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Mitophagy

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Synonyms

Mitochondrial selective autophagy

Definition

Mitophagy is an evolutionary conserved cellular process that sustains mitochondrial homeostasis by mediating the elimination of superfluous and/or defective mitochondria. Mitophagy can be classified as basal, stress-induced, and programmed based on physiological context. Failure to properly carry out mitophagy deregulates mitochondrial metabolism and causes progressive accumulation of defective organelles leading to the deterioration of biological systems, often culminating in tissue collapse.

Basic Characteristics

Molecular Mechanisms of Mitophagy

Cells have evolved several molecular signaling pathways to eliminate defective organelles and adjust their mitochondrial pool in response to environmental and/or intracellular stimuli. Hence, different signals can promote mitophagy via multiple signaling cascades in distinct cellular contexts. Although mitophagy pathways are classified as ubiquitin-dependent or -independent, several studies highlight the intricate interplay between different signaling and execution mechanisms, and emphasize the conservation of mitophagy regulators in eukaryotes (Table 1) (Harper et al. 2018; Palikaras et al. 2018).

The PINK1/Parkin Pathway

The PINK1 (phosphatase and tensin homolog (PTEN)-induced putative kinase 1)/Parkin is the most well-studied molecular pathway known to mediate ubiquitin-dependent mitochondrial removal. Several aspects of mitochondrial metabolism, such as mitochondrial dynamics, biogenesis, transport, and recruitment of autophagic machinery, are associated with the induction of the PINK1/Parkin pathway to assure the degradation of damaged organelles.

In healthy mitochondria, the PINK1 kinase is transported into the inner mitochondrial membrane (IMM), where it is processed and cleaved by mitochondrial proteases. In turn, the truncated form of PINK1 is degraded by the proteasome

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Mitophagy, Table 1 Mitophagy components are evolutionary conserved

Model organisms					
S. serevisiae	*C. elegans*	*D. melanogaster*	*M. musculus*	*H. sapiens*	Molecular function
–	SQST-1	Ref(2)a	p62/SQST1	p62/SQST1	Adaptor protein
–	PINK-1	Pink1	PINK1	PINK1	Mitochondrial kinase
–	PDR-1	Park	Parkin/PARK2	Parkin/PARK2	E3 ubiquitin ligase
Atg8	LGG-1/-2	Atg8a	MAP1LC3	MAP1LC3	Autophagosomal protein
–	DCT-1	–	BNIP3	BNIP3	Mitophagy receptor
–	–	–	NIX	NIX	Mitophagy receptor
Atg32		–	BCL2L13	BCL2L13	Mitophagy receptor
–	FNDC-1	–	FUNDC1	FUNDC1	Mitophagy receptor
Phb2	PHB-2	Phb2	PHB2	PHB2	Mitophagy receptor
–	FZO-1	Fzo, Dmfn	MFN2	MFN2	Mitochondrial GTPase
Dnm1	DRP-1	Drp1	DRP1	DRP1	Mitochondrial GTPase
Mgm1	EAT-3	DmeI/Opa1	OPA1	OPA1	Mitochondrial GTPase

system. Upon stress conditions, mitochondrial membrane is depolarized mediating the stabilization of PINK1 on the outer mitochondrial membrane (OMM). Subsequently, the enzymatic activity of PINK1 is enhanced by its auto-phosphorylation and promotes the recruitment of the E3 ubiquitin ligase Parkin to mitochondrial surface (Sekine and Youle 2018). PINK1 phosphorylates both Parkin triggering its E3 ligase activity and ubiquitin molecules or poly-ubiquitin chains on depolarized mitochondria. Inactive Parkin is translocated and bound to phopho-ubiquitin molecules or chains leading to its subsequent activation by PINK1. Then, Parkin mediates a feed-forward mechanism generating poly-ubiquitin chains, which are substrates for PINK1, amplifying mitophagy signal (Harper et al. 2018). In addition to its beneficial effects on Parkin activation and recruitment, PINK1-dependent phosphorylation of ubiquitin and poly-ubiquitin chains ameliorates hydrolysis by deubiquitinating enzymes (Harper et al. 2018). Several deubiquitinases, such as USP15, USP30, and USP35, block mitophagy by removing Parkin-generated ubiquitin chains from its mitochondrial substrates (Harper et al. 2018). Hence, a fine-tuned balance between ubiquitination and deubiquitination events regulates energy metabolism and underlines poly-ubiquitination as an "eat-me" signal for defective mitochondria.

