bacterial infection (Gao et al. 2019). Together, these studies suggest that bacterial infection activates the UPR$^{mt}$ response in worms in a regulated fashion and that mitochondrial damage upregulates antimicrobial signaling pathways, analogous to what has been reported in fly PD models.

## 4. MITOCHONDRIAL DYSFUNCTION AND INFLAMMATION CONTRIBUTE TO DISEASE PATHOLOGY

### 4.1. Parkinson's Disease May Result from Unmitigated Mitochondrial Damage

PD is attributed both to defects in mitochondrial physiology and to the accumulation of protein aggregates (Lewy bodies), which result in degeneration of dopaminergic neurons in the substantia nigra (Kalia & Lang 2015). From clinical studies, the genes for Parkin, PINK1, and DJ-1 were implicated in autosomal recessive juvenile-onset PD (Bonifati et al. 2003, Kitada et al. 1998, Valente et al. 2004) (**Table 1**). Foundational studies in *D. melanogaster* established the products of these genes as being important for maintaining mitochondrial health, which led to the discovery of the roles of PINK1 and Parkin in initiating mitophagy (Youle 2019). However, patients with familial disease–linked mutations make up only a small percentage of all PD cases; the majority of PD cases are sporadic, with no known causative gene mutation (Kalia & Lang 2015). Patients with PD often display high levels of mtDNA mutations, which are attributed to the failure of the mitochondrial surveillance mechanisms and to accumulating oxidative damage (Giannoccaro et al. 2017). Deletion of mtDNA in mice with a mitochondria-targeted restriction enzyme, PstI, results in OXPHOS dysfunction and the onset of neurodegenerative disease symptoms (Pickrell et al. 2011). In addition to aging, the accumulation of mtDNA mutations might be a driving factor in the development of some sporadic PD; this has been modeled in rodents with a proofreading-deficient, *PolG*$^{mutator}$ transgene (Trifunovic et al. 2004). Additionally, the use of OXPHOS-inhibiting pesticides and industrial fertilizers is clinically linked to sporadic PD (Kalia & Lang 2015). Of the genetically inherited forms of PD, mutations in LRRK2 are the most common (Kalia & Lang 2015). LRRK2 has various functions in endosomal, autophagic, and lysosomal trafficking that are attributed to its kinase activity toward Rab proteins (Steger et al. 2016). The mechanisms through which mutations in LRRK2 lead to neurodegeneration remain unclear; however, the common mutant variant LRRK2$^{G2019S}$ causes trafficking defects, increased proinflammation signaling (Alessi & Sammler 2018), and impairment of PINK1- and Parkin-dependent mitophagy (Bonello et al. 2019).

### 4.2. Inflammation Is Implicated in Multiple Neurodegenerative Diseases

A critical role for inflammatory responses in neurodegeneration has become much more apparent in the past two decades, with a large body of research that is beyond the scope of this review. In this final section, we provide a brief overview of the state of this field. Many neurodegenerative diseases feature aggregating proteins; some examples of these are α-synuclein (SNCA)-induced Lewy bodies in certain types of PD, amyloid beta (Aβ) and tau fibrils in Alzheimer's disease (AD), Huntingtin (HTT) protein aggregates in Huntington's disease, and inclusion bodies containing TAR DNA-binding protein 43 (TDP43) observed in amyotrophic lateral sclerosis (ALS) (Hammond et al. 2019) (**Table 1**). Some of these protein aggregates are localized to the mitochondrial network and disrupt mitochondrial health, which activates oxidative stress pathways and potentially triggers an inflammatory response (Rodolfo et al. 2018). Increased

**Table 1 Mitochondrial quality control and disease**

| Disease | Genes/pathways | Summary | References |
|---|---|---|---|
| **Parkinson's disease** | *PRKN* (also called *Parkin* or *PARK2*)<br>*PINK1* (*PARK6*)<br>DJ-1 (*PARK7*) | Autosomal recessive mutations in *PRKN*, *PINK1*, and *PARK7* are linked to early-onset hereditary PD. | Kitada et al. 1998 (Parkin), Valente et al. 2004 (PINK1), Bonifati et al. 2003 (DJ-1) |
| | *LRRK2*<br>*SNCA* | The most commonly mutated gene in PD is *LRRK2*. SNCA protein aggregates form neurotoxic Lewy bodies. | Kalia & Lang 2015 |
| | *CHCHD2* | *CHCHD2* mutations are found in autosomal dominant late-onset hereditary PD. | Funayama et al. 2015 |
| **Alzheimer's disease** | Aβ, APP | Accumulation of Aβ and APP peptides leads to defects in mitochondrial dynamics and metabolism. | Rodolfo et al. 2018 |
| | Aβ, *tau*<br>Presenilin-1 (*PS-1*) | Aβ and phospho-tau induce mitochondrial fragmentation. *PS-1* mutations linked to early-onset AD induce mitophagy and mitochondrial mobility defects. | Hammond et al. 2019 |
| **Amyotrophic lateral sclerosis** | *SOD1*, *FUS*, *TDP-43*<br>*OPTN*<br>Ubiquitin–proteosome | Accumulation of mutant SOD1 or aggregates of FUS or TDP-43 in inclusion bodies impairs mitochondrial function and dynamics in motor neurons. Defects in autophagy or the ubiquitin–proteasome system reduce clearance of these aggregates. | Rodolfo et al. 2018, Hammond et al. 2019 |
| | *CHCHD10* | *CHCHD10* mutations are associated with mitochondrial dysfunction and ALS-related neurodegeneration. | Hammond et al. 2019 |
| **Huntington's disease** | *Huntingtin* (*HTT*) | Aggregating mutant HTT causes mitochondrial damage and defects in mitophagy. | Rodolfo et al. 2018 |
| | p62 (*SQSTM1*)<br>*ULK1* | HTT facilitates selective autophagy pathways and interacts with p62 and ULK1. | Hammond et al. 2019 |
| **Stroke/ischemia** | *PINK1/PRKN* | Stroke-induced injuries lead to a neuroprotective increase in Parkin-dependent mitophagy. | Shi et al. 2018 |
| **Myocardial infarction and heart failure** | *PINK1/PRKN*<br>*BNIP3/NIX*<br>*ATG* genes | Defects in mitochondrial dynamics and mitophagy (both PINK1/Parkin–dependent and BNIP3/NIX pathways) are implicated in cardiac myopathy. | Shi et al. 2018 |
| **Cancer** | *BCL-2*, *BAX*<br>*BNIP3*<br>*PINK1/PRKN* | Antiapoptotic oncogenes promote cancer cell survival and tumor progression. Elevated mitophagy is observed in multiple cancers. | Shi et al. 2018 |

