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Mechanisms of mitophagy in cellular homeostasis, physiology and pathology

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Mitophagy is an evolutionarily conserved cellular process to remove dysfunctional or superfluous mitochondria, thus fine-tuning mitochondrial number and preserving energy metabolism. In this Review, we survey recent advances towards elucidating the molecular mechanisms that mediate mitochondrial elimination and the signalling pathways that govern mitophagy. We consider the contributions of mitophagy in physiological and pathological contexts and discuss emerging findings, highlighting the potential value of mitophagy modulation in therapeutic intervention.

Mitochondria are essential to cellular metabolism and physiology, and mitochondrial damage is associated with a broad spectrum of pathologies[1]. As defective mitochondria can be detrimental to cellular homeostasis, quality control mechanisms have evolved to restore and preserve energy metabolism[2]. Although homeostatic pathways mediate cellular responses to mitochondrial damage, persistent defects trigger elimination of the entire organelle through mitophagy[2]. Mitophagy impairment perturbs mitochondrial function and causes progressive accumulation of defective organelles, leading to cell and tissue damage. Here, we focus on the molecular pathways that orchestrate mitochondrial degradation, underlining challenges in our understanding. We discuss the pivotal roles of mitophagy in physiological and pathological conditions, highlighting potential therapeutic implications of mitophagy modulation.

Molecular pathways of mitophagy

Cells possess several mitophagy mechanisms, and different stimuli can promote mitophagy through multiple signalling cascades in distinct cellular contexts[1]. Mitophagy regulatory pathways are classified as ubiquitin-dependent or -independent[3]. Recent studies have revealed an intricate crosstalk between signalling and execution pathways, and underscore the conservation of mitophagy factors from yeast to mammals[4].

PINK1–Parkin-mediated mitophagy. The phosphatase and tensin homologue (PTEN)-induced putative kinase 1 (PINK1)–Parkin pathway regulates ubiquitin-dependent mitophagy[4]. Multiple aspects of mitochondrial physiology, including mitochondrial dynamics, biogenesis, transport and recruitment of autophagic machinery, converge on the PINK1–Parkin pathway to ensure elimination of defective organelles[1,2,4,5]. In functional mitochondria, PINK1 is transported into the inner mitochondrial membrane (IMM), where it is processed and cleaved by several proteases[5,6]. The truncated form of PINK1 is degraded by the ubiquitin–proteasome system[5,6]. Following membrane potential dissipation, mitochondrial import is prevented, facilitating PINK1 stabilization on the outer mitochondrial membrane (OMM)[5–7]. PINK1 is activated by auto-phosphorylation leading to Parkin translocation to the mitochondrial surface[4–6]. Emerging findings suggest a causative relationship between PINK1 kinase activity and Parkin stimulation during mitochondrial damage. PINK1-dependent phosphorylation alters Parkin conformation, promoting its association with the mitochondrial surface and triggering its E3 ligase activity[5,8]. PINK1 also phosphorylates ubiquitin (Ub) and poly-Ub chains on dysfunctional mitochondria. Inactive Parkin binds phospho-Ub facilitating its activation by PINK1, and Parkin mediates a feed-forward mechanism generating poly-Ub chains, which are substrates for PINK1, thereby amplifying mitophagy signals[5,9]. The majority of these findings are based on experiments performed under artificial conditions using immortal model cell systems, combined with the overexpression of *PINK1* or *Parkin* genes, which could interfere with mitophagy activation. Several studies show controversial results, making it difficult to dissect the molecular signalling of this feed-forward process in a physiological context.

In addition to their beneficial role in Parkin stimulation and retention on the OMM, phosphorylated Ub and poly-Ub chains display diminished hydrolysis by deubiquitinases[5]. Deubiquitinating enzymes, such as USP15, USP30 and USP35, counteract mitophagy by eliminating Parkin-generated Ub chains from the mitochondrial surface[10–14]. Therefore, a fine-tuned balance between ubiquitination and deubiquitination events regulates energy homeostasis, suggesting poly-Ub chains may function as an 'eat-me' signal for damaged organelles.