Following its translocation, Parkin mediates poly-ubiquitination of several OMM proteins leading eventually to their recognition by autophagy adaptors or proteasomal degradation. Parkin-dependent proteasomal turnover of mitofusins (MFN1/2) diminishes mitochondrial fusion mediating the isolation of damaged organelles from the healthy mitochondrial population (Harper et al. 2018). MFN2 is also phosphorylated by PINK1 and thereby functions as a scaffold protein for Parkin recruitment upon mitochondrial stress. Additionally, PINK1 influences indirectly DRP1 activity promoting fission of defective mitochondria for their autophagic elimination. Furthermore, mitochondrial motility is also reduced during energetic stress. Mitochondria Rho-GTPase (Miro), an OMM protein that anchors mitochondria to cytoskeleton, is degraded upon Parkin-driven ubiquitination. Miro turnover mediates inhibition of mitochondrial transport in response to mitochondrial membrane dissipation (Harper et al. 2018; Palikaras et al. 2018; Sekine and Youle 2018). Collectively, inhibition of mitochondrial motility and enhanced fission might promote mitophagy, presumably by generating smaller organelles that can easily be engulfed by autophagosomes.

Although Parkin is a major mitophagy regulator, mitochondrial elimination does not exclusively rely on its activity. Several ubiquitin E3 ligases, such as Gp78, SMURF1, SIAH, MUL1,

and ARIH1, have been reported to ubiquitinate mitochondrial proteins facilitating mitophagy (Harper et al. 2018; Montava-Garriga and Ganley 2019; Palikaras et al. 2018). Once poly-ubiquitin chains have been generated on mitochondrial surface, they stimulate the recruitment of autophagy adaptor molecules, including p62/SQST1, optineurin 1 (OPTN1), nuclear dot protein 52 (NDP52) among others. In turn, autophagy adaptors interact directly with the autophagosomal light chain 3 (LC3) protein via their LIR (LC3-interacting region) motifs promoting autophagosome biogenesis and sequestration of dysfunctional mitochondria (Harper et al. 2018; Montava-Garriga and Ganley 2019; Palikaras et al. 2018).

Receptor-Mediated Mitophagy

Multiple OMM proteins serve as mitophagy receptors interacting directly with autophagosomal machinery and fine-tuning mitochondrial number in response to various stimuli in mammalian cells. Mitophagy receptors bind directly to autophagosomal membrane proteins via their LIR motifs promoting mitochondrial degradation.

Genetic studies in *Saccharomyces cerevisiae* have identified Atg32 as an essential protein for mitochondrial removal. Atg32 is an OMM protein associating with cargo-specific protein Atg8 and Atg11 adaptor to facilitate autophagosome formation. The kinases Hog1 and CK2 (casein kinase 2) regulate Atg32 phosphorylation status promoting its association with Atg11 adaptor (Montava-Garriga and Ganley 2019). Interestingly, Atg11 recruits also Dnm1 (the homologue of DRP1 in yeast) to enhance mitochondrial fission leading to the isolation of damaged organelles. Hence, Atg32 mediate the formation of a multiprotein complex together with Atg8 and Atg11 regulating mitochondrial network morphology and mitophagy stimulation. Recently, BCL-2-like protein 13 (BCL2L13) has been characterized as a functional homologue of Atg32 in mammals. BCL2L13 is localized on the OMM and interacts directly with LC3 via its LIR motif upon mitochondrial stress (Sekine and Youle 2018; Montava-Garriga and Ganley 2019).

NIX has a pivotal role in the elimination of entire mitochondrial population during erythrocytes differentiation. Indeed, NIX-deficient cells display mitochondrial accumulation leading to elevated apoptotic events and subsequently to developmental deficits (Montava-Garriga and Ganley 2019; Palikaras et al. 2018). In addition to NIX, BNIP3 (BCL2 interacting protein 3) acts as a mitophagy receptor mediating mitochondrial removal. Interestingly, BNIP3 also regulates mitochondrial fission and fusion through its direct association with OPA1 and DRP1 in response to mitochondrial damage. Mutations in the LIR motifs of both NIX and BNIP3 disturb their physical interaction with autophagosomal proteins and deregulate mitophagy. Multiple lines of experimental evidence indicate a complex interplay between mitophagy receptors and the PINK1/Parkin signaling pathway. Indeed, both NIX and BNIP3 require Parkin recruitment to preserve mitochondrial metabolism. Notably, Parkin targets and ubiquitinates NIX promoting its recognition by autophagy adaptors to enhance mitochondrial clearance (Montava-Garriga and Ganley 2019; Palikaras et al. 2018). Recently, DCT-1, the mammalian homologue of BNIP3 and NIX in nematodes, is underlined as a key mediator of mitophagy sustaining cellular homeostasis and viability during challenged conditions. In congruent with its mammalian counterparts, DCT-1 is also ubiquitinated by PDR-1 (the nematode Parkin homologue) in a PINK1-dependent manner during stress-induced mitophagy. Interestingly, BNIP3 prevents PINK1 proteolytic cleavage and mediates its stabilization on the mitochondrial surface (Montava-Garriga and Ganley 2019; Palikaras et al. 2018).