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; HTT, Huntingtin; PD, Parkinson's disease; PINK1, PTEN-induced putative kinase protein 1; PS-1, presenilin-1.

inflammation is a clinical signature of these diseases. However, much work remains to be done on uncovering the benefits and costs of these cytokine-signaling pathways in each disease; rodent studies have demonstrated that, in the case of PD models, suppressing inflammatory pathways is beneficial (Frank-Cannon et al. 2008, Matheoud et al. 2019, Sliter et al. 2018). For many neuroinflammatory disorders, it is controversial whether suppressing inflammation impacts disease progression (Hammond et al. 2019); however, meta-analysis has shown that the usage of nonsteroidal anti-inflammatory drugs is negatively correlated with PD (Noyce et al. 2012). In AD research, boosting the innate immune response has been shown to decrease amyloid fibrils, but inhibiting immune pathways results in fewer tau aggregates and better cognitive performance in mouse models, further complicating the question of whether this inflammation is harmful or beneficial (Van Eldik et al. 2016). Regardless of current limitations, the study of inflammation in neurodegenerative diseases is an exciting field, with the ultimate goal of helping to discover cures to the most debilitating and costly diseases in the aging population.

## SUMMARY POINTS

1. The failure to maintain mitochondrial network health is implicated in neurodegenerative disorders, cancer, and cardiac disease.
2. Multiple pathways of mitochondrial quality control exist to sense and remove damaged mitochondria; these include mitophagy, piecemeal degradation, mitochondrial fission, mitochondrial proteostasis pathways, and the mitochondrial unfolded protein response ($UPR^{mt}$).
3. Disruptions in mitochondrial quality control invoke innate immune responses due to the similarity of mitochondrial damage-associated molecular patterns (DAMPs) to pathogen-associated molecular patterns (PAMPs).
4. Release of mitochondrial DNA (mtDNA) to the cytosol triggers the AIM2 and NLRP3 inflammasomes as well as a cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING)–mediated type 1 interferon response.
5. Recent rodent studies of Parkinson's disease reveal the importance of cGAS/STING–mediated cytokine signaling in dopaminergic neuron loss in *PARK*$^{-/-}$; *PolG*$^{mutator}$ mice and a novel role for the gut microbiome in the onset of Parkinsonian symptoms in PINK1-deficient mice.
6. *Drosophila melanogaster* and *Caenorhabditis elegans* are powerful model systems for studying mitochondrial diseases and immunity.

## FUTURE ISSUES

1. The detailed mechanisms and roles for PINK1/Parkin–dependent mitophagy and Parkin-independent mitophagy pathways in vivo require further delineation.
2. Mitochondrial dysfunction and inflammation cause specific motor neuron defects in Parkinson's disease, but why dopaminergic neurons are selectively affected remains unclear.

3. What are the mechanisms underlying sporadic (nonhereditary) neurodegenerative diseases, and are there as-yet-unknown risk factors, either genetic or lifestyle derived, that can be identified?
4. Targeting inflammatory pathways is an exciting therapeutic strategy for treating mitochondrial disorders, yet more basic and applied research is needed to determine the costs and benefits of inhibiting inflammation.
5. Artificially increasing the targeting of protein aggregates to the autophagy pathway and increasing mitophagy via pharmaceutical intervention are promising future avenues of treatment for mitochondrial disorders and neurodegenerative diseases.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

## ACKNOWLEDGMENTS

We wish to thank members of the Youle lab for feedback on content and assistance with editing, particularly Tara Fischer for suggestions. We also recognize the large body of work that has been performed in these fields, and we recognize that some work may have been omitted due to space limitations. This work is supported by the National Institute of Neurological Disorders and Stroke (NINDS) Intramural Research Program (to R.J.Y.).

## LITERATURE CITED

Ablasser A, Chen ZJ. 2019. cGAS in action: expanding roles in immunity and inflammation. *Science* 363(6431):eaat8657

Ablasser A, Schmid-Burgk JL, Hemmerling I, Horvath GL, Schmidt T, et al. 2013. Cell intrinsic immunity spreads to bystander cells via the intercellular transfer of cGAMP. *Nature* 503(7477):530–34

Agaisse H, Perrimon N. 2004. The roles of JAK/STAT signaling in *Drosophila* immune responses. *Immunol. Rev.* 198:72–82

Alessi DR, Sammler E. 2018. LRRK2 kinase in Parkinson's disease. *Science* 360(6384):36–37

Allen GFG, Toth R, James J, Ganley IG. 2013. Loss of iron triggers PINK1/Parkin-independent mitophagy. *EMBO Rep.* 14(12):1127–35

Andreazza S, Samstag CL, Sanchez-Martinez A, Fernandez-Vizarra E, Gomez-Duran A, et al. 2019. Mitochondrially-targeted APOBEC1 is a potent mtDNA mutator affecting mitochondrial function and organismal fitness in *Drosophila*. *Nat. Commun.* 10:3280

