



Novel Insights Into the Anti-aging Role of Mitophagy

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Contents

1. Introduction	170
2. The Pathways of Mitophagy	172
2.1 The Phosphatase and Tensin Homolog—Induced Putative Kinase 1/Parkin Pathway	174
2.2 Parkin-independent Pathways and More	178
2.2.1 <i>The Phosphatase and Tensin Homolog—Induced Putative Kinase 1—Synphilin-1—Seven in Absentia Homolog 1 Complex</i>	178
2.2.2 <i>ARIH1-Mediated Mitophagy</i>	179
2.3 Receptor-Mediated Mitophagy Pathways	179
3. Signaling Cascades That Modulate Aging Through Mitophagy and Mitochondrial Biogenesis Coordination	184
3.1 Mitochondrial Homeostasis Interfaces With Mammalian Target of Rapamycin and AMP-Activated Protein Kinase Signaling	184
3.2 Sirtuins: Critical Regulators of Energy Metabolism	188
4. Mitophagy Deficiency During Aging: Lessons From Model Organisms	190
5. Synthetic and Natural Compounds to Modulate Mitophagy during Aging	193
6. Conclusions	194
Acknowledgments	196
References	196

Abstract

Aging is a complex biological process affecting almost all living organisms. Although its detrimental effects on animals' physiology have been extensively documented, several aspects of the biology of aging are insufficiently understood. Mitochondria, the central energy producers of the cell, play vital roles in a wide range of cellular processes,

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including regulation of bioenergetics, calcium signaling, metabolic responses, and cell death, among others. Thus, proper mitochondrial function is a prerequisite for the maintenance of cellular and organismal homeostasis. Several mitochondrial quality control mechanisms have evolved to allow adaptation to different metabolic conditions, thereby preserving cellular homeostasis and survival. A tight coordination between mitochondrial biogenesis and mitochondrial selective autophagy, known as mitophagy, is a common characteristic of healthy biological systems. The balanced interplay between these two opposing cellular processes dictates stress resistance, healthspan, and lifespan extension. Mitochondrial biogenesis and mitophagy efficiency decline with age, leading to progressive accumulation of damaged and/or unwanted mitochondria, deterioration of cellular function, and ultimately death. Several regulatory factors that contribute to energy homeostasis have been implicated in the development and progression of many pathological conditions, such as neurodegenerative, metabolic, and cardiovascular disorders, among others. Therefore, mitophagy modulation may serve as a novel potential therapeutic approach to tackle age-associated pathologies. Here, we review the molecular signaling pathways that regulate and coordinate mitophagy with mitochondrial biogenesis, highlighting critical factors that hold promise for the development of pharmacological interventions toward enhancing human health and quality of life throughout aging.



1. INTRODUCTION

Aging is associated with marked alterations in cellular and organismal metabolism that are critically linked to whole-organism homeostasis. Research over the last 3 decades has culminated in the identification of conserved signaling pathways and transcription factors that tightly regulate the aging process. Mitochondria, mostly known as the energy-producing organelles in eukaryotic cells, are dynamic structures that play crucial roles in various cellular processes including apoptosis, Ca^{2+} signaling, fatty acid β -oxidation, iron-sulfur cluster synthesis, cell signaling, and interorganellar interactions, particularly with the endoplasmic reticulum (ER) (Saraste, 1999; Parsons and Green, 2010; Seo et al., 2010; Marchi et al., 2012). Accumulating findings from studies in model organisms indicate that changes in mitochondrial network morphology and function influence mitochondrial bioenergetics efficiency in a quite highly conserved manner, contributing to aging and the pathophysiology of age-related diseases such as obesity, diabetes, cancer, neurodegenerative disorders, among others (Liesa and Shirihai, 2013; Sonntag et al., 2017). Although, age-related deterioration of mitochondrial function is a universal phenomenon, its cellular and molecular underpinnings remain largely unknown. Given that the consequences

of mitochondrial dysfunction might be particularly detrimental for terminally differentiated cells such as the postmitotic neurons and cardiomyocytes, affected cells develop quality control systems to counteract this damage. Increasing evidence suggests that failure of mitochondrial quality control mechanisms accelerates aging. Macroautophagy (hereafter, referred to as autophagy) is the main process for bulk protein and organelle recycling in cells. It involves the formation of a double-membrane structure, the autophagosome, which engulfs the material destined for degradation in the lysosome (Klionsky and Emr, 2000). Besides the ATG5/ATG7 canonical pathway, autophagy in mammals can occur through an ATG5/ATG7-independent pathway, which also relies on several autophagic proteins, including Unc-51-like kinase 1 (ULK1) and Beclin1. This alternative autophagy requires the small GTPase RAB9 for fusion of isolation membranes with vesicles derived from the *trans*-Golgi and late endosomes. Interestingly, ATG5/ATG7 unconventional autophagy has been shown to mediate mitochondrial elimination during erythrocyte differentiation *in vivo* (Nishida et al., 2009) and is essential for induced pluripotent stem cell reprogramming (Ma et al., 2015).

Autophagy is considered to be a cellular quality control mechanism whose efficiency declines with age (Rubinsztein et al., 2011). While autophagy is primarily nonselective, selective autophagy pathways that mediate the removal of unwanted cellular cargo or damaged organelles and intracellular pathogens also exist. Selective autophagy relies on the core autophagy machinery and requires specific receptors or adaptors bearing one or more LC3-interacting regions (LIRs) for binding intracellular cargo to proteins of the LC3/GABARAP (microtubule-associated protein 1A/1B-light chain 3/gamma-aminobutyric acid receptor-associated protein) family (Svenning and Johansen, 2013; Stolz et al., 2014). Based on the type of autophagy receptor and the mechanism mediating cargo recognition, selective autophagy can be distinguished into ubiquitin (Ub)-dependent and Ub-independent pathways (Khaminets et al., 2016).

One type of selective autophagy with more or less clearly delineated steps is the selective mitochondrial autophagy (mitophagy). Mitophagy primarily serves a housekeeping function by modulating turnover of mitochondria under steady-state conditions and appropriately adjusting the number of mitochondria according to changing metabolic needs of the cell. In response to stress, mitophagy induction leads to selective elimination of functionally impaired mitochondria, thus contributing to the maintenance of a healthy mitochondrial population. Studies in model organisms

have shown that mitophagy is an evolutionarily conserved process subjected to tight regulation (Youle and Narendra, 2011). In mammals, although mitophagy has been studied mainly in cell lines under artificial conditions, conserved molecular mediators of this process have been identified (Table 1) (Liu et al., 2012; Ordureau et al., 2014). Most notably, increasing evidence suggests that impairment of mitophagy is implicated in aging and age-related diseases (Fang et al., 2014; Menzies et al., 2015). Conversely, its coordination with the opposing process of mitochondrial biogenesis is crucial for promoting stress resistance, healthspan and lifespan in the nematode *Caenorhabditis elegans* (Palikaras et al., 2015b). Besides its role in removing damaged or dysfunctional mitochondria, mitophagy is also responsible for mitochondria elimination in diverse developmental contexts such as during red blood cell differentiation (Schweers et al., 2007; Kundu et al., 2008) and brain development in mammalian cells (Deczkowska and Schwartz, 2017; Esteban-Martinez et al., 2017) or following fertilization for degrading sperm-derived paternal mitochondria in the nematode *C. elegans* and in early mouse embryos (Sato and Sato, 2011; Rojansky et al., 2016). In this review, we focus on the role of mitophagy in aging. We first describe the molecular mechanisms that govern the mitophagy process as well as its complex interplay with mitochondrial dynamics. Furthermore, we discuss the age-related decline of mitophagy efficiency and the coordination of mitochondrial biogenesis with mitochondrial degradation to compensate for defects in mitochondria quality control and function. Better understanding of mitochondrial turnover mechanisms is a key requirement for the development of more efficient strategies to battle numerous pathological conditions in humans. Finally, we end with recent advances on the potential of pharmacological and nutritional interventions or the physical exercise to modulate mitophagy for health and/or longevity benefits.



2. THE PATHWAYS OF MITOPHAGY

Different mechanisms of selective mitochondrial autophagy have been identified to date suggesting that different stimuli may induce mitophagy via multiple signaling pathways in distinct cell contexts (Frank et al., 2012; Jin and Youle, 2013; Melser et al., 2013). However, numerous important connections exist between these pathways, and notably, emerging findings indicate that key mitophagy players are functionally conserved (Table 1). In the following sections, we describe the fundamental mechanisms involved in this process.