FUN14 domain-containing protein 1 (FUNDC1) is a highly conserved OMM protein that facilitates the selective elimination of damaged organelles during hypoxic stress (Georgakopoulos et al. 2017; Montava-Garriga and Ganley 2019; Palikaras et al. 2018). The Sc and CK2 kinases regulate the activation of FUNDC1 by modulating the phosphorylation status of its LIR motif and thereby inhibiting the recruitment of autophagic machinery under non-stress conditions. FUNDC1 influences

mitochondrial network morphology through its associations with both fission and fusion components in response to hypoxia. Indeed, the mitochondrial phosphatase PGAM5 dephosphorylates FUNDC1 promoting its dissociation with OPA1 and eventually preventing mitochondrial fusion. Then, FUNDC1 is recruited to ER-mitochondrial contact sites stimulating DRP1 translocation and subsequently mitochondrial network fragmentation. In addition to FUNDC1, both NIX and BNIP3 have been involved in the execution of mitochondrial removal upon low oxygen levels (Georgakopoulos et al. 2017; Montava-Garriga and Ganley 2019; Palikaras et al. 2018). Interestingly, HIF1 (hypoxia inducible factor 1) regulates transcriptionally both BNIP3 and NIX enhancing mitophagy during hypoxic-like conditions (Palikaras et al. 2018).

Recent studies revealed an unexpected role of IMM proteins and phospholipids in mitochondrial turnover. OMM is disrupted in response to excessive stress conditions resulting in elevated ROS levels, mtDNA release, and cytoplasmic externalization of several IMM proteins. Notably, prohibitin 2 (PHB2) and cardiolipin, which are primarily distributed in IMM, are exposed to the cytoplasm and trigger mitophagy through the direct association with their LIR motifs with LC3 autophagosomal protein (Montava-Garriga and Ganley 2019; Palikaras et al. 2018).

Taken together, this experimental evidence underscores that the coordinated and compensatory action of OMM and IMM mitophagy receptors might assure the efficiency of mitochondrial surveillance and energy metabolism in response to multiple stimuli.

Basal, Stress-Induced, and Programmed Mitophagy

Until recently, information on basal mitophagy levels was limited. However, the generation of transgenic animals expressing mitophagy reporters endorsed the in vivo investigation of mitochondrial degradation under physiological conditions (Palikaras et al. 2018; Montava-Garriga and Ganley 2019). Although most cell types undergo continuously basal mitophagy during routine mitochondrial quality control, the extent differs across tissues or even between cells within the same tissue. Notably, mitophagy is highly induced in heart, skeletal muscles, brain, liver, and kidney, whereas thymus and spleen display low levels of mitochondrial removal (Palikaras et al. 2018; Montava-Garriga and Ganley 2019). Surprisingly, the PINK1/Parkin pathway does not regulate basal mitophagy both in mammals and flies, highlighting the existence of different mitophagy regulators under physiological and stress conditions (Montava-Garriga and Ganley 2019).