Bobela W, Zheng L, Schneider BL. 2014. Overview of mouse models of Parkinson's disease. *Curr. Protoc. Mouse Biol.* 4(3):121–39

Bonello F, Hassoun SM, Mouton-Liger F, Shin YS, Muscat A, et al. 2019. LRRK2 impairs PINK1/Parkin-dependent mitophagy via its kinase activity: pathologic insights into Parkinson's disease. *Hum. Mol. Genet.* 28(10):1645–60

Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, et al. 2003. Mutations in the *DJ-1* gene associated with autosomal recessive early-onset parkinsonism. *Science* 299(5604):256–59

Boyer PD, Chance B, Ernster L, Mitchell P, Racker E, Slater EC. 1977. Oxidative phosphorylation and photophosphorylation. *Annu. Rev. Biochem.* 46:955–66

Bratic A, Kauppila TES, Macao B, Grönke S, Siibak T, et al. 2015. Complementation between polymerase- and exonuclease-deficient mitochondrial DNA polymerase mutants in genomically engineered flies. *Nat. Commun.* 6:8808

Brokatzky D, Dörflinger B, Haimovici A, Weber A, Kirschnek S, et al. 2019. A non-death function of the mitochondrial apoptosis apparatus in immunity. *EMBO J.* 38(11):e2018100907

Buchon N, Silverman N, Cherry S. 2014. Immunity in *Drosophila melanogaster*—from microbial recognition to whole-organism physiology. *Nat. Rev. Immunol.* 14(12):796–810

Burman JL, Pickles S, Wang C, Sekine S, Vargas JNS, et al. 2017. Mitochondrial fission facilitates the selective mitophagy of protein aggregates. *J. Cell Biol.* 216(10):3231–47

Carneiro LAM, Travassos LH, Girardin SE. 2007. Nod-like receptors in innate immunity and inflammatory diseases. *Ann. Med.* 39(8):581–93

Carp H. 1982. Mitochondrial *N*-formylmethionyl proteins as chemoattractants for neutrophils. *J. Exp. Med.* 155(1):264–75

Castanier C, Garcin D, Vazquez A, Arnoult D. 2010. Mitochondrial dynamics regulate the RIG-I-like receptor antiviral pathway. *EMBO Rep.* 11(2):133–38

Cha GH, Kim S, Park J, Lee E, Kim M, et al. 2005. Parkin negatively regulates JNK pathway in the dopaminergic neurons of *Drosophila*. *PNAS* 102(29):10345–50

Cho JH, Park JH, Chung CG, Shim H-J, Jeon KH, et al. 2015. Parkin-mediated responses against infection and wound involve TSPO-VDAC complex in *Drosophila*. *Biochem. Biophys. Res. Commun.* 463(1–2):1–6

**Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, et al. 2006. *Drosophila pink1* is required for mitochondrial function and interacts genetically with *parkin*. *Nature* 441(7097):1162–66**

**Established that *pink1* functions epistatically with *parkin*; published concurrently with Park et al. (2006).**

Cornelissen T, Vilain S, Vints K, Gounko N, Verstreken P, Vandenberghe W. 2018. Deficiency of *parkin* and *pink1* impairs age-dependent mitophagy in *Drosophila*. *eLife* 7:e35878

Deng H, Dodson MW, Huang H, Guo M. 2008. The Parkinson's disease genes *pink1* and *parkin* promote mitochondrial fission and/or inhibit fusion in *Drosophila*. *PNAS* 105(38):14503–8

Deng P, Naresh NU, Du Y, Lamech LT, Yu J, et al. 2019. Mitochondrial UPR repression during *Pseudomonas aeruginosa* infection requires the bZIP protein ZIP-3. *PNAS* 116(13):6146–51

Dhir A, Dhir S, Borowski LS, Jimenez L, Teitell M, et al. 2018. Mitochondrial double-stranded RNA triggers antiviral signaling in humans. *Nature* 560(7717):238–42

Dolezal P, Likic V, Tachezy J, Lithgow T. 2006. Evolution of the molecular machines for protein import into mitochondria. *Science* 313(5785):314–18

Dyall SD, Brown MT, Johnson PJ. 2004. Ancient invasions: from endosymbionts to organelles. *Science* 304(5668):253–57

Dzamko N, Geczy CL, Halliday GM. 2015. Inflammation is genetically implicated in Parkinson's disease. *Neuroscience* 302:89–102

Fiorese CJ, Schulz AM, Lin YF, Rosin N, Pellegrino MW, Haynes CM. 2016. The transcription factor ATF5 mediates a mammalian mitochondrial UPR. *Curr. Biol.* 26(15):2037–43

Frank-Cannon TC, Tran T, Ruhn KA, Martinez TN, Hong J, et al. 2008. Parkin deficiency increases vulnerability to inflammation-related nigral degeneration. *J. Neurosci.* 28(43):10825–34

Friedman JR, Lackner LL, West M, DiBenedetto JR, Nunnari J, Voeltz GK. 2011. ER tubules mark sites of mitochondrial division. *Science* 334(6054):358–62

Funayama M, Ohe K, Amo T, et al. 2015. *CHCHD2* mutations in autosomal dominant late-onset Parkinson's disease: a genome-wide linkage and sequencing study. *Lancet Neurol.* 14(3):274–82

Gao K, Li Y, Hu S, Liu Y. 2019. SUMO peptidase ULP-4 regulates mitochondrial UPR-mediated innate immunity and lifespan extension. *eLife* 8:e41792

Giannoccaro MP, La Morgia C, Rizzo G, Carelli V. 2017. Mitochondrial DNA and primary mitochondrial dysfunction in Parkinson's disease. *Mov. Disord.* 32(3):346–63