Table 1 Mitophagy Components are Evolutionarily Conserved in Eukaryotes

Organisms					
<i>Saccharomyces cerevisiae</i>	<i>Caenorhabditis elegans</i>	<i>Drosophila melanogaster</i>	<i>Mus musculus</i>	<i>Homo sapiens</i>	Molecular Function
—	PINK-1	Pink1	PINK1	PINK1	Serine/threonine protein kinase
—	PDR-1	Park	PARK2	PARK2	E3 ubiquitin ligase
ATG32	—	—	—	—	—
ATG8	LGG-1, LGG-2	Atg8a	GABARAP/LC3	GABARAP/LC3	Autophagosomal protein
—	UNC-51	—	ULK1	ULK1	Serine/threonine protein kinase
—	DCT-1	—	BNIP3, NIX/BNIP3L	BNIP3, NIX/BNIP3L	Mitophagy receptor
CG5676	FUNDC-1	—	FUNDC1	FUNDC1	Mitophagy receptor
—	—	—	OPTN	OPTN	Mitophagy receptor
—	—	—	NDP52	NDP52	Mitophagy receptor
—	SQST-1	Ref(2)P	SQST1	p62/SQSTM1	Autophagy adaptor
—	FZO-1	FZO1, DMFN	MFN1/2	MFN1/2	Mitochondrial GTPase
PHB2	PHB-2	PHB2	PHB2	PHB2	Mitophagy receptor
—	FKB-6	CG5482	FKBP8	FKBP8	Mitophagy receptor

2.1 The Phosphatase and Tensin Homolog–Induced Putative Kinase 1/Parkin Pathway

The first pathway shown to mediate mitophagy in animals is the phosphatase and tensin homolog–induced putative kinase 1 (PINK1)/Parkin signaling cascade (Pickrell and Youle, 2015). Several processes, such as mitochondrial dynamics, trafficking, and recruitment of core autophagy components converge into PINK1/Parkin-mediated mitophagy to ensure removal of functionally impaired mitochondria. Its cellular and molecular underpinnings are described below.

The Ser/Thr PINK1 and the E3 ubiquitin ligase Parkin (encoded by *PARK2* in humans) are the key players of this pathway. Parkin is a member of the RING-between-RING E3 ubiquitin family. It consists of an N-terminal ubiquitin-like (Ubl) domain and 4 zinc-coordinating RING-like domains, namely RING0, RING1, a cysteine-rich in-between RING domain (IBR), and RING2 (Pickrell and Youle, 2015). Structural analysis revealed that Parkin normally exists in an autoinhibited state in the cytosol (Chaugule et al., 2011; Trempe et al., 2013; Wauer and Komander, 2013). Following mitochondrial uncoupling, Parkin translocates to damaged mitochondria where it is activated to ubiquitinate mitochondrial substrates for initiating organelle clearance by mitophagy. It is now well established that Parkin recruitment to mitochondria and its subsequent activation depends on the stabilization of PINK1 on the outer membrane of depolarized mitochondria (Matsuda et al., 2010; Narendra et al., 2010). What is the sequence of events that link latent Parkin activation with PINK1 processing and stabilization upon mitochondrial uncoupling or unfolded protein overload?

In healthy mitochondria, PINK1 is imported into the inner mitochondrial membrane, in a membrane potential-dependent manner (Fig. 1). It is processed into a 60-kDa form by the mitochondrial processing peptidase (MPP) (Greene et al., 2012) and then cleaved between Ala103 and Phe104 residues of its amino-terminus, giving rise to an unstable 52-kDa fragment. The cleavage is mediated mainly by the inner membrane rhomboid protease presenilin-associated rhomboid-like protein (PARL) (Jin et al., 2010; Deas et al., 2011) and AFG3L2, a subunit of the m-AAA protease (Greene et al., 2012). The 52-kDa truncated PINK1 is rapidly degraded by the proteasome (Jin et al., 2010; Narendra et al., 2010b).

In depolarized mitochondria, however, the import of proteins, including PINK1, across the inner mitochondrial membrane is abolished.

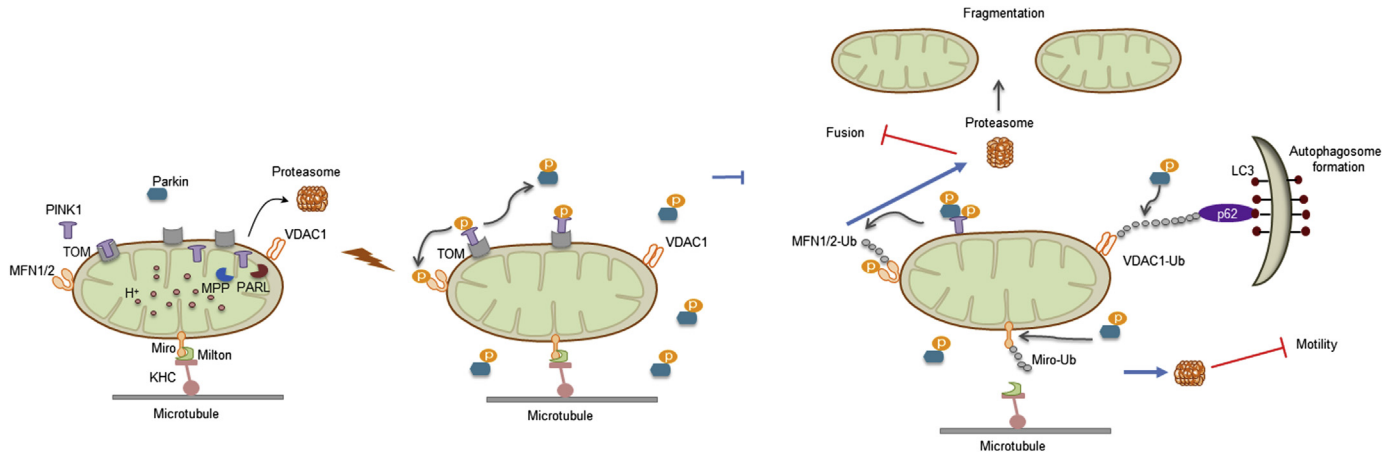


Figure 1 *The phosphatase and tensin homolog-induced putative kinase 1 (PINK1)/Parkin pathway at a glance.* In healthy mitochondria, phosphatase and tensin homolog-induced putative kinase 1 (PINK1) is imported across the inner mitochondrial membrane by the translocase of the outer membrane (TOM) complex, processed by mitochondrial processing peptidase (MPP) and presenilins-associated rhomboid-like protein (PARL), and subsequently degraded by the proteasome. Upon mitochondrial membrane depolarization, PINK1 is stabilized on the outer mitochondrial membrane and activated by autophosphorylation, triggering Parkin recruitment and activation. Then, Parkin ubiquitinates several mitochondrial surface proteins, such as voltage-dependent anion-selective channel protein 1 (VDAC1), mitofusins (MFN1/2), and mitochondrial ras homologous (RHO) guanine triphosphate (GTP) ase Miro, coordinating mitochondrial motility, dynamics, and recruitment of autophagic machinery components. *KHC*, kinesin heavy chain; *Ub*, ubiquitin.

As a consequence, unprocessed (63-kDa) PINK1 accumulates on the outer mitochondrial membrane, with its kinase domain facing the cytosol (Fig. 1) (Jin et al., 2010; Matsuda et al., 2010; Narendra et al., 2010b). In turn, PINK1 is assembled in a 700-kDa complex that includes also core components of the translocase of the outer membrane (TOM) complex, but not Parkin (Lazarou et al., 2012). Notably, PINK1 artificially tethered to other compartments that lack TOM complex, such as peroxisomes or lysosomes, is able to induce Parkin recruitment, organelle ubiquitination, and, at least in the case of peroxisomes, autophagic clearance, indicating that the TOM complex is dispensable for PINK1-mediated Parkin recruitment and activation (Lazarou et al., 2012). On the other hand, Parkin artificially recruited to the mitochondria in the absence of PINK1 is unable to ubiquitinate mitochondrial proteins and initiate mitophagic clearance (Matsuda et al., 2010; Narendra et al., 2010b). Hence, PINK1 has a prominent role in activation of Parkin E3 ligase activity and subsequent mitophagy induction.