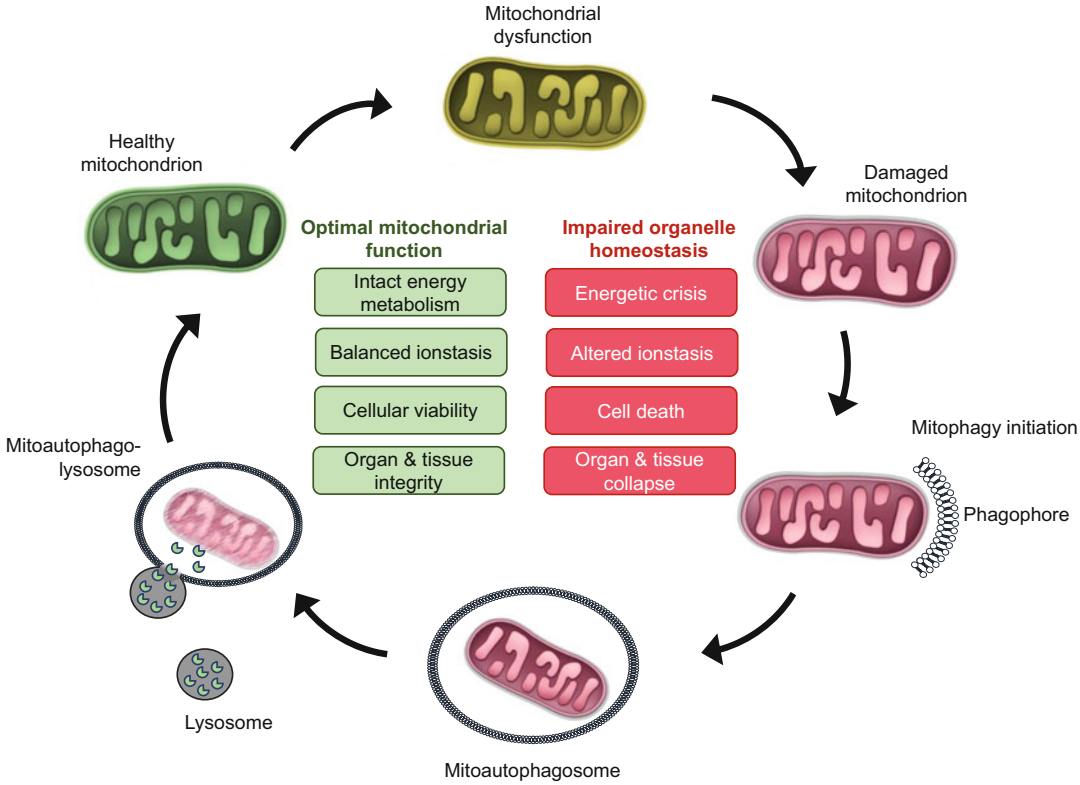
Mitochondrial homeostasis is impaired by extracellular and/or environmental stimuli that induce acute clearance of damaged mitochondria, by stress-induced mitophagy. Nutrient starvation, hypoxia, insecticides, and mitochondrial toxins deregulate electron transport chain (ETC) function and subsequently trigger energetic stress. In turn, stress-induced mitophagy facilitates mitochondrial quality control mediating the adjustment and adaptation of cellular metabolism to the external challenge. Nutrient deprivation promotes re-shaping of mitochondrial network and autophagy induction to degrade cytoplasmic materials and supply cells with building blocks for re-use and metabolism (Murphy and Hartley 2018; Palikaras et al. 2018; Montava-Garriga and Ganley 2019). Damaged and small sized mitochondria are initially removed through mitophagy to sustain a healthy mitochondrial pool during short period of starvation. Then, mitochondrial fission is prevented leading to unopposed fusion events. Elongated mitochondrial network is protected against autophagic degradation and display enhanced capacity of energy production (Montava-Garriga and Ganley 2019). On the other hand, prolonged starvation stimulates excessive mitochondrial elimination to renew and optimize mitochondrial population to the new environmental conditions. These observations underline the ability of eukaryotic cells to differentially regulate mitophagy under certain conditions of starvation. Indeed, yeast cells induce or inhibit mitophagy depending on carbon source in response to nitrogen starvation (Palikaras et al. 2018). General toxicants and mitochondrial uncouplers, including paraquat, valinomycin,

oligomycin, and CCCP among others, have been widely used to induce mitochondrial depolarization and subsequently mediate PINK1/Parkin-dependent mitophagy (Georgakopoulos et al. 2017). Alternatively, hypoxia mainly engages receptor-mediated mitophagy to mediate mitochondrial quality and quantity (Georgakopoulos et al. 2017; Montava-Garriga and Ganley 2019; Palikaras et al. 2018). Lack of oxygen results in defective mitochondrial respiration and energetic stress. Thus, a metabolic switch from oxidative phosphorylation (OXPHOS) to glycolysis is taking place attenuating the features of mitochondrial population. HIF1, the master regulator of hypoxic responses, orchestrates and coordinates both metabolic switch and mitophagy through the transcriptional regulation of glycolytic and mitophagic genes (Georgakopoulos et al. 2017; Montava-Garriga and Ganley 2019; Palikaras et al. 2018). Cytoplasmic iron chelation is an alternative procedure to trigger hypoxic-like response (Georgakopoulos et al. 2017). Although the impact of iron deprivation on mitochondrial physiology is not well studied, iron chelating agents, such as deferiprone (DFP) and 2′,2-bipyridyl (BP), are potent mitophagy inducers both in vitro and in vivo. Interestingly, DFP and BP activate different molecular signaling pathways to mediate mitochondrial removal underlining the complexity and diversity of mitophagy induction even under similar cellular responses (Georgakopoulos et al. 2017).