Glauser L, Sonnay S, Stafa K, Moore DJ. 2011. Parkin promotes the ubiquitination and degradation of the mitochondrial fusion factor Mitofusin1. *J. Neurochem.* 118(4):636–45

Goto A, Okado K, Martins N, Cai H, Barbier V, et al. 2018. The kinase IKKβ regulates a STING- and NF-κB-dependent antiviral response pathway in *Drosophila*. *Immunity* 49(2):225–34.e4

Gottlieb E, Armour SM, Harris MH, Thompson CB. 2003. Mitochondrial membrane potential regulates matrix configuration and cytochrome *c* release during apoptosis. *Cell Death Differ.* 10(6):709–17

Greene JC, Whitworth AJ, Andrews LA, Parker TJ, Pallanck LJ. 2005. Genetic and genomic studies of *Drosophila parkin* mutants implicate oxidative stress and innate immune responses in pathogenesis. *Hum. Mol. Genet.* 14(6):799–811

**First description of fly *parkin* mutants, establishing a role for Parkin in mitochondrial homeostasis.**

**Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ. 2003. Mitochondrial pathology and apoptotic muscle degeneration in *Drosophila parkin* mutants. *PNAS* 100(7):4078–83**

Gui X, Yang H, Li T, Tan X, Shi P, et al. 2019. Autophagy induction via STING trafficking is a primordial function of the cGAS pathway. *Nature* 567(7747):262–66

Hammond TR, Marsh SE, Stevens B. 2019. Immune signaling in neurodegeneration. *Immunity* 50(4):955–74

Hatefi Y. 1985. The mitochondrial electron transport and oxidative phosphorylation system. *Annu. Rev. Biochem.* 54:1015–69

He Y, Hara H, Núñez G. 2016. Mechanism and regulation of NLRP3 inflammasome activation. *Trends Biochem. Sci.* 41(12):1012–21

Heo JM, Ordureau A, Paulo JA, Rinehart J, Harper JW. 2015. The PINK1-PARKIN mitochondrial ubiquitylation pathway drives a program of OPTN/NDP52 recruitment and TBK1 activation to promote mitophagy. *Mol. Cell* 60(1):7–20

Hill JH, Chen Z, Xu H. 2014. Selective propagation of functional mitochondrial DNA during oogenesis restricts the transmission of a deleterious mitochondrial variant. *Nat. Genet.* 46(4):389–92

Hoppins S, Lackner L, Nunnari J. 2007. The machines that divide and fuse mitochondria. *Annu. Rev. Biochem.* 76:751–80

Hsu YT, Wolter KG, Youle RJ. 1997. Cytosol-to-membrane redistribution of Bax and Bcl-$X_L$ during apoptosis. *PNAS* 94(8):3668–72

Hubert V, Peschel A, Langer B, Gröger M, Rees A, Kain R. 2016. LAMP-2 is required for incorporating syntaxin-17 into autophagosomes and for their fusion with lysosomes. *Biol. Open* 5(10):1516–29

Hughes AL, Hughes CE, Henderson KA, Yazvenko N, Gottschling DE. 2016. Selective sorting and destruction of mitochondrial membrane proteins in aged yeast. *eLife* 5:e13943

Ishikawa H, Ma Z, Barber GN. 2009. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature* 461(7265):788–92

Issa A-R, Sun J, Petitgas C, Mesquita A, Dulac A, et al. 2018. The lysosomal membrane protein LAMP2A promotes autophagic flux and prevents SNCA-induced Parkinson disease-like symptoms in the *Drosophila* brain. *Autophagy* 14(11):1898–910

Iyer SS, He Q, Janczy JR, Elliott EI, Zhong Z, et al. 2013. Mitochondrial cardiolipin is required for Nlrp3 inflammasome activation. *Immunity* 39(2):311–23

Jin SM, Youle RJ. 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(11):1750–57

Johansen T, Lamark T. 2011. Selective autophagy mediated by autophagic adapter proteins. *Autophagy* 7(3):279–96

Kalia LV, Lang AE. 2015. Parkinson's disease. *Lancet* 386(9996):896–912

Kane LA, Lazarou M, Fogel AI, Li Y, Yamano K, et al. 2014. PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J. Cell Biol.* 205(2):143–53

Kanneganti TD, Lamkanfi M, Núñez G. 2007. Intracellular NOD-like receptors in host defense and disease. *Immunity* 27(4):549–59

Karbowski M, Youle RJ. 2011. Regulating mitochondrial outer membrane proteins by ubiquitination and proteasomal degradation. *Curr. Opin. Cell Biol.* 23(4):476–82

Katayama H, Hama H, Nagasawa K, Kurokawa H, Sugiyama M, et al. 2020. Visualizing and modulating mitophagy for therapeutic studies of neurodegeneration. *Cell* 181(5):1176–87.e16

Katayama H, Kogure T, Mizushima N, Yoshimori T, Miyawaki A. 2011. A sensitive and quantitative technique for detecting autophagic events based on lysosomal delivery. *Chem. Biol.* 18(8):1042–52

Kauppila TES, Bratic A, Jensen MB, Baggio F, Partridge L, et al. 2018. Mutations of mitochondrial DNA are not major contributors to aging of fruit flies. *PNAS* 115(41):E9620–29

Kazlauskaite A, Kondapalli C, Gourlay R, Campbell DG, Ritorto MS, et al. 2014. Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at $Ser^{65}$. *Biochem. J.* 460(1):127–41

Khan NA, Nikkanen J, Yatsuga S, Jackson C, Wang L, et al. 2017. mTORC1 regulates mitochondrial integrated stress response and mitochondrial myopathy progression. *Cell Metab.* 26(2):419–28.e5