Parkin-mediated poly-ubiquitination of several OMM proteins leads to their recognition by autophagy adaptors or their proteasomal degradation[15–19]. However, the fate of Parkin substrates on the mitochondrial surface remains enigmatic. A recent study argues against extensive degradation of Parkin targets following mitochondrial depolarization in cancer and neuronal cells[17]. These results suggest either that the OMM proteins may be differentially ubiquitinated by Parkin and degraded in a cell- and/or tissue-specific manner, or that alterations in PINK1 or Parkin overexpression levels may generate different ubiquitination profiles and OMM protein degradation rates. Future studies with in vivo models or primary mammalian cells will further define the mechanisms that regulate Parkin activity and OMM proteins turnover rates following energetic stress.

The PINK1–Parkin pathway interferes with other mitochondrial quality control mechanisms, such as mitochondria-derived vesicles (MDVs) and mitochondrial dynamics, to sustain energy homeostasis[16,20–23]. Regarding mitochondrial dynamics, PINK1 indirectly triggers dynamin-related protein 1 (DRP1) activity, promoting fission of dysfunctional mitochondria, which enables their autophagic degradation[21]. Although DRP1 has an essential role in mitophagy, recent studies indicate the existence of DRP1-independent mitochondrial clearance[24]. Proteotoxic stress generates misfolded

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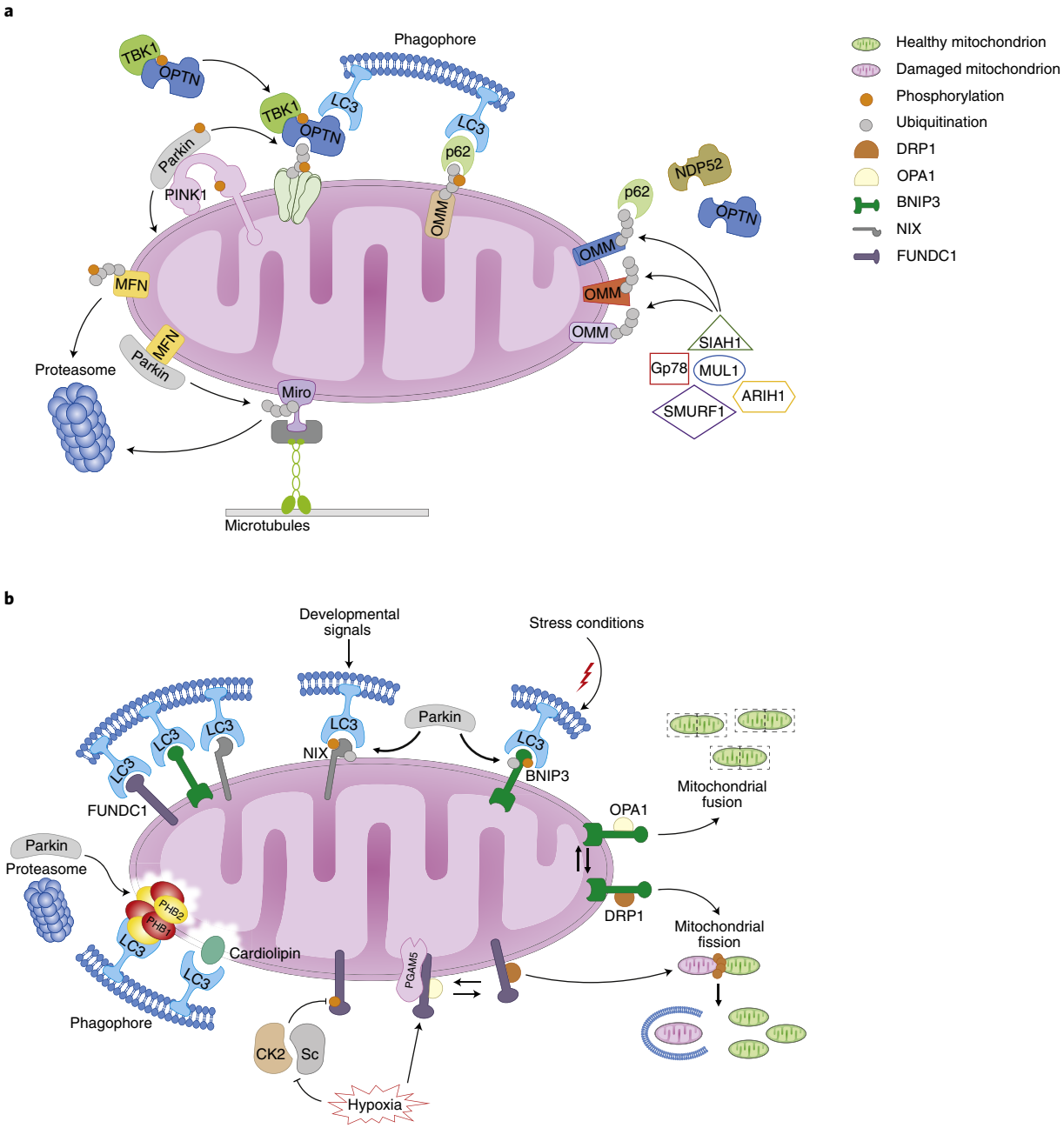