Besides its ability to detect mitochondrial uncoupling, PINK1 can sense the accumulation of misfolded proteins in the mitochondrial matrix of healthy mitochondria. Its subsequent stabilization on the outer mitochondrial membrane results in Parkin recruitment and mitophagy activation, ultimately leading to a reduction in unfolded protein overload. PINK1/Parkin-mediated mitophagy is enhanced by the knockdown of Lon protease (Jin and Youle, 2013), an adenosine triphosphate (ATP)-stimulated mitochondrial matrix protein, which recognizes and selectively degrades oxidatively damaged proteins (Bota and Davies, 2002). The effect of Lon depletion does not originate from mitochondrial depolarization (Jin and Youle, 2013; Burbulla et al., 2014). Further supporting the protective role of PINK1 and Parkin against intramitochondrial proteotoxicity, a recent study showed that impaired mitochondrial homeostasis caused by the loss of mortalin function is rescued by the PINK1/Parkin-mediated clearance of dysfunctional mitochondria (Burbulla et al., 2014).

The question then arises: how does PINK1 trigger the E3 ligase activity of Parkin? It was shown that PINK1 becomes activated upon membrane depolarization by autophosphorylation at Thr257, Ser228, and Ser402. These phosphorylation events are essential for the recruitment of Parkin to damaged mitochondria (Kondapalli et al., 2012; Okatsu et al., 2012). Then, activated PINK1 directly phosphorylates Parkin on conserved Ser65 of the Ubl domain. Although this phosphorylation event is not sufficient for driving mitochondrial translocation of Parkin, it reportedly releases

the autoinhibitory configuration of Parkin, unleashing its enzymatic activity (Sha et al., 2010; Kondapalli et al., 2012; Shiba-Fukushima et al., 2012; Fu et al., 2013; Caulfield et al., 2014). The RING1 domain of Parkin is required for the physical interaction of Parkin with PINK1 (Sha et al., 2010), while the RING2 domain is essential for ubiquitin-thioester transfer through the formation of an ubiquitin-thioester intermediate on Cys431 (Iguchi et al., 2013; Lazarou et al., 2013). Under basal conditions, RING0 occludes the ubiquitin-acceptor site Cys431 in RING2, ensuring autoinhibition of E3 ligase activity (Trempe et al., 2013). Moreover, PINK1 was demonstrated to drive self-association of Parkin through its IBR domain. This self-association is essential for the activation of Parkin ubiquitin ligase activity upstream of its mitochondrial translocation (Lazarou et al., 2013).

Previous studies have shown that ubiquitin itself is phosphorylated by PINK1 on Ser65, similar to the Ubl domain of Parkin (Kane et al., 2014; Kazlauskaitė et al., 2014; Koyano et al., 2014; Shiba-Fukushima et al., 2014). Although this phosphorylation was initially suggested to activate the E3 ligase activity of Parkin, it has been later proposed that phosphorylation of polyubiquitin follows ubiquitin conjugation onto Parkin substrates (Ordureau et al., 2014; Stolz et al., 2014). Parkin catalyzes the formation of K6-, K11-, K27-, K48-, and K63-linked ubiquitin chains on its substrates (Geisler et al., 2010; Ordureau et al., 2014). The role of each of these different polyubiquitin structures in substrate degradation and subsequent mitochondrial fate determination remains poorly understood. A recent study identified a binding switch between the phosphoubiquitin (pUb) and the Ubl domain of Parkin as the key in PINK1-mediated Parkin activation on the mitochondria. According to this work, pUb binds to RING1 of Parkin at a site formed by His302 and Arg305, promoting disengagement of the Ubl domain from RING1 and subsequent Parkin phosphorylation, which leads to the activation of its ligase activity (Sauve et al., 2015).

A recent report suggests that PINK1 serves as a pro-fission signal upon uncoupling. By disrupting the protein kinase A (PKA) - A-kinase-anchoring protein 1 (AKAP1) axis, it activates DRP1, promoting fission of damaged organelles for their autophagic clearance, independently of Parkin. Thus, PINK1 is proposed to be a master mitophagy regulator (Pryde et al., 2016).

Following its translocation to damaged mitochondria, Parkin ubiquitinates outer mitochondrial membrane proteins, including mitofusins mitochondrial assembly regulatory factor (MARF) in flies (Poole et al., 2010; Ziviani et al., 2010), mitofusins (MFN1 and MFN2) (Gegg et al., 2010; Tanaka et al., 2010), voltage-dependent anion-selective channel protein 1

(VDAC1) (Geisler et al., 2010), and Miro (Wang et al., 2011; Shlevkov et al., 2016), among others, promoting their degradation. Parkin-mediated polyubiquitination of mitofusins, in particular, leads to their proteasome-dependent degradation (Gegg et al., 2010; Tanaka et al., 2010; Glauser et al., 2011). As a consequence, mitochondrial fusion is blocked, preventing functional organelles to fuse with their damaged counterparts. Another model supports the idea that MFN2 but not MFN1 acts as the receptor for Parkin on the surface of damaged mitochondria. MFN2–Parkin binding provokes the ubiquitination of mitochondrial proteins, thus targeting defective organelles for autophagic degradation (Chen and Dorn, 2013). Since mitochondrial fission is uninterrupted, it may facilitate mitophagy, presumably by supplying smaller organelles of manageable sizes that can easily be engulfed by autophagosomes. Regarding Miro, a component of the conserved primary motor/adaptor complex that anchors kinesin to the mitochondrial surface, PINK1-mediated phosphorylation triggers its proteasomal degradation in a Parkin-dependent manner and the subsequent release of kinesin from mitochondria. As a consequence, mitochondrial movement is inhibited in both *Drosophila* and mammalian neurons, where damaged organelles may be first sequestered locally within an autophagosome prior to their clearance (Wang et al., 2011). Together, these reports highlight the complex interplay between mitochondrial dynamics and mitophagy, both of which critically contribute to mitochondrial homeostasis.

Interestingly, a new study highlights a previously unappreciated role for reactive oxygen species (ROS) in the completion of PINK/Parkin-mediated mitophagy following Parkin recruitment to damaged mitochondria. Indeed, superoxide rather than hydrogen peroxide is proposed to be the driving force for the execution of mitophagy, although it alone is not able to modulate Parkin dynamics or mitochondrial elimination via autophagy (Xiao et al., 2017).

2.2 Parkin-independent Pathways and More

Although the PINK1/Parkin mitophagy pathway has attracted much attention in recent years, additional pathways capable of mediating the selective clearance of damaged mitochondria have been characterized.

2.2.1 *The Phosphatase and Tensin Homolog–Induced Putative Kinase 1–Synphilin-1–Seven in Absentia Homolog 1 Complex*

A recent study revealed a novel Parkin-independent mitophagy pathway that involves the PINK1/synphilin-1/seven in absentia homolog 1

(SIAH1) complex. In this specialized case, PINK1 recruits synphilin-1 to the mitochondria both in rat brain tissues and cultured cells, promoting mitochondrial depolarization and stabilization of uncleaved PINK1 at the organelle. In turn, synphilin-1 recruits the E3 ubiquitin ligase SIAH1, which ubiquitinates mitochondrial proteins, resulting in LC3 recruitment for autophagosome formation. Notably, while PINK1 is required for synphilin-1 mitochondrial translocation and mitophagy, the PINK1-synphilin-1 pathway is independent of PINK1 kinase activity. This pathway might represent a new drug target to enhance degradation of damaged mitochondria, thus compensating for mitophagy failure in Parkinson's disease patients carrying mutations in genes encoding PINK1 and Parkin (Szargel et al., 2016).

2.2.2 ARIH1-Mediated Mitophagy

The E3 ubiquitin ligase Ariadne RBR E3 Ub protein ligase 1 (ARIH1) (also known as HHARI), which is extensively expressed in several cancer cell types, especially in breast and lung adenocarcinomas, has recently evolved as a novel mitophagy regulator. In cancer cells where Parkin is frequently downregulated, ARIH1 polyubiquitinates damaged mitochondria, inducing mitophagy. ARIH1-mediated mitophagy depends on its ubiquitin ligase activity and PINK1 stabilization. This mechanism contributes to chemotherapy resistance, challenging the prevailing view that the main mitophagy regulators act as tumor suppressors (Villa et al., 2017).