Kim J, Gupta R, Blanco LP, Yang S, Shteinfer-Kuzmine A, et al. 2019. VDAC oligomers form mitochondrial pores to release mtDNA fragments and promote lupus-like disease. *Science* 366(6472):1531–36

Kim YY, Um J-H, Yoon J-H, Kim H, Lee D-Y, et al. 2019. Assessment of mitophagy in mt-Keima *Drosophila* revealed an essential role of the PINK1-Parkin pathway in mitophagy induction *in vivo*. *FASEB J.* 33(9):9742–51

Kirienko NV, Ausubel FM, Ruvkun G. 2015. Mitophagy confers resistance to siderophore-mediated killing by *Pseudomonas aeruginosa*. *PNAS* 112(6):1821–26

**Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, et al. 1998. Mutations in the *parkin* gene cause autosomal recessive juvenile parkinsonism. *Nature* 392(6676):605–8**

**First report of *parkin* mutations in early-onset recessive Parkinson's disease.**

Kitada T, Tong Y, Gautier CA, Shen J. 2009. Absence of nigral degeneration in aged parkin/DJ-1/PINK1 triple knockout mice. *J. Neurochem.* 111(3):696–702

Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD. 1997. The release of cytochrome c from mitochondria: a primary site for Bcl- 2 regulation of apoptosis. *Science* 275(5303):1132–36

Koehler CL, Perkins GA, Ellisman MH, Jones DL. 2017. Pink1 and Parkin regulate *Drosophila* intestinal stem cell proliferation during stress and aging. *J. Cell Biol.* 216(8):2315–27

Koppen M, Langer T. 2007. Protein degradation within mitochondria: versatile activities of AAA proteases and other peptidases. *Crit. Rev. Biochem. Mol. Biol.* 42(3):221–42

Koyano F, Okatsu K, Kosako H, Tamura Y, Go E, et al. 2014. Ubiquitin is phosphorylated by PINK1 to activate parkin. *Nature* 510(7503):162–66

Koyano F, Yamano K, Kosako H, Tanaka K, Matsuda N. 2019. Parkin recruitment to impaired mitochondria for nonselective ubiquitylation is facilitated by MITOL. *J. Biol. Chem.* 294(26):10300–14

Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, et al. 2015. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature* 524(7565):309–14

Le Guerroué F, Eck F, Jung J, Starzetz T, Mittelbronn M, et al. 2017. Autophagosomal content profiling reveals an LC3C-dependent piecemeal mitophagy pathway. *Mol. Cell* 68(4):786–96.e6

Lee JJ, Sanchez-Martinez A, Zarate AM, Benincá C, Mayor U, et al. 2018. Basal mitophagy is widespread in *Drosophila* but minimally affected by loss of Pink1 or Parkin. *J. Cell Biol.* 217(5):1613–22

Lemasters JJ. 2005. Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. *Rejuvenation Res.* 8(1):3–5

Lemasters JJ, Nieminen A-L, Qian T, Trost LC, Elmore SP, et al. 1998. The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. *Biochim. Biophys. Acta Bioenerg.* 1366(1–2):177–96

Lieber T, Jeedigunta SP, Palozzi JM, Lehmann R, Hurd TR. 2019. Mitochondrial fragmentation drives selective removal of deleterious mtDNA in the germline. *Nature* 570(7761):380–84

Liston A, Masters SL. 2017. Homeostasis-altering molecular processes as mechanisms of inflammasome activation. *Nat. Rev. Immunol.* 17(3):208–14

Liu Y, Gordesky-Gold B, Leney-Greene M, Weinbren NL, Tudor M, Cherry S. 2018. Inflammation-induced, STING-dependent autophagy restricts Zika virus infection in the *Drosophila* brain. *Cell Host Microbe* 24(1):57–68.e3

Liu Y, Olagnier D, Lin R. 2017. Host and viral modulation of RIG-I-mediated antiviral immunity. *Front. Immunol.* 7:663

Lugrin J, Martinon F. 2018. The AIM2 inflammasome: sensor of pathogens and cellular perturbations. *Immunol. Rev.* 281(1):99–114

Luteijn RD, Zaver SA, Gowen BG, Wyman SK, Garelis NE, et al. 2019. SLC19A1 transports immunoreactive cyclic dinucleotides. *Nature* 573(7774):434–38

Ma H, Xu H, O'Farrell PH. 2014. Transmission of mitochondrial mutations and action of purifying selection in *Drosophila melanogaster*. *Nat. Genet.* 46(4):393–97

Ma P, Yun J, Deng H, Guo M. 2018. Atg1-mediated autophagy suppresses tissue degeneration in *pink1/parkin* mutants by promoting mitochondrial fission in *Drosophila*. *Mol. Biol. Cell* 29(26):3082–92

Manor U, Bartholomew S, Golani G, Christenson E, Kozlov M, Higgs H, Spudich J, Lippincott-Schwartz J. 2015. A mitochondria-anchored isoform of the actin-nucleating spire protein regulates mitochondrial division. *eLife* 4:e08828

Martin M, Hiroyasu A, Guzman RM, Roberts SA, Goodman AG. 2018. Analysis of *Drosophila* STING reveals an evolutionarily conserved antimicrobial function. *Cell Rep.* 23(12):3537–50.e6

**Matheoud D, Cannon T, Voisin A, Penttinen A-M, Ramet L, et al. 2019. Intestinal infection triggers Parkinson's disease-like symptoms in *Pink1*$^{-/-}$ mice. *Nature* 571(7766):565–69**

Reported that *PINK1*$^{-/-}$ mice are sensitive to gut infection, leading to development of parkinsonian pathology.

Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, et al. 2010. PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. *J. Cell Biol.* 189(2):211–21

McArthur K, Whitehead LW, Heddleston JM, Li L, Padman BS, et al. 2018. BAK/BAX macropores facilitate mitochondrial herniation and mtDNA efflux during apoptosis. *Science* 359(6378):eaao6047

McPhee CK, Baehrecke EH. 2009. Autophagy in *Drosophila melanogaster*. *Biochim. Biophys. Acta Mol. Cell Res.* 1793(9):1452–60

McWilliams TG, Prescott AR, Allen GFG, Tamjar J, Munson MJ, et al. 2016. *mito*-QC illuminates mitophagy and mitochondrial architecture in vivo. *J. Cell Biol.* 214(3):333–45

McWilliams TG, Prescott AR, Montava-Garriga L, Ball G, Singh F, et al. 2018. Basal mitophagy occurs independently of PINK1 in mouse tissues of high metabolic demand. *Cell Metab.* 27(2):439–49.e5

Melber A, Haynes CM. 2018. UPR$^{mt}$ regulation and output: a stress response mediated by mitochondrial-nuclear communication. *Cell Res.* 28(3):281–95

Mishra P, Chan DC. 2016. Metabolic regulation of mitochondrial dynamics. *J. Cell Biol.* 212(4):379–87

Morais VA, Haddad D, Craessaerts K, De Bock P-J, Swerts J, et al. 2014. PINK1 loss-of-function mutations affect mitochondrial complex I activity via NdufA10 ubiquinone uncoupling. *Science* 344(6180):203–7

Morais VA, Verstreken P, Roethig A, Smet J, Snellinx A, et al. 2009. Parkinson's disease mutations in PINK1 result in decreased Complex I activity and deficient synaptic function. *EMBO Mol. Med.* 1(2):99–111

Moreno E, Yan M, Basler K. 2002. Evolution of TNF signaling mechanisms: JNK-dependent apoptosis triggered by Eiger, the *Drosophila* homolog of the TNF superfamily. *Curr. Biol.* 12(14):1263–68

Murley A, Lackner LL, Osman C, West M, Voeltz GK, Walter P, Nunnari J. 2013. ER-associated mitochondrial division links the distribution of mitochondria and mitochondrial DNA in yeast. *eLife* 2:e00422

Narendra DP, Jin SM, Tanaka A, Suen D-F, Gautier CA, et al. 2010a. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLOS Biol.* 8(1):e1000298

Narendra DP, Kane LA, Hauser DN, Fearnley IM, Youle RJ. 2010b. p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. *Autophagy* 6(8):1090–106

**Narendra D, Tanaka A, Suen D-F, Youle RJ. 2008. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J. Cell Biol.* 183(5):795–803**

Demonstrated the role of Parkin in promoting mitophagy of depolarized mitochondria.

Nargund AM, Pellegrino MW, Fiorese CJ, Baker BM, Haynes CM. 2012. Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. *Science* 337(6094):587–90

Neupert W, Brunner M. 2002. The protein import motor of mitochondria. *Nat. Rev. Mol. Cell Biol.* 3(8):555–65

Nomura J, So A, Tamura M, Busso N. 2015. Intracellular ATP decrease mediates NLRP3 inflammasome activation upon nigericin and crystal stimulation. *J. Immunol.* 195(12):5718–24

Novak I, Kirkin V, McEwan DG, Zhang J, Wild P, et al. 2010. Nix is a selective autophagy receptor for mitochondrial clearance. *EMBO Rep.* 11(1):45–51

Noyce AJ, Bestwick JP, Silveira-Moriyama L, Hawkes CH, Giovannoni G, et al. 2012. Meta-analysis of early nonmotor features and risk factors for Parkinson disease. *Ann. Neurol.* 72(6):893–901

Nunnari J, Suomalainen A. 2012. Mitochondria: in sickness and in health. *Cell* 148(6):1145–59

Oka T, Hikoso S, Yamaguchi O, Taneike M, Takeda T, et al. 2012. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. *Nature* 485(7397):251–55

Okatsu K, Oka T, Iguchi M, Imamura K, Kosako H, et al. 2012. PINK1 autophosphorylation upon membrane potential dissipation is essential for Parkin recruitment to damaged mitochondria. *Nat. Commun.* 3:1016

Otera H, Wang C, Cleland MM, Setoguchi K, Yokota S, et al. 2010. Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells. *J. Cell Biol.* 191(6):1141–58

Palikaras K, Lionaki E, Tavernarakis N. 2015. Coordination of mitophagy and mitochondrial biogenesis during ageing in *C. elegans*. *Nature* 521(7553):525–28

**Park J, Lee SB, Lee S, Kim Y, Song S, et al. 2006. Mitochondrial dysfunction in *Drosophila PINK1* mutants is complemented by *parkin*. *Nature* 441(7097):1157–61**

Established that *pink1* functions epistatically with *parkin*; published concurrently with Clark et al. (2006).

Pellegrino MW, Nargund AM, Kirienko NV, Gillis R, Fiorese CJ, Haynes CM. 2014. Mitochondrial UPR-regulated innate immunity provides resistance to pathogen infection. *Nature* 516(7531):414–17

Pickrell AM, Fukui H, Wang X, Pinto M, Moraes CT. 2011. The striatum is highly susceptible to mitochondrial oxidative phosphorylation dysfunctions. *J. Neurosci.* 31(27):9895–904

Pogson JH, Ivatt RM, Sanchez-Martinez A, Tufi R, Wilson E, et al. 2014. The complex I subunit *NDUFA10* selectively rescues *Drosophila pink1* mutants through a mechanism independent of mitophagy. *PLOS Genet.* 10(11):e1004815

Poltorak A, He X, Smirnova I, Liu M-Y, Van Huffel C, et al. 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* 282(5396):2085–88

Poole AC, Thomas RE, Yu S, Vincow ES, Pallanck L. 2010. The mitochondrial fusion-promoting factor Mitofusin is a substrate of the PINK1/Parkin pathway. *PLOS ONE* 5(4):e10054