The induction of programmed mitophagy is a critical event during organismal development. Erythrocytes maturation requires the removal of all the internal organelles from immature erythroblast. NIX-mediated mitochondrial elimination is essential for erythrocytes differentiation and organismal physiology, since NIX deficient mice retain mitochondria in their peripheral blood cells displaying defective erythroid maturation and anemia (Montava-Garriga and Ganley 2019; Palikaras et al. 2018). Furthermore, mitophagy regulates the degradation of sperm-derived mitochondria upon oocytes fertilization in nematodes, flies, and mice preventing paternal mtDNA inheritance (Palikaras et al. 2018). Mitophagy has a prominent role in cardiomyocyte maturation. Adult heart is an organ with enhanced metabolic demands that preferentially utilizes fatty acids for energy production in mitochondria. During cardiomyocyte maturation, programmed mitophagy is taking place to eliminate fetal mitochondria, which primarily use glucose for energy generation. Therefore, mitochondrial population is functionally and morphologically altered to meet the contractile demands of the adult heart (Palikaras et al. 2018). Re-shaping and re-adjusting mitochondrial network fine-tune the transition from OXPHOS to glycolysis and vice versa. Indeed, mitophagy-mediated glycolytic shift is pivotal both for retinal ganglion cells differentiation and macrophage polarization (Montava-Garriga and Ganley 2019). Moreover, mitophagy-driven mitochondrial rejuvenation dictates several features of stem cell biology. Although stem cells rely on glycolytic metabolism and exhibit few spherical mitochondria, accumulating evidence underscores the impact of mitochondrial homeostasis and mitophagy on stem cells activation, fate, and senescence (Zhang et al. 2018).

Drugs

Mitochondria are cellular organelles specialized for energy production and critically influence several features of cell metabolism and physiology. The maintenance of healthy mitochondrial population is a prerequisite for cellular and tissue homeostasis. Compromised mitochondrial function results in the transformation of cellular powerhouses to “hotspots” of metabolic stress. Hence, it is not surprising that mitochondrial damage is associated with a broad spectrum of pathologies, such as ageing, myopathies, cardiovascular, metabolic and neurodegenerative diseases (Fig. 1). Given that the consequences of defective mitochondrial function can be detrimental for cellular viability, interventions modulating mitochondrial turnover hold a promise for considerable therapeutic potential and have been in the spotlight of scientific research. Pharmacological screenings are taking place to identify novel chemical compounds that may be used to manipulate the



Mitophagy, Fig. 1 Contribution of proper mitochondrial function to cellular and tissue homeostasis. In physiological conditions, intact mitochondrial population sustain energy metabolism, ionstasis, and cellular survival resulting in subsequent organ and tissue integrity. Excessive mitochondrial damage disrupts energy production and promotes ROS elevation and ionic imbalance, which trigger cell death pathways and often culminating in tissue collapse. Efficient elimination of dysfunctional organelles via mitophagy ensures mitochondrial homeostasis protecting against deterioration of biological systems

clearance of defective mitochondria and restore cellular energetic status. To this direction, several synthetic and natural chemical molecules have been utilized to modulate mitophagy (Georgakopoulos et al. 2017; Murphy and Hartley 2018; Palikaras et al. 2017).

Recently, mitophagy modulators, including general mitochondrial targeting agents, NAD^+ precursor molecules, and naturally occurring products among others, have been shown to enhance energy metabolism, healthspan, and organismal survival.

General Mitochondrial Targeting Agents to Manipulate Mitochondrial Turnover

Various pharmacological agents have been shown to interfere with mitochondrial ETC function and trigger mitophagy. Mitochondrial toxicants, such as paraquat, carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), and carbonyl cyanide-*p*-(trifluoromethoxy) phenyl hydrazone (FCCP), have been widely utilized to induce mitochondrial elimination through initiation of the PINK1/Parkin signaling cascade in mammalian cells and nematodes (Georgakopoulos et al. 2017). On the other hand, well-established ROS-generators, including rotenone, 6-OHDA (6-hydroxyldopamine) and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), are not able to

promote PINK1/Parkin-mediated mitophagy due to their moderate effects on mitochondrial membrane potential (Georgakopoulos et al. 2017). Notably, cardiolipin is released and binds to LC3 facilitating mitochondrial removal upon rotenone and 6-OHDA treatment. Despite the widespread application of general mitochondrial toxicants in basal research, their nonselective properties and off-target effects limit their use for therapeutic interventions (Fig. 2).