Fig. 1 | Mechanisms of mitochondrial selective autophagy. a, The PINK1–Parkin pathway of mitophagy. Following stress, PINK1 is stabilized on the OMM, promoting Parkin recruitment. Parkin ubiquitinates several outer membrane components. Poly-Ub chains are subsequently phosphorylated by PINK1 serving as an 'eat me' signal for the autophagic machinery. Adaptor proteins (p62, OPTN, NDP52) recognize phosphorylated poly-Ub chains on mitochondrial proteins and initiate autophagosome formation through binding with LC3. TBK1 phosphorylates OPTN, thereby enhancing its binding affinity to Ub chains. The OPTN–TBK1 complex establishes a feed-forward mechanism promoting mitochondrial clearance. Gp78, SMURF1, MUL1, SIAH1 and ARIH1 represent alternative E3 ubiquitin ligases targeting OMM proteins prior to mitophagy. The PINK1–Parkin pathway modulates mitochondrial dynamics and motility by targeting MFN and Miro for proteasomal degradation. **b**, Receptor-mediated mitophagy. BNIP3, NIX and FUNDC1 mitophagy receptors localize to the OMM and interact directly with LC3 to mediate mitochondrial elimination. PHB2 and cardiolipin are externalized to OMM and interact with LC3 following mitochondrial impairment. Different receptors ensure specificity of the process in different tissues and following diverse stimuli. NIX and BNIP3 phosphorylation enhances their association with LC3. Both CK2 and Sc kinases and PGAM5 phosphatase influence FUNDC1 phosphorylation status, regulating mitochondrial dynamics during hypoxia. Mitophagy receptors promote fission of damaged organelles through the disassembly and release of OPA1, and the recruitment of DRP1 on the mitochondrial surface. Parkin-dependent ubiquitination of NIX and BNIP3 highlights an intricate crosstalk between receptor-mediated mitophagy and the PINK1–Parkin pathway.

protein aggregates segregated into mitochondrial subdomains, which are removed through piece-meal mitophagy[24]. DRP1 may serve as an 'insulator' of piece-meal mitophagy, spatially restricting PINK1–Parkin activity to eliminate local mitochondrial damage. Although this model is consistent with the differential turnover rates of several mitochondrial proteins modulated by the PINK1–Parkin pathway, the involvement of DRP1-mediated fission requires further investigation in physiological contexts.