2.3 Receptor-Mediated Mitophagy Pathways

In recent years, the role of selective autophagy receptors in mammalian mitophagy has been revisited. Based on current evidence, PINK1 phosphorylates ubiquitin, and this phosphorylation event subsequently triggers the recruitment of the autophagy receptors nuclear dot protein 52 (NDP52) and optineurin (OPTN), but not p62, to mitochondria, thereby stimulating mitophagy in a Parkin-independent manner (Fig. 2). Once localized on mitochondria, receptors recruit the autophagy components ULK1, double FYVE-domain-containing protein 1 (DFCP1), and WD repeat domain phosphoinositide-interacting protein 1 (WIPI1) that function upstream of LC3, to initiate autophagosome formation proximal to dysfunctional organelles. When Parkin is present, it acts to amplify the PINK1-generated phosphoubiquitin signal, resulting in robust mitophagy induction (Lazarou et al., 2015). Considerable controversy has ensued concerning the role of p62 in mitophagy (Fig. 2). Certain reports suggested that p62 is crucial for the

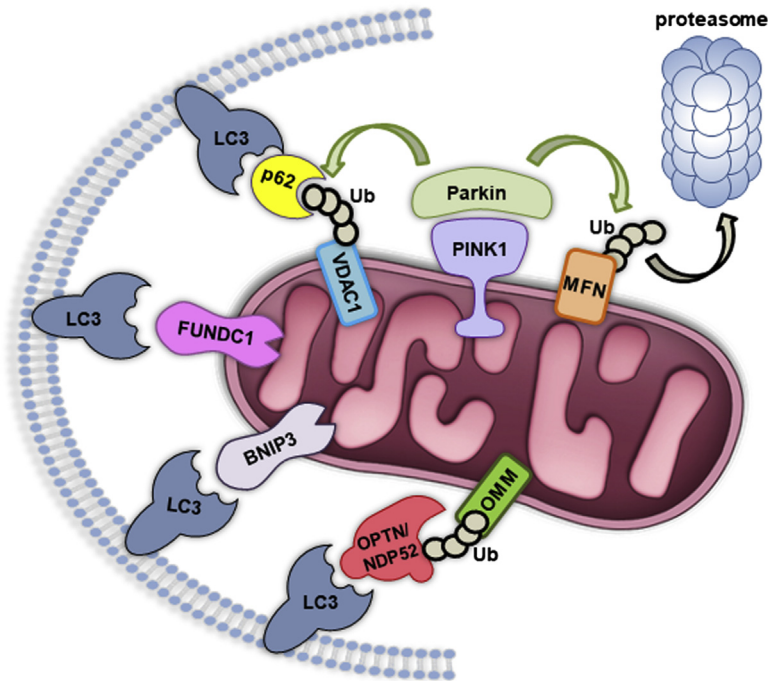


Figure 2 Receptor-mediated mitophagy. Phosphatase and tensin homolog-induced putative kinase 1 (PINK1) is stabilized on the outer mitochondrial membrane and recruits Parkin in response to mitochondrial damage. In turn, Parkin ubiquitinates several outer mitochondrial membrane proteins leading both to their proteasomal degradation [e.g., mitofusin (MFN)] and association with the adaptor molecule p62. Then, p62 interacts with the autophagosomal protein LC3, mediating mitochondrial removal. In parallel to the PINK1/Parkin signaling pathway, impaired mitochondria could be also removed by receptor-mediated mitophagy. FUN14 domain-containing protein 1 (FUNDC1), BNIP3, optineurin (OPTN), and nuclear dot protein 52 (NDP52) mitophagy receptors interact directly or indirectly with LC3, promoting the sequestration of defective organelles. The existence of multiple mitophagy mechanisms ensures the efficient quality control of mitochondrial population under stress conditions. *OMM*, outer mitochondrial membrane protein; *Ub*, ubiquitin; *VDAC1*, voltage-dependent anion-selective channel protein 1.

elimination of depolarized mitochondria (Ding et al., 2010; Youle and Narendra, 2011; Zhang et al., 2015), whereas other studies have led to the conclusion that p62 is dispensable for mitophagy (Narendra et al., 2010a; Lazarou et al., 2015).

Recently, the serine/threonine kinase TANK-binding kinase 1 (TBK1) has emerged as a critical regulator of the mitophagy network, as it has been shown to phosphorylate the OPTN, NDP52, and p62 autophagy receptors

on several autophagy-relevant sites (Wild et al., 2011; Heo et al., 2015; Richter et al., 2016). In mammals, phosphorylation of selective autophagy receptors enhances their binding to both the cargo and the autophagic machinery (Fig. 2) (Heo et al., 2015; Richter et al., 2016). TBK1-mediated phosphorylation of OPTN, in particular, reinforces its binding to the ubiquitin chains of outer mitochondrial membrane proteins, TBK activation, and OPTN retention on damaged mitochondria, thereby promoting their removal (Heo et al., 2015).

As a mode of selective autophagy, mitophagy, in most cases, relies on specific receptors that recognize and direct damaged mitochondria for degradation. In this sense, mitochondrial proteins that engage core autophagy machinery components are considered as mitophagy receptors. Like yeast receptors, which have one or more Atg8 family interacting motifs (AIMs), mammalian receptors have one or more LIRs to associate with LC3/GABARAP family proteins. Functional homologs of mitophagy receptors have been identified in diverse species ranging from yeast to mammals (Table 1). In yeast, two independent screens for mitophagy-defective mutants identified autophagy-related protein 32 (Atg32) as essential for respiratory growth-induced mitophagy in post-log phase cells (Kanki et al., 2009; Okamoto et al., 2009). Atg32 is a mitochondrial-anchor protein with an N-terminal domain facing the cytosol, a transmembrane domain, and a C-terminal domain exposed to mitochondrial intermembrane space. The cytoplasmic domain of Atg32 contains a tetrapeptide sequence, W/YXXI/L/V (AIM), which is conserved among proteins that physically associate with the Atg8 family members. Furthermore, Atg32 has been shown to interact with Atg11, a scaffold protein required for selective autophagy pathways. Together, these findings support the idea that Atg32 forms a complex with Atg8 and Atg11. This interaction precedes and is independent of isolation membrane generation and subsequent autophagosome formation (Kanki et al. 2009, 2010; Kondo-Okamoto et al., 2012). Atg32 is phosphorylated by the conserved serine and/or threonine casein kinase-2 (CK2). This phosphorylation event reinforces Atg32-Atg11 interaction, ultimately inducing mitophagy (Kanki et al., 2013).

Until recently, no Atg32 homolog was known in metazoans (Tolkovsky, 2009). A new study just showed that BCL-2-like protein 13 (BCL2L13) is an Atg32 functional homolog in mammalian cells. Interestingly, BCL2L13 is involved in mitochondrial fragmentation and in mitophagy. It consists of a C-terminal single transmembrane domain, four conserved BCL-2 homology domains (BH1-4), and 2 WXXL/I motifs. The BH domains are crucial

for fragmentation, while the WXXI motif mediates the interaction of BCL2L13 with LC3, thus stimulating mitophagy. Notably, BCL2L13 is able to promote mitochondrial fragmentation in DRP1-depleted cells as well as mitophagy in Parkin-deficient cells. However, the molecular mechanism by which BCL2L13 coordinates mitochondrial fission and mitophagy remains elusive (Murakawa et al., 2015).

In mammals, mitochondria are eliminated during terminal differentiation of red blood cells through mitophagy by a mechanism that relies on the outer mitochondrial membrane NIP3-like protein X (NIX; also referred to as BNIP3L). NIX/BNIP3L-deficient mice retain mitochondria in peripheral blood erythrocytes, displaying impaired erythroid maturation and anemia (Schweers et al., 2007; Sandoval et al., 2008). NIX/BNIP3L is a typical BCL-2 homology domain 3 (BH3)-only protein initially suggested to act by engaging the BCL-2 apoptotic pathway. It is now clear that NIX/BNIP3L, which has a WXXL-like motif facing the cytosol, binds to LC3 and GABARAP proteins on autophagosomes, thus mediating, at least partially, mitophagy (Schwarten et al., 2009; Novak et al., 2010). In this case, NIX/BNIP3L functions as a regulated receptor for selective mitochondrial removal (Novak et al., 2010; Rogov et al., 2017). Although NIX/BNIP3L has a crucial role in elimination of mitochondria during reticulocyte maturation, mitophagy can be also induced to some extent without NIX/BNIP3L and also independently of core autophagy proteins such as ATG5 and ATG7 (Nishida et al., 2009; Honda et al., 2014). Similar to NIX/BNIP3L, its relative BNIP3 has been reported to function as a mitophagy receptor. Specifically, it has been shown that phosphorylation of Ser17 and 24 flanking the BNIP3 LIR domain enhances its interaction with specific Atg8 members LC3B and GATE-16, thereby inducing mitophagy versus apoptosis (Zhu et al., 2013). Intriguingly, NIX/BNIP3L was recently identified as a substrate of Parkin in *Drosophila melanogaster*. Moreover, the ubiquitination of NIX/BNIP3L by Parkin on mitochondria recruits the autophagy adaptor neighbor of *BRCA1* gene 1 (NBR1), ultimately leading to NIX/BNIP3L-mediated mitophagy in human cell lines (Gao et al., 2015). Another study showed that DAF-16/FOXO controlled, germline tumor affecting-1 (DCT-1) (Oh et al., 2006; Pinkston-Gosse and Kenyon, 2007) is the *C. elegans* homolog of mammalian BNIP3 and NIX/BNIP3L and serves as a key mediator of mitophagy, promoting survival under stress conditions. Importantly, DCT-1 is ubiquitinated on Lys26, in particular, under mitophagy-inducing conditions. This ubiquitination event requires the activity of PINK-1. Furthermore, DCT-1 ubiquitination

depends on PDR-1, the nematode Parkin homolog. Together, these findings indicate that DCT-1, PINK-1, and PDR-1 function in the same genetic pathway to mediate elimination of impaired mitochondria, thus contributing to cell and whole-organism homeostasis (Palikaras et al., 2015b).