Kinetin triphosphate (KTP; 6-furfurylaminopurine) is a modified analog of ATP that binds and amplifies PINK1 catalytic activity both in vitro and in vivo. Supplementation of KTP diminishes mitochondrial motility, enhances Parkin translocation on depolarized organelles, and ameliorates apoptosis in a PINK1-dependent manner. Although KTP was initially identified and applied in clinical trials as mRNA splicing moderator, its safety and ability to cross the blood-brain barrier highlight its therapeutic potential in pathological conditions with defective PINK1 enzymatic activity, such as Parkinson's disease (Georgakopoulos et al. 2017).

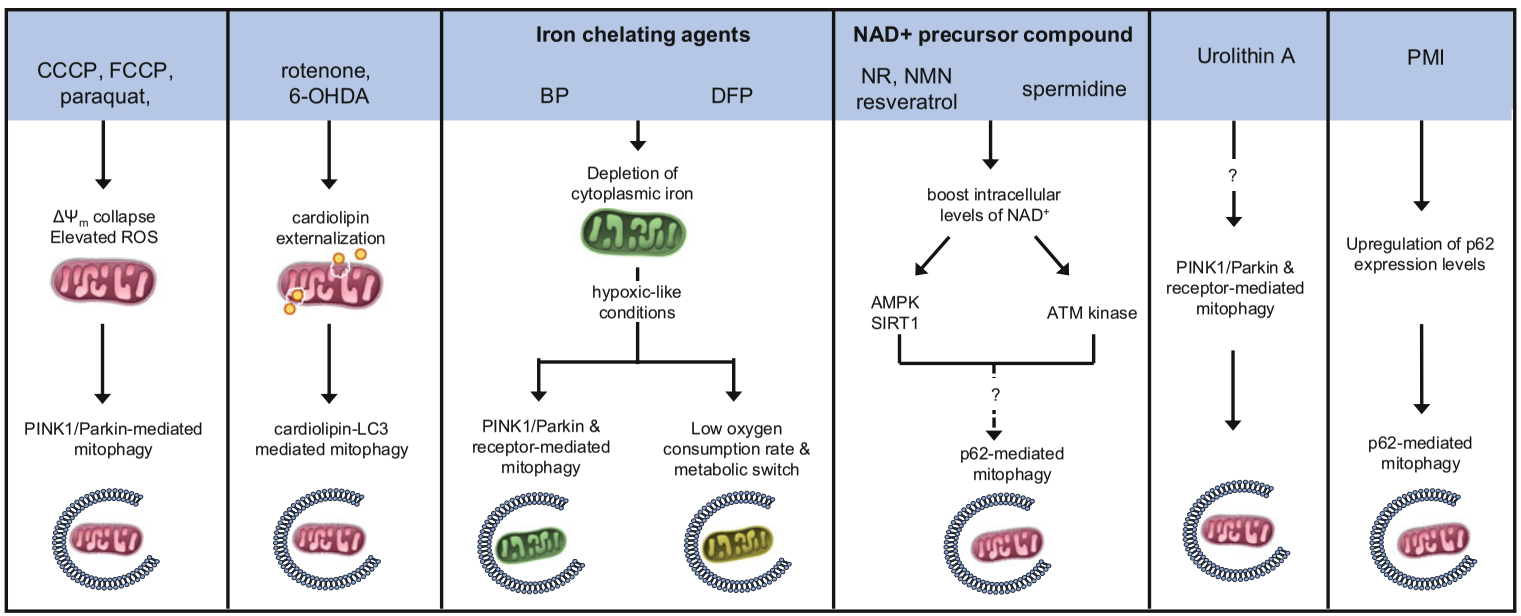
To avoid nonspecific and toxic effects of uncontrolled mitochondrial membrane potential collapse by proton ionophores, a synthesized chemical compound called p62-mediated mitophagy inducer (PMI) was developed. PMI promotes the expression of p62/SQST1 adaptor protein forcing mitochondria to be sequestered and degraded by autophagosomes (Fig. 2). Interestingly, PMI does not affect mitochondrial bioenergetics and network morphology suggesting that its supplementation enhances the levels of basal mitophagy. This notion is further supported by the fact that PMI-induced mitophagy does not require the presence of PINK1 and/or Parkin (Georgakopoulos et al. 2017; Palikaras et al. 2017).

Maintenance of Mitochondrial Homeostasis Through the Regulation of NAD^+ Metabolism

Nicotinamide adenine dinucleotide (NAD^+) is an intracellular metabolite and a critical denominator of energy metabolism and organismal fitness. Age-dependent reduction of NAD^+ concentration is associated with premature ageing and several age-related pathologies, including cancer, metabolic syndrome, and neurodegeneration (Fang et al. 2017; Katsyuba and Auwerx 2017).