Puri R, Cheng X-T, Lin M-Y, Huang N, Sheng Z-H. 2019. Mul1 restrains Parkin-mediated mitophagy in mature neurons by maintaining ER-mitochondrial contacts. *Nat. Commun.* 10:3645

Quirós PM, Prado MA, Zamboni N, D'Amico D, Williams RW, et al. 2017. Multi-omics analysis identifies ATF4 as a key regulator of the mitochondrial stress response in mammals. *J. Cell Biol.* 216(7):2027–45

Raetz CRH, Whitfield C. 2002. Lipopolysaccharide endotoxins. *Annu. Rev. Biochem.* 71:635–700

Reczek CR, Chandel NS. 2015. ROS-dependent signal transduction. *Curr. Opin. Cell Biol.* 33:8–13

Rikka S, Quinsay MN, Thomas RL, Kubli DA, Zhang X, et al. 2011. Bnip3 impairs mitochondrial bioenergetics and stimulates mitochondrial turnover. *Cell Death Differ.* 18(4):721–31

Riparbelli MG, Callaini G. 2007. The *Drosophila parkin* homologue is required for normal mitochondrial dynamics during spermiogenesis. *Dev. Biol.* 303(1):108–20

Ritchie C, Cordova AF, Hess GT, Bassik MC, Li L. 2019. SLC19A1 is an importer of the immunotransmitter cGAMP. *Mol. Cell* 75(2):372–81.e5

Rodolfo C, Campello S, Cecconi F. 2018. Mitophagy in neurodegenerative diseases. *Neurochem. Int.* 117:156–66

Samstag CL, Hoekstra JG, Huang C-H, Chaisson MJ, Youle RJ, et al. 2018. Deleterious mitochondrial DNA point mutations are overrepresented in *Drosophila* expressing a proofreading-defective DNA polymerase γ. *PLOS Genet.* 14(11):e1007805

Sánchez-Aparicio MT, Ayllón J, Leo-Macias A, Wolff T, García-Sastre A. 2017. Subcellular localizations of RIG-I, TRIM25, and MAVS complexes. *J. Virol.* 91(2):e01155-16

Sarraf SA, Raman M, Guarani-Pereira V, Sowa ME, Huttlin EL, et al. 2013. Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. *Nature* 496(7445):372–76

Schiavi A, Maglioni S, Palikaras K, Shaik A, Strappazzon F, et al. 2015. Iron-starvation-induced mitophagy mediates lifespan extension upon mitochondrial stress in *C. elegans*. *Curr. Biol.* 25(14):1810–22

Schindler C, Levy DE, Decker T. 2007. JAK-STAT signaling: from interferons to cytokines. *J. Biol. Chem.* 282(28):20059–63

Seth RB, Sun L, Ea CK, Chen ZJ. 2005. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-κB and IRF3. *Cell* 122(5):669–82

Shi R, Guberman M, Kirshenbaum LA. 2018. Mitochondrial quality control: the role of mitophagy in aging. *Trends Cardiovasc. Med.* 28(4):246–60

Shimada K, Crother TR, Karlin J, Dagvadorj J, Chiba N, et al. 2012. Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity* 36(3):401–14

Shukla AK, Spurrier J, Kuzina I, Giniger E. 2019. Hyperactive innate immunity causes degeneration of dopamine neurons upon altering activity of Cdk5. *Cell Rep.* 26(1):131–44.e4

**Sliter DA, Martinez J, Hao L, Chen X, Sun N, et al. 2018. Parkin and PINK1 mitigate STING-induced inflammation. *Nature* 561(7722):258–62**

**Showed that PINK1/Parkin suppresses STING-dependent inflammation; loss of STING alleviated neurodegeneration in Parkin-knockout, *PolG*$^{mutator}$ mice.**

Steger M, Tonelli F, Ito G, Davies P, Trost M, et al. 2016. Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases. *eLife* 5:e12813

Sun L, Wu J, Du F, Chen X, Chen ZJ. 2013. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science* 339(6121):786–91

Sun N, Yun J, Liu J, Malide D, Liu C, et al. 2015. Measuring in vivo mitophagy. *Mol. Cell* 60(4):685–96

Sun Y, Vashisht AA, Tchieu J, Wohlschlegel JA, Dreier L. 2012. Voltage-dependent anion channels (VDACs) recruit Parkin to defective mitochondria to promote mitochondrial autophagy. *J. Biol. Chem.* 287(48):40652–60

Tait SWG, Green DR. 2013. Mitochondrial regulation of cell death. *Cold Spring Harb. Perspect. Biol.* 5(9):a008706

Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, et al. 1999. Differential roles of TLR2 and TLR4 in recognition of Gram-negative and Gram-positive bacterial cell wall components. *Immunity* 11(4):443–51

Tanaka A, Cleland MM, Xu S, Narendra DP, Suen D-F, et al. 2010. Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. *J. Cell Biol.* 191(7):1367–80

Tieu Q, Okreglak V, Naylor K, Nunnari J. 2002. The WD repeat protein, Mdv1p, functions as a molecular adaptor by interacting with Dnm1p and Fis1p during mitochondrial fission. *J. Cell Biol.* 158(3):445–52

Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, et al. 2004. Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* 429(6990):417–23

First report linking *PINK1* mutations with early-onset recessive Parkinson's disease.