The mitochondrial outer membrane protein FUN14 domain-containing protein 1 (FUNDC1) also acts as a mitophagy receptor to recruit autophagosomes for mitochondrial clearance (Fig. 2). FUNDC1 is highly conserved from flies to humans (Table 1). It has three putative transmembrane domains near the C-terminus which face the intermembrane space, and a typical LIR mediating the direct interaction with LC3 under hypoxic conditions (Liu et al., 2012). Further analysis showed that FUNDC1 regulation is quite complex. Specifically, phosphorylation of FUNDC1 LIR motif by Src kinases and CK2 blocks mitophagy under physiological conditions in mammalian cells (Liu et al., 2012). In response to hypoxia or uncoupling, however, the mitochondrial phosphatase phosphoglycerate mutase family member 5 (PGAM5) dephosphorylates FUNDC1, initiating mitophagy (Chen et al., 2014). In a different level of regulation, ULK1-mediated phosphorylation of FUNDC1 at Ser17 renders this receptor capable of responding to a general stimulus for autophagy (Wu et al., 2014). Together, these findings suggest that FUNDC1 is activated upon both general and selective autophagy stimuli through ULK1 and PGAM5 signaling, respectively. As recently shown, the hypoxia-responsive microRNA mir-137 is also implicated in FUNDC1 and NIX/BNIP3L regulation. Downregulation of mir-137 increases FUNDC1 expression, enhancing its interaction with LC3, and ultimately promoting mitophagy during hypoxia (Li et al., 2014). Further delineating the role of FUNDC1 in mitophagy, FUNDC1 is considered as a central node of a newly identified pathway that interfaces mitochondrial dynamics and mitophagy under hypoxic conditions. Specifically in hypoxic cells, FUNDC1 relocates to the ER-mitochondrial contact site (MAM) where it accumulates by binding to the ER membrane protein calnexin. As mitophagy proceeds, FUNDC1/calnexin association attenuates allowing FUNDC1 to interact with DRP1, promoting its recruitment to the MAM and the consequent mitochondrial fission (Wu et al., 2016). A parallel study showed that FUNDC1 interacts with both DRP1 and OPA1, a mitochondrial fusion protein in the intermembrane space to coordinate mitochondrial dynamics and mitophagy. Indeed, FUNDC1 normally interacts with OPA1, but this association is abolished under mitochondrial stress, promoting the interaction of FUNDC1 with DRP1, which then leads to mitochondrial fission. Furthermore, FUNDC1

dephosphorylation under stress conditions promotes the disassembly of the FUNDC1-OPA1 complex while inducing FUNDC1-DRP1 association. Together, these findings suggest that FUNDC1 has a regulatory role in both mitochondrial dynamics and mitophagy (Chen et al., 2016).

Surprisingly, the inner mitochondrial membrane protein prohibitin 2 (PHB2) was lately proposed to act as a mitophagy receptor. Following mitochondrial depolarization or proteasome-dependent outer membrane rupture, PHB2 interacts with LC3 via its LIR domain, inducing mitophagy. This process is responsible for paternal mitochondria elimination after embryonic fertilization in *C. elegans* and Parkin-induced mitophagy in mammalian cells, highlighting its conservation in eukaryotes (Wei et al., 2017).

The anti-apoptotic FK506-binding protein 8 (FKBP8, also known as FKBP38) has been very recently added to the repertoire of known Parkin-independent mitophagy receptors. FKBP8 is an anti-apoptotic protein normally localized to the outer mitochondrial membrane. It contains a canonical N-terminal LIR motif via which it associates with LC3A and related GABARAP members to initiate mitophagy in response to membrane potential dissipation, independently of Parkin. Intriguingly, FKBP8 escapes from the mitochondria to avoid its own degradation, thereby preventing apoptosis as mitophagy progresses (Bhujabal et al., 2017; Lim and Lim, 2017).



3. SIGNALING CASCADES THAT MODULATE AGING THROUGH MITOPHAGY AND MITOCHONDRIAL BIOGENESIS COORDINATION

Emerging observations suggest that alterations in mitophagy leading to accumulation of damaged mitochondria may have an important role in aging and age-related diseases (Madeo et al., 2015). Several molecular mechanisms have shown to mitigate mitophagy, adjusting mitochondrial number in response to energy demands, and intracellular and/or environmental stimuli. Perturbations of these signaling pathways deregulate the tight interplay between mitochondrial biogenesis and mitophagy resulting in impaired energy metabolism and eventually in cellular and tissue degeneration (Palikaras et al., 2015a).

3.1 Mitochondrial Homeostasis Interfaces With Mammalian Target of Rapamycin and AMP-Activated Protein Kinase Signaling

The mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) are master regulators of cellular and organismal metabolism,

and their activity is associated with the coupling of mitochondrial biogenesis and mitophagy in eukaryotic cells. Previous work has extensively documented that mTOR activity attenuates autophagy (Kim and Guan, 2015). Indeed, several studies have shown an inverse interplay between autophagy initiation and mTOR function (Zoncu et al., 2011). Importantly, it has been reported that mTOR acts as a negative autophagy regulator, since mTOR-dependent ULK1 phosphorylation inhibits autophagosome biogenesis under nutrient deprivation (Ganley et al., 2009; Hosokawa et al., 2009; Jung et al., 2009).

AMPK is a conserved master regulator of cellular and energy metabolism (Herzig and Shaw, 2017). Various stress stimuli, including starvation, hypoxia, and DNA damage, among others, result in energy depletion and AMPK activation (Laderoute et al., 2006). In turn, AMPK promotes an intricate signaling cascade leading to a complex transcriptional network fine-tuning energy homeostasis (Hardie, 2013). Furthermore, AMPK triggers autophagy through the modulation of multiple signaling pathways underlining its pivotal role in autophagy regulation. Firstly, AMPK phosphorylates and inhibits mTOR activity in response to energy depletion, resulting in autophagy induction (Gwinn et al., 2008). Additionally, glucose deprivation leads to AMPK-dependent phosphorylation and activation of ULK1 (Kim et al., 2011). In turn, ULK1 phosphorylates Beclin1 and enhances the activity of VPS34 lipid kinase, promoting selective mitochondrial removal under nutrient limitation and exercise training (Egan et al., 2011; Laker et al., 2017). Although ULK1 mediates autophagy initiation, it serves also as a negative regulator of autophagy. ULK1 phosphorylates AMPK, inhibiting its enzymatic activity and setting up a negative feedback loop, thereby terminating the signaling events and preserving energy homeostasis (Loffler et al., 2011). Thus, AMPK activation could remove defective mitochondria and be beneficial in response to harmful conditions contributing to various age-related pathologies.

Indeed, both genetic and pharmacological AMPK stimulation promotes longevity via systemic remodeling of mitochondrial dynamics and metabolism, highlighting a considerable communication between AMPK activity and mitochondrial homeostasis (Apfeld et al., 2004; Mair et al., 2011; Burkewitz et al., 2015; Fontana and Partridge, 2015; Toyama et al., 2016). Congruently, a recent study demonstrates that AMPK-mediated longevity depends on mitochondrial network morphology, since inhibition of both fission and fusion machinery abolishes lifespan extension driven by AMPK stimulation and caloric restriction (Weir et al., 2017). Interestingly,

experimental evidence indicates a direct effect of AMPK on mitochondrial morphology, since it is reported that mitochondrial fission factor (MFF) is phosphorylated and activated by AMPK, which subsequently results in DRP1-mediated mitochondrial fission and mitophagy stimulation (Ducommun et al., 2015; Toyama et al., 2016).