NAD^+ acts as a co-factor regulating the enzymatic activity of several proteins, including sirtuins and poly ADP-ribose polymerase 1 (PARP1) among others. Sirtuins are deacetylating enzymes and their activity relies mainly on NAD^+ intracellular levels. Sirtuins are involved in the regulation of multiple cellular processes, such as DNA damage responses, autophagy, and mitochondrial function (Katsyuba and Auwerx 2017). Supplementation of the NAD^+ precursor molecules, nicotinamide (NAM), nicotinamide riboside (NR), and nicotinamide mononucleotide (NMN), boosts the intracellular NAD^+ levels enhancing energy metabolism, neuroprotection, and lifespan extension (Lou et al. 2019). Notably, NAM and NMN is recently documented to induce mitophagy in mammals and nematodes. The beneficial effects of NAD^+ replenishment on mitochondrial metabolism and organismal homeostasis are mediated, at least in part, by sirtuins (Fang et al. 2017). Indeed, NR and NMN treatment have been shown to induce NAD^+ – SIRT1 axis preventing protein aggregates accumulation and cognitive decline in Alzheimers' disease (AD) animal models via the restoration of mitochondrial activity and mitophagy (Lou et al. 2019). Moreover, administration of NR stimulates both the expression and activity of additional sirtuins, such as SIRT3 and SIRT6, ameliorating the hearing-loss defects in AD mouse models (Fang et al. 2017; Lou et al. 2019).

PARP1 is a NAD^+-consuming enzyme that is activated to maintain genome integrity through the recruitment of DNA repairing complexes during genotoxic stress. Accumulation of age-dependent DNA damage triggers the overstimulation of PARP1 resulting in diminished NAD^+ levels, reduced SIRT1 activity, and impairment of mitochondrial metabolism and mitophagy (Fang et al. 2017). Olaparib is a very well-studied inhibitor of PARP1 enzymatic activity. Interestingly, olaparib



Mitophagy, Fig. 2 Overview of mitochondrial targeting agents that stimulates mitophagy. The molecular mechanisms and mitophagy-inducing properties of several synthetic and natural small molecules, such as CCCP, FCCP, paraquat, rotenone, 6-OHDA, iron chelating agents, NAD^+ precursors, spermidine, urolithin A, and PMI, are described

administration promotes mitophagy and restores mitochondrial homeostasis. Congruently, genetic ablation of PME-1 (the homolog of the mammalian PARP1 in *Caenorhabditis elegans*) enhances mitochondrial homeostasis and lifespan extension by augmenting intracellular NAD^+ content and sirtuins activity (Fang et al. 2017; Katsyuba and Auwerx 2017).

Altogether, these results underscore that genetic and chemical-induced modulation of intracellular NAD^+ pool might propel the development of novel therapeutic strategies to sustain energy homeostasis and cellular viability by stimulating the degradation of damaged mitochondria (Fig. 2). Although the beneficial outcome of NAD^+ replenishment methods has been investigated in several animal models, their off-target effects are questioned, highlighting the crucial and multifactorial role of NAD^+ in several cellular processes, including mitochondrial biogenesis, general autophagy, and tumorigenesis among others (Fang et al. 2017; Katsyuba and Auwerx 2017).

Natural Chemical Agents with Mitophagic Capacities

Mitophagy-inducing abilities of natural small-molecule drugs have been assessed in several model organisms, including yeast, flies, nematodes, and mice. Resveratrol, spermidine, urolithin A are first-in class mitophagy inducers that have been shown to adjust, preserve, and restore mitochondrial population and energy metabolism (Berman et al. 2017; Lou et al. 2019; Madeo et al. 2018; Palikaras et al. 2018).

Resveratrol is a natural phenol compound, which is highly concentrated in red grapes skin. Resveratrol has been characterized as a caloric restriction mimetic exerting anti-inflammatory and anti-ageing effects. This natural product has attracted the attention of scientific community due to its potential therapeutic properties against several human pathologies, such as neurological disorders, cardiovascular diseases, and diabetes (Berman et al. 2017). Experimental evidence indicates that resveratrol increases the intracellular NAD^+ concentration via AMP-protein kinase (APMK) activity resulting in the subsequent stimulation of SIRT1 (Fig. 2). Moreover, mitochondrial metabolism and biogenesis is upregulated in response to resveratrol treatment alleviating muscular and metabolic defects in obese mice (Palikaras et al. 2017).