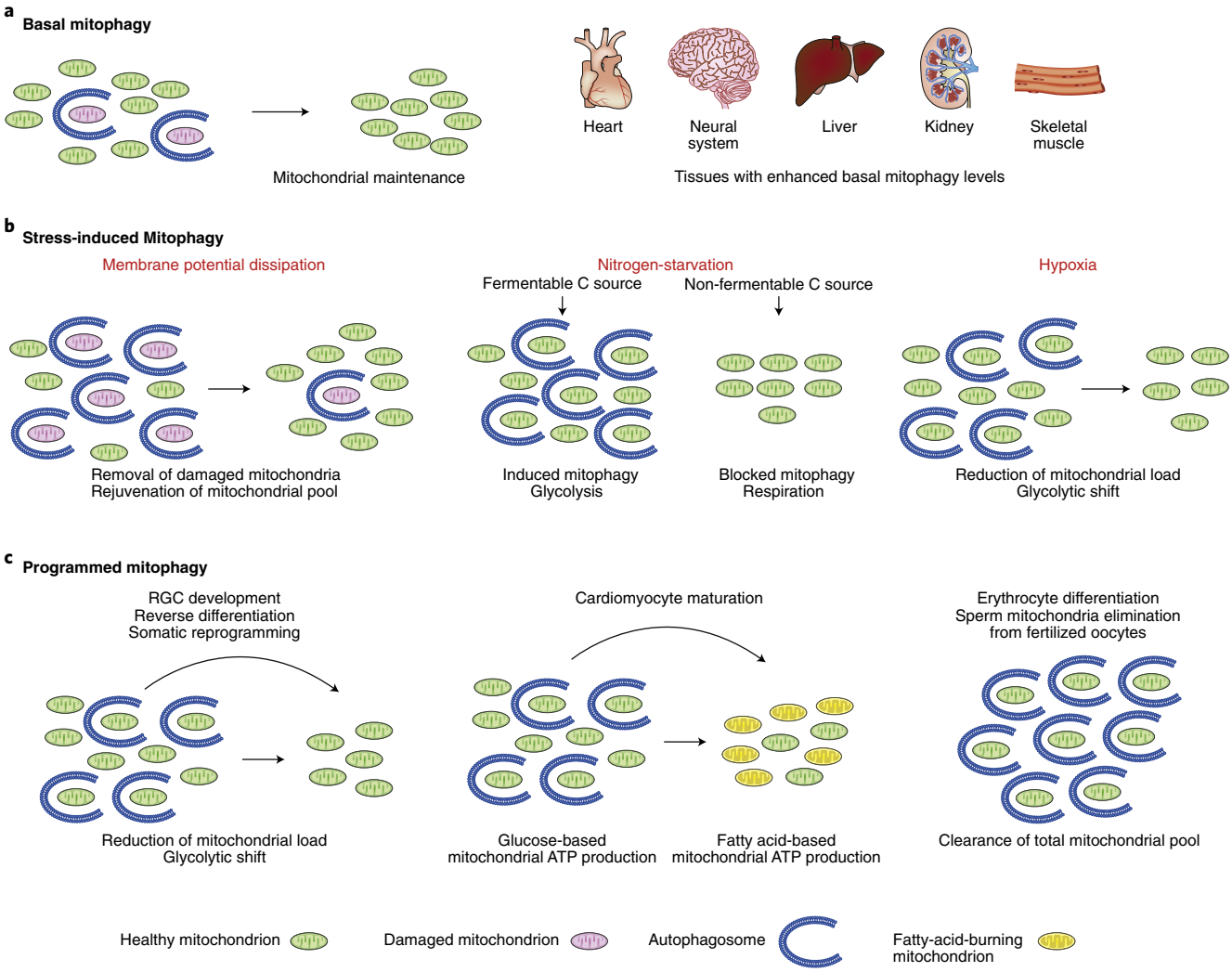

Fig. 2 | Physiological roles of mitophagy. Examples of mitophagy in physiological contexts. Effects of mitophagy on mitochondrial and cellular metabolism are noted in each case. **a**, Basal mitophagy; tissues of enhanced mitophagy levels, including heart, neural system, liver, kidney and skeletal muscle, are depicted. **b**, Stress-induced mitophagy; mitophagic effects during dissipation of mitochondrial membrane potential, nitrogen starvation and hypoxia are noted. **c**, Programmed mitophagy; mitophagic effects during retinal ganglion cell development (RGC), reverse differentiation towards pluripotency during somatic reprogramming, cardiomyocyte maturation, erythrocyte differentiation and sperm mitochondria elimination, are depicted. For simplicity, information on mitochondrial morphology alterations is not depicted.

Parkin-dependent proteasomal turnover of mitofusins (MFNs) abolishes mitochondrial fusion, resulting in the isolation of defective organelles from the healthy mitochondrial network[16,23]. Additionally, MFN2 may also function as a scaffold protein for Parkin translocation, in response to mitochondrial damage (Fig. 1a). PINK1 phosphorylates MFN2 triggering its association with Parkin following mitochondrial uncoupling[25]. PINK1–Parkin-mediated MFN2 degradation disrupts endoplasmic reticulum (ER)–mitochondria contact sites, separating damaged organelles, thereby facilitating their removal[26]. These results suggest that ER–mitochondria contacts may represent critical loci for mitophagy, where PINK1–Parkin activation and deubiquitination events occur. Notably, PINK1 and Beclin1 are recruited to mitochondria-associated membranes, enhancing autophagosomal formation following mitophagic stimulus[27]. Hence, ER–mitochondria contact sites could negatively regulate mitophagy by protecting specific OMM proteins from PINK1–Parkin-dependent degradation, thereby repressing organelle removal.

Mitochondrial motility is decreased during impaired energy metabolism[28]. Dissipation of the mitochondrial membrane potential stimulates PINK1-dependent phosphorylation of Miro, a Rho–GTPase of