Despite the undoubted association of AMPK and mTOR with mitochondrial metabolism and mitophagy regulation, it is unclear whether they could mediate specific modifications on mitophagy-related proteins, such as mitophagy receptors. To this direction, there is evidence that the BNIP3 mitophagy receptor inactivates mTOR signaling through its direct association with Ras homolog enriched in brain (Rheb), an upstream mTOR activator, upon hypoxia (Li et al., 2007). Interestingly, AMPK phosphorylates and triggers ULK1 activity in response to hypoxic stress. In turn, ULK1 is recruited to dysfunctional mitochondria mediating their removal via mitophagy (Tian et al., 2015). However, whether AMPK alters the phosphorylation status of BNIP3 has not been examined yet. On the contrary, it has been shown that the mitophagy receptor FUNDC1 is phosphorylated by ULK1, enhancing its strong association with the autophagosomal protein LC3 under hypoxia (Wu et al., 2014). However, the exact mechanism of ULK1 mitochondrial transport, whether this translocation occurs under other stress conditions as well, how it triggers mitophagy, and whether it targets other outer mitochondrial membrane proteins are insufficiently understood.

In addition to its role in mitochondrial selective autophagy, AMPK orchestrates also mitochondrial biogenesis in various cell types including muscle cells, adipocytes, hepatic cells, and macrophages, among others (Galic et al., 2011; Hasenour et al., 2014; Mottillo et al., 2016). Increased energy requirements signal for more ATP production leading to mitochondrial biogenesis. Generation of newly synthesized mitochondria requires the upregulation of several mitochondrial proteins and lipids, which in their majority are encoded by nuclear genes. AMPK is shown to be the central regulator of this retrograde signaling cascade (Fig. 3). Many studies have demonstrated that genetic and/or pharmacological AMPK activation promotes mitochondrial biogenesis (Hardie, 2013). Furthermore, exercise training expands mitochondrial content in an AMPK-dependent manner (Narkar et al., 2008). On the contrary, genetic ablation of AMPK subunits results in decreased mitochondrial population and cellular inability to induce mitochondrial biogenesis under energetic stress, highlighting the essential

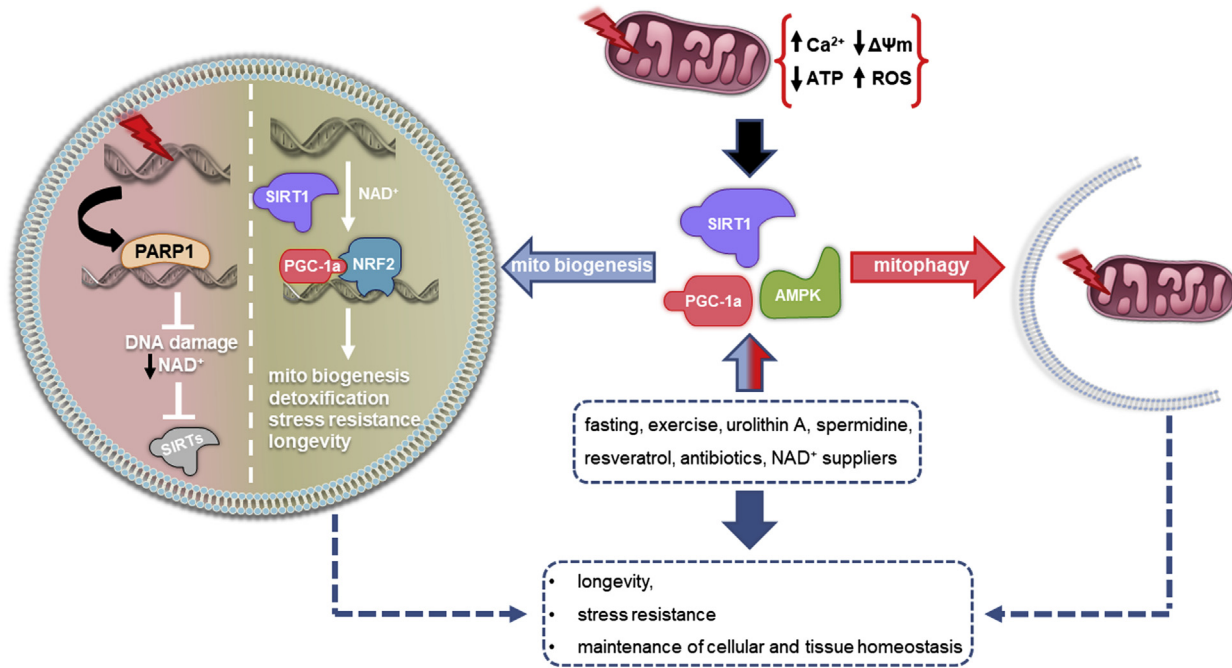


Figure 3 *Coupling of mitophagy and mitochondrial biogenesis preserves energy metabolism and organismal homeostasis.* Mitochondrial dysfunction, characterized by elevated cytoplasmic calcium levels, mitochondrial membrane potential collapse, adenosine triphosphate (ATP) depletion, and increased reactive oxygen species (ROS) production, is detrimental for cellular viability. Uncontrolled mitochondrial dysfunction could lead to genotoxic stress and progeria syndromes. Poly (adenosine diphosphate-ribose) polymerase 1 (PARP1) activation protects against DNA damage. However, persistent PARP1 activity results in nicotinamide adenine dinucleotide (NAD⁺) depletion and subsequent SIRT1 inhibition. Pharmacological interventions, fasting and exercise promote degradation of impaired organelles through mitophagy. Simultaneously, peroxisome proliferator activated receptor- γ coactivator 1 α (PGC-1 α) is induced by AMP-activated protein kinase (AMPK) and Sirtuin 1 (SIRT1) activity to mediate mitochondrial biogenesis. PGC-1 α serves as a transcriptional co-activator and interacts with multiple transcription factors, such as nuclear factor-erythroid 2-related factor 2 (NRF2), to orchestrate the expression of numerous mitochondrial, detoxification, stress resistance and longevity genes. This intricate interplay between mitophagy and mitochondrial biogenesis upholds energy metabolism, survival and tissue homeostasis during stress.

role of AMPK in mitochondrial metabolism (Jeppesen et al., 2013; Tanner et al., 2013; Lantier et al., 2014).

AMPK mediates mitochondrial biogenesis through the regulation of multiple signaling pathways converging on peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC-1 α) stimulation. PGC-1 α serves as a co-activator of numerous transcription factors, including estrogen-related receptors (ERRs), peroxisome proliferator-activated receptors (PPARs), nuclear respiratory factor 1 (NRF1), myocyte enhancer factor 2 (MEF2), and forkhead box O (FOXO), among others. Thus, PGC-1 α orchestrates an intricate transcriptional program, which promotes the expression of several mitochondrial-related genes (Fernandez-Marcos and Auwerx, 2011; Scarpulla, 2011; Ploumi et al., 2017). Notably, PGC-1 α drives the expression of several genes involved in oxidative metabolism upon AMPK induction. Moreover, AMPK is found to phosphorylate PGC-1 α in vitro (Jager et al., 2007). Although, experimental evidence indicates a direct association between AMPK and PGC-1 α activation, whether AMPK interacts and phosphorylates PGC-1 α directly in vivo is still unclear.

Another layer of mitochondrial biogenesis regulation through an indirect AMPK activity involves Sirtuin 1 (SIRT1). SIRT1 is a nicotinamide adenine dinucleotide (NAD⁺)-dependent histone deacetylase, which participates in various cellular processes. AMPK influences SIRT1 enzymatic activity through elevation of intracellular NAD⁺ levels (Canto et al., 2009). Recently, it is also demonstrated that AMPK phosphorylates glyceraldehyde 3-phosphate dehydrogenase (GAPDH) leading to SIRT1 activation and autophagy induction upon glucose deprivation (Chang et al., 2015). Additionally, AMPK could also promote directly SIRT1 phosphorylation and stimulation in vitro (Lau et al., 2014). In turn, SIRT1 deacetylates and activates PGC-1 α , promoting mitochondrial biogenesis (Canto and Auwerx, 2009).

Collectively, AMPK holds an essential role in mitochondrial homeostasis, and defining its substrates and effects in response to energetic stress would be beneficial with potential therapeutic implications in several pathological states.

3.2 Sirtuins: Critical Regulators of Energy Metabolism

NAD is a crucial metabolite for cellular homeostasis (Fang et al., 2017a). It has been reported that NAD⁺/NADH ratio declines with age in multiple organs, such as the brain, liver, muscles, and adipose tissue (Yoshino et al., 2011; Zhu et al., 2015; Zhang et al., 2016). Notably, decreased NAD⁺ levels

shorten nematodes lifespan, whereas genetic and/or pharmacological augmentation of intracellular NAD^+ levels protects against age-dependent metabolic impairment and promotes lifespan extension (Mouchiroud et al., 2013).