Polyamines, putrescine, spermidine, and spermine, are involved in the regulation of multiple cellular processes, such as mitochondrial homeostasis, innate immunity, cell growth, and proliferation. Therefore, the biosynthetic and metabolic pathways of polyamines are tightly associated with organismal survival and viability. Indeed, the intracellular content of spermidine is gradually reduced in several cell types with age leading to the deterioration of biological systems (Madeo et al. 2018). Chronic supplementation of spermidine enhances memory and lifespan in yeast, flies, nematodes, and mice, in an autophagy-dependent manner. Recent studies demonstrate that chronic administration of spermidine promotes also mitophagy sustaining heart function and renal homeostasis in aged rodents (Madeo et al. 2018). Ataxia telangiectasia mutated (ATM) kinase stimulates the PINK1/Parkin pathway upon spermidine treatment. ATM is involved in DNA damage responses preserving cellular physiology by altering mitochondrial activity and mitophagy upregulation. Notably, spermidine-induced mitochondrial membrane potential collapse leads to ATM activation and, in turn, PINK is stabilized on OMM and recruits Parkin on mitochondrial surface to initiate autophagosomal generation (Fig. 2) (Palikaras et al. 2018).

Urolithin A (UA) is the most abundant ellagitannin-derived metabolite in human body and is mainly generated by intestinal microflora upon consumption of fruits and nuts, such as pomegranate, raspberries, strawberries, walnuts, and almonds. Although the molecular mechanism of UA function is poorly understood, its antioxidant, anti-inflammatory, and anti-tumor properties are very well established (Palikaras et al. 2017). Recently, supplementation of UA is shown to improve muscle function and lifespan by stimulating mitophagy in both nematodes and mice

(Palikaras et al. 2017, 2018). Furthermore, UA-induced mitophagy is shown to ameliorate cognitive defects, inflammatory responses, and protein aggregates in neuronal and microglia cells of AD nematode and mouse models (Lou et al. 2019). Interestingly, genetic studies in *C. elegans* uncovered that UA relies on BEC-1, PINK-1, SQST-1, and DCT-1 (the mammalian homologs of Beclin, PINK1, p62/SQST1, and BNIP3/NIX, respectively) to promote longevity, whereas PINK-1 and PDR-1 (the mammalian homolog of Parkin) are required for its neuroprotective effects indicating that there is an intricate communication between mitophagy regulators to sustain energy metabolism and cellular homeostasis in a cell-type and tissue-specific manner (Fig. 2). Moreover, the UA beneficial effects on cellular and organismal physiology are independent of dietary conditions and age. Thus, UA treatment could be used as a novel therapeutic strategy to rejuvenate mitochondrial metabolism and protect against age-dependent decline of muscular activity and mobility deficits (Palikaras et al. 2017, 2018). Indeed, the report of the first-in-human clinical study in which UA is orally administrated to healthy, sedentary elderly individuals highlights the successful translation of its benefits to humans, underlined by UA-mediated improvements on mitochondrial homeostasis and muscle activity together with its safety and bioavailability profile (Andreux et al. 2019).

Mitophagy Inhibitors

Despite the beneficial effects of mitophagy in mitochondrial metabolism and cellular homeostasis, runaway mitochondrial clearance promotes shrinkage of mitochondrial pool, overstressing the remaining organelles, triggering energetic crisis, and eventually leading to cell death (Kubli and Gustafsson 2012). Hence, several chemical substances have been identified and used to prevent mitophagy.

The most commonly used method to inhibit mitophagy relies on lysosomal inhibitors, such as bafilomycin, chloroquine, and berbamine, which could impair lysosomal acidification or the process of fusion between autophagosomal and lysosomal membranes (Georgakopoulos et al. 2017). An alternative indirect approach to block mitochondrial removal is via the regulation of fission/fusion machinery. Mitochondrial network fragmentation is a perquisite event for mitophagy under stress conditions. Administration of mitochondrial division inhibitor-1 (mdivi-1) disturbs mitochondrial morphology by preventing fission and subsequently mitophagy in yeast and mammalian cells (Georgakopoulos et al. 2017; Murphy and Hartley 2018). Mechanistically mdivi-1 disturbs enzymatic activity of DRP1 protecting against oxygen-glucose deprivation and glutamate driven neurotoxicity. Recently, a novel peptide inhibitor was generated to manipulate FUNDC1-meidated mitochondrial clearance. This specific peptide is cell-permeable and associates directly with the LIR motif of FUNDC1. Indeed, this LIR mimetic peptide diminishes the physical interaction between FUNDC1 and LC3 preventing FCCP- and hypoxia-induced mitophagy (Georgakopoulos et al. 2017). Notably, general autophagy was not affected underlining peptide selectivity and endorsing the rationale for the designing of specific peptides mimetics to modulate mitophagy execution.

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