**Valente EM, Abou-Sleiman PM, Caputo V, Muqit MMK, Harvey K, et al. 2004. Hereditary early-onset Parkinson's disease caused by mutations in *PINK1*. *Science* 304(5674):1158–60**

Van Eldik LJ, Carrillo MC, Cole PE, Feuerbach D, Greenberg BD, et al. 2016. The roles of inflammation and immune mechanisms in Alzheimer's disease. *Alzheimer's Dement. Transl. Res. Clin. Interv.* 2(2):99–109

Vincow ES, Merrihew G, Thomas RE, Shulman NJ, Beyer RP, et al. 2013. The PINK1-Parkin pathway promotes both mitophagy and selective respiratory chain turnover in vivo. *PNAS* 110(16):6400–5

Voigt A, Berlemann LA, Winklhofer KF. 2016. The mitochondrial kinase PINK1: functions beyond mitophagy. *J. Neurochem.* 139(S1):232–39

Walter P, Ron D. 2011. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 334(6059):1081–86

Wang C. 2020. A sensitive and quantitative mKeima assay for mitophagy via FACS. *Curr. Protoc. Cell Biol.* 86(1):e99

Wang X, Winter D, Ashrafi G, Schlehe J, Wong YL, et al. 2011. PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. *Cell* 147(4):893–906

Weidberg H, Amon A. 2018. MitoCPR—a surveillance pathway that protects mitochondria in response to protein import stress. *Science* 360(6385):eaan4146

Weinberg SE, Sena LA, Chandel NS. 2015. Mitochondria in the regulation of innate and adaptive immunity. *Immunity* 42(3):406–17

Established that mitochondrial DNA can activate DNA-sensing innate antiviral pathways.

**West AP, Khoury-Hanold W, Staron M, Tal MC, Pineda CM, et al. 2015. Mitochondrial DNA stress primes the antiviral innate immune response. *Nature* 520(7548):553–57**

West AP, Shadel GS. 2017. Mitochondrial DNA in innate immune responses and inflammatory pathology. *Nat. Rev. Immunol.* 17(6):363–75

Whitworth AJ, Pallanck LJ. 2017. PINK1/Parkin mitophagy and neurodegeneration—what do we really know *in vivo*? *Curr. Opin. Genet. Dev.* 44:47–53

Whitworth AJ, Theodore DA, Greene JC, Beneš H, Wes PD, Pallanck LJ. 2005. Increased glutathione *S*-transferase activity rescues dopaminergic neuron loss in a *Drosophila* model of Parkinson's disease. *PNAS* 102(22):8024–29

Wiedemann N, Pfanner N. 2017. Mitochondrial machineries for protein import and assembly. *Annu. Rev. Biochem.* 86:685–714

Wong YC, Holzbaur ELF. 2014. Optineurin is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation. *PNAS* 111(42):E4439–48

Wrobel L, Topf U, Bragoszewski P, Wiese S, Sztolsztener ME, et al. 2015. Mistargeted mitochondrial proteins activate a proteostatic response in the cytosol. *Nature* 524(7566):485–88

Yamano K, Youle RJ. 2013. PINK1 is degraded through the N-end rule pathway. *Autophagy* 9(11):1758–69

Yamashita SI, Jin X, Furukawa K, Hamasaki M, Nezu A, et al. 2016. Mitochondrial division occurs concurrently with autophagosome formation but independently of Drp1 during mitophagy. *J. Cell Biol.* 215(5):649–65

Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, et al. 1997. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science* 275(5303):1129–32

Yaron JR, Gangaraju S, Rao MY, Kong X, Zhang L, et al. 2015. $K^+$ regulates $Ca^{2+}$ to drive inflammasome signaling: dynamic visualization of ion flux in live cells. *Cell Death Dis.* 6(10):e1954

Yoneda M, Miyatake T, Attardi G. 1994. Complementation of mutant and wild-type human mitochondrial DNAs coexisting since the mutation event and lack of complementation of DNAs introduced separately into a cell within distinct organelles. *Mol. Cell Biol.* 14(4):2699–712

Yoshii SR, Kishi C, Ishihara N, Mizushima N. 2011. Parkin mediates proteasome-dependent protein degradation and rupture of the outer mitochondrial membrane. *J. Biol. Chem.* 286(22):19630–40

Yoshii SR, Mizushima N. 2015. Autophagy machinery in the context of mammalian mitophagy. *Biochim. Biophys. Acta Mol. Cell Res.* 1853(10):2797–801

Youle RJ. 2019. Mitochondria—striking a balance between host and endosymbiont. *Science* 365(6454):9855

Yun J, Puri R, Yang H, Lizzio MA, Wu C, et al. 2014. MUL1 acts in parallel to the PINK1/parkin pathway in regulating mitofusin and compensates for loss of PINK1/parkin. *eLife* 3:e01958

Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, et al. 2010. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* 464(7285):104–7

Zhang Y, Wang ZH, Liu Y, Chen Y, Sun N, et al. 2019. PINK1 inhibits local protein synthesis to limit transmission of deleterious mitochondrial DNA mutations. *Mol. Cell* 73(6):1127–37.e5

Zhao Q, Wang J, Levichkin IV, Stasinopoulos S, Ryan MT, Hoogenraad NJ. 2002. A mitochondrial specific stress response in mammalian cells. *EMBO J.* 21(17):4411–19

Zhong Z, Liang S, Sanchez-Lopez E, He F, Shalapour S, et al. 2018. New mitochondrial DNA synthesis enables NLRP3 inflammasome activation. *Nature* 560(7717):198–203

**Zhong Z, Umemura A, Sanchez-Lopez E, Liang S, Shalapour S, et al. 2016. NF-κB restricts inflammasome activation via elimination of damaged mitochondria. *Cell* 164(5):896–910**

Ziviani E, Tao RN, Whitworth AJ. 2010. *Drosophila* Parkin requires Pink1 for mitochondrial translocation and ubiquitinates Mitofusin. *PNAS* 107(11):5018–23

**Reported that biomolecules released during mitochondrial stress, such as mitochondrial DNA, can activate the NLRP3 inflammasome.**