The beneficial effects of NAD^+ on cellular metabolism are mediated, at least in part, by sirtuins (Verdin, 2015; Fang et al., 2017a). As mentioned above, sirtuins are NAD^+ -dependent enzymes and their function has been implicated in the regulation of multiple cellular processes, including autophagy, energy metabolism, cell death, and aging. In mammalian cells, the sirtuin family consists of seven proteins, SIRT1–SIRT7. SIRT3, SIRT4, and SIRT5 are mitochondria-targeted proteins, SIRT1, SIRT6, and SIRT7 are mainly nuclear localized, and SIRT2 displays a broad cytoplasmic distribution pattern (Houtkooper et al., 2012). In congruent with the age-associated decrease of NAD^+ levels, sirtuins levels and activity decline also with age. Both mitochondrial SIRT3 and SIRT7 protein levels are reduced in aged tissues (Brown et al., 2013; Mohrin et al., 2015). Upregulation of their expression restores energy metabolism and regenerative capacity, and abolishes age-related deterioration of hematopoietic stem cells (Brown et al., 2013; Mohrin et al., 2015). Additionally, SIRT3 regulates the acetylation status of several mitochondrial proteins mediating adaptive neuronal responses to physiological challenges and protecting against neurodegeneration (Cheng et al., 2016). A recent study has implicated SIRT4 activity in the maintenance of mitochondrial homeostasis via mitophagy stimulation. Interestingly, SIRT4 associates with OPA1, an inner mitochondrial membrane GTPase, influencing mitochondrial morphology in response to mitochondrial damage (Lang et al., 2017).

The most well-studied member of sirtuins is SIRT1. Attenuation of SIRT1 enzymatic activity affects cellular metabolism and aging (Sebastian et al., 2012). Although the lifespan-extending properties of SIRT1 homolog in flies and nematodes have been challenged, its conserved role in longevity is now widely accepted, and has been verified also in mammals (Burnett et al., 2011; Viswanathan and Guarente, 2011; Satoh et al., 2013). In *C. elegans*, NAD^+ supplementation extends lifespan and protects mitochondrial metabolism in an SIR-2.1-dependent manner (Mouchiroud et al., 2013). Furthermore, there is evidence indicating that SIRT1 activity regulates autophagy (Lee et al., 2008). Caloric restriction is shown to mediate longevity through autophagy. Knocking down of SIR-2.1 diminishes autophagy and longevity in response to nutrient deprivation in nematodes (Morselli et al., 2010). Moreover, SIRT1 depletion results in accumulation of dysfunctional mitochondria and increased protein levels of the autophagy

substrate p62 in multiple organs (Lee et al., 2008). Additionally, *Sirt1*^{-/-} mice display excessive mitochondrial damage and increased lipid oxidation levels in hepatic cells, indicating a possible role of SIRT1 in mitochondrial selective autophagy (Boily et al., 2008). Indeed, it is demonstrated that defective NAD⁺–SIRT1–PGC-1 α axis leads to deregulation of PINK1 and subsequently to impaired mitophagy upon genotoxic stress (Fang et al., 2014). SIRT1, initially identified as a histone deacetylase, might serve as a cellular sensor translating metabolic imbalance to transcriptional outputs (Lin et al., 2004). Several transcription factors, including FOXO, NRF1, and PGC-1 α , among others, which participate in mitochondrial biogenesis, are modulated by SIRT1 enzymatic activity (Fig. 3) (Brunet et al., 2004; Rodgers et al., 2005; Kim et al., 2010). Notably, SIRT1 physically interacts and deacetylates PGC-1 α , enhancing its transcriptional activity during fasting (Rodgers et al., 2005). Several studies underline the pivotal role of SIRT1-dependent modulation of PGC-1 α activity as a detrimental regulatory step in cellular adaptation in response to energetic stress (Rodgers et al., 2005; Lagouge et al., 2006; Gerhart-Hines et al., 2007; Rodgers and Puigserver, 2007). Interestingly, impaired SIRT1 function influences also mtDNA metabolism. A recent study demonstrates that SIRT1 depletion reduced mitochondrial transcription factor A (TFAM) activity through its transcriptional regulation (Gomes et al., 2013). Therefore, decreased SIRT1, due to age-dependent decline of NAD⁺ intracellular levels, exacerbates deterioration of energy homeostasis.

Altogether, these results demonstrate that influencing the intracellular NAD⁺ concentration by using caloric restriction mimetics, fasting, exercise, or extracellular NAD⁺ supplementation could stimulate SIRT1–PGC-1 α axis, leading to mitophagy induction and subsequent upregulation of mitochondrial biogenesis (Fang et al., 2017a). In turn, the cells could adjust their mitochondrial content and fulfil their energy requirements in response to stress conditions. Although the delineation of SIRT1–PGC-1 α interplay represents a milestone in the field of energy metabolism, there are several mechanistic questions to be addressed, including how SIRT1/PGC-1 α association is driven in response to various stressors and whether it displays any tissue-specific pattern.



4. MITOPHAGY DEFICIENCY DURING AGING: LESSONS FROM MODEL ORGANISMS

Impaired mitochondrial metabolism, signified by elevated mtDNA mutation rate, decreased electron transport chain function, increased ROS

production, defective cytoplasmic calcium buffering, and uncontrolled release of pro-apoptotic factors, is a hallmark of aging, highlighting the pivotal role of mitochondria in organismal fitness (Lopez-Otin et al., 2013). Furthermore, cellular inability to remove dysfunctional organelles, because of the age-dependent autophagy decline, results in accumulation of defective mitochondrial population exacerbating energy metabolism collapse (Palikaras et al., 2017). Therefore, mitochondrial activity is under a constant control to maintain cellular homeostasis and survival.

Recent studies demonstrate the essential role of mitophagy throughout life. For example, it is implicated in paternal mitochondria elimination upon fertilization, in developmental processes such as retina ganglion cell differentiation, erythrocyte, and T lymphocytes maturation, and in the prevention of age-associated pathologies (Pua et al., 2009; Sato and Sato, 2011; Palikaras et al. 2015b, 2017; Esteban-Martinez and Boya, 2017; Fang et al., 2017a). Aberrant accumulation of defective mitochondria has been observed in various tissues and organisms during aging (Wallace, 1999; Preston et al., 2008; Palikaras et al., 2015b). Accelerated mutation rates and deletions of mtDNA have been associated with impairment of energy metabolism resulting in age-related disorders (Vafai and Mootha, 2012; Bratic and Larsson, 2013). Moreover, mitochondrial damage is shown to be accompanied by a senescence response underlining its association with aging phenotypes (Wiley et al., 2016). Thus, elimination of damaged mitochondria seems to be a “safe-guard” process preserving cellular function, tissue homeostasis, and organismal healthspan.

Several conserved longevity pathways modulate aging via mitophagy stimulation. In *C. elegans*, mitophagy induction is required for lifespan extension of several long-lived mutants, including animals with impaired mitochondrial function, reduced activity of insulin/IGF-1 signaling, and caloric restricted mutants, among others (Palikaras et al., 2015b). Furthermore, moderate mitochondrial defects and hypoxia-like responses promote longevity in a mitophagy-dependent manner (Schiavi et al., 2015). In congruent with the cytoprotective role of mitophagy in nematodes, a recent study uncovered that mitochondrial removal is diminished with age in mice (Sun et al., 2015). Cells with high-energy demands, such as hepatic and kidney cells, require constitutively active and fine-tuned mitochondrial function to sustain their homeostasis. Additionally, postmitotic cells, including neurons and cardiomyocytes that need to survive throughout the lifetime of an organism, display increased mitochondrial population and enhanced sensitivity to oxidative damage with age (Palikaras et al., 2017). Notably,

old mice display approximately 70% decrease of mitophagy in the hippocampus, a brain area essential for memory and learning (Sun et al., 2015 Mol Cell). Furthermore, mitophagy is reduced in a mouse model of Huntington's disease, highlighting the importance of mitochondrial turnover under pathological conditions (Sun et al., 2015). Satellite cells are muscle-specific stem cells characterized by elevated regenerative capacity and long lifespan. Hence, age-dependent failure of mitochondrial elimination leads to decreased number of muscle satellite stem cells in mice and human due to proteostasis collapse and excessive mitochondrial damage (Garcia-Prat et al., 2016). Age-associated myopathy and sarcopenia are defined by increased mitochondrial dysfunction, impaired energy metabolism, and decreased mitochondrial degradation rate. Chronic inflammation is accompanied by muscle degeneration and diminished anti-inflammatory responses during aging (Woods et al., 2012). Thus, mitophagy levels could be influenced by chronic inflammation leading to sarcopenia. Indeed, a recent study demonstrates that the anti-inflammatory cytokine interleukin 10 (IL-10) regulates immune responses by promoting mitophagy (Ip et al., 2017). IL-10 inhibits mTOR via AMPK activation and mediates the removal of dysfunctional mitochondrial, preventing inflammasome stimulation and the progression of inflammatory diseases (Sag et al., 2008; Ko et al., 2016; Ip et al., 2017).

Mitophagy deficiency results in uncontrolled accumulation of damaged organelles, which are characterized mainly by impaired mitochondrial proteostasis and function (Palikaras et al., 2015b; Schiavi et al., 2015). In turn, defective mitochondria set up an intricate communication with the nucleus sustaining cellular homeostasis and survival. In *C. elegans*, SKN-1, the homolog of the mammalian nuclear factor-erythroid 2-related factor 2 (NRF2), is activated and orchestrates the transcription of several nuclear genes regulating both mitophagy and mitochondrial biogenesis in response to mitochondrial damage (Ghose et al., 2013; Palikaras et al., 2015b). The importance of this intricate interplay between the mitochondria and nucleus is also underlined in progeria diseases, such as Cockayne syndrome, xeroderma pigmentosum group A, and ataxia telangiectasia, characterized by defective DNA repair mechanisms (Fang et al., 2014, 2016). Age-dependent accretion of nuclear DNA damage leads to permanent activation of poly (adenosine diphosphate ribose) polymerase 1 (PARP1), which detects and corrects DNA lesions by recruiting DNA repair complexes (Fig. 3). Although, the role of PARP1 stimulation in DNA maintenance is beneficial, its persistent activation diminishes NAD^+ levels and SIRT1 enzymatic activity, resulting in mitophagy and mitochondrial biogenesis defects

(Fang et al., 2014). Similarly, depletion of PME-1, the nematode homolog of PARP1, improves energy metabolism and promotes longevity by enhancing intracellular NAD^+ concentration and SIR-2.1 activity (Mouchiroud et al., 2013).



5. SYNTHETIC AND NATURAL COMPOUNDS TO MODULATE MITOPHAGY DURING AGING

Since a wide range of human pathologies, including cardiovascular disorders, hepatic failure, autoimmune diseases, and neurodegeneration, among others, are linked with aberrant mitochondrial accumulation and defective energy metabolism, selective priming of impaired organelles could be detrimental for the cellular and tissues physiology. Mitophagy regulation holds a promise for the development of novel therapeutic strategies to tackle several age-associated diseases through the maintenance of mitochondrial homeostasis. Therefore, many synthetic and/or natural compounds have been proposed to regulate mitochondrial elimination promoting cellular viability and enhancing healthspan during aging.

Inducers of sirtuins enzymatic activity, including sirtuin activating compounds (STACs), NAD^+ precursors (e.g., nicotinamide mononucleotide, NMN and nicotinamide riboside, NR) and resveratrol, among others, have been shown to ameliorate age-related pathologies by modulating mitophagy and repairing mitochondrial function (Howitz et al., 2003; Feige et al., 2008; Canto and Auwerx, 2009; Yoshino et al., 2011; Mouchiroud et al., 2013; Bonkowski and Sinclair, 2016; Park et al., 2016). Furthermore, NAD^+ supplementation sustains mitochondrial metabolism, protects against several neurodegenerative phenotypes, and promotes healthspan and lifespan in nematodes and mouse models of progeria syndromes (Mouchiroud et al., 2013; Fang et al. 2014, 2016). Although it has been reported that resveratrol and other sirtuins activators display beneficial effects on cellular metabolism and age-related deterioration, several studies have challenged the lifespan extending properties of these compounds (Pearson et al., 2008; Zarse et al., 2010; Poulsen et al., 2013).

Given the proposed endosymbiotic theory, mitochondria homeostasis is severely affected by antibiotics (Richter et al., 2013). Recent studies report that antibiotics, such as actinonin and doxycycline, interfere with energy metabolism and mediate mitophagy in mammalian cells (Sun et al., 2015; Xing et al., 2017). Thus, controlled use of antibiotics could be beneficial for mitochondrial homeostasis maintenance and prevention of healthspan-limiting diseases.

Furthermore, naturally occurring compounds, including spermidine and urolithin A, preserve mitochondrial function and prolong longevity through mitophagy induction. It is demonstrated that spermidine supplementation promotes lifespan extension in many model organisms, including yeast, nematodes, flies, and mice (Eisenberg et al., 2009). Autophagy has a pivotal role in spermidine-mediated longevity. Notably, dietary supplementation of spermidine induces autophagy, whereas genetic and/or pharmacological inhibition of autophagic pathway abolishes its beneficial effects on animals' physiology during aging (Eisenberg et al., 2009; Morselli et al., 2010). Moreover, spermidine administration ameliorates cardiovascular pathologies and age-associated cardiac alterations, such as hypertrophy, arterial stiffness, impaired diastolic function, maintaining arterial physiology, and cardiac activity in old animals (LaRocca et al., 2013; Eisenberg et al., 2016). Interestingly, the cardioprotective effects of spermidine are mediated through mitophagy stimulation and restoration of cardiomyocytes energy homeostasis (Eisenberg et al., 2016).

An additional first-in-class mitophagy inducing compound is urolithin A. Urolithin A is an ellagitannins-derived metabolite from pomegranate seeds, which initiates mitochondrial removal upon its dietary supplementation (Ryu et al., 2016). The potential therapeutic activity of urolithin A is mainly highlighted by general improvements of mitochondrial metabolism resulting in increased exercise capacity, elevated muscle function, and lifespan extension both in nematodes and rats (Ryu et al., 2016). Interestingly, both SKN-1-dependent lifespan extension and enhanced mitochondrial biogenesis, which are documented in response to long-term urolithin A administration, indicate that urolithin A might coordinate mitochondrial biogenesis and mitophagy to sustain energy homeostasis. In addition to urolithin A, tomatadine, a natural substance found in unripe tomatoes, stimulates mitochondrial elimination leading to increased longevity and improved muscular function both in nematodes and mice (Fang et al., 2017b). Tomatadine treatment mediates mitohormesis resulting in elevated ROS levels and SKN-1/NRF2 activation (Fang et al., 2017b). Thereby, mitophagy and mitochondrial biogenesis in coordination contribute to preserving tissue homeostasis and organismal survival.



6. CONCLUSIONS

Since genetic manipulations could influence the organismal lifespan, awareness has grown that aging is a fine-tuned biological process rather

than a passive damage accumulation that occurs in a haphazard manner (Klass, 1983; Friedman and Johnson, 1988; Kenyon et al., 1993). Therefore, there is an extensive effort in the scientific community to delineate the molecular underpinnings and interfere with the rate and quality of organismal aging. The global aged population is projected to substantially rise the next decades. Notably, it is expected that 25% of the world population will be older than 65 years by the year 2100 (Fang et al., 2015). This represents an international concern because of the severe socioeconomic and healthcare challenges associated with aging. Although impressive progress has been made in understanding the physiological and molecular requirements for aging, many questions remain elusive with regard to how this biological process is regulated.

Mitochondria are placed in the center of aging research. Energy homeostasis is impaired during aging, affecting survival and longevity. Furthermore, accrual of defective organelles is underlined as a key feature of multiple age-related pathologies, including neurodegenerative disorders, myopathies, inflammatory diseases, and metabolic syndromes, among others. Thus, maintenance of mitochondrial function, through a fine-tuned mitochondrial quality control system, is a critical factor of cellular and organismal homeostasis. The tight interplay between mitophagy and mitochondrial biogenesis preserves a healthy mitochondrial population, promoting stress resistance and lifespan extension (Fig. 3). Although major milestones in mitophagy research have been achieved, several uncertainties remain to be elucidated, such as the tissue-specificity component and dependency on the disease context.

Taken together, several recent studies suggest that rejuvenation of mitochondrial network or boosting energy metabolism could be an efficient strategy to tackle aging and age-associated disorders. Alongside, numerous screenings are taking place to identify synthetic or natural molecules that can be used to promote healthspan through the coordination of mitophagy and mitochondrial biogenesis (Fig. 3) (Suliman and Piantadosi, 2016; Palikaras et al., 2017). Although the results obtained from recent animal studies by using novel mitophagy modulators are encouraging, the consequences on human physiology and their therapeutic potential remain unclear. Therefore, interventional clinical trials should be organized and promoted to investigate the therapeutic potential of mitophagy- and mitochondrial biogenesis-inducers against age-related pathologies.

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Conflict of interest

The authors declare no conflict of interest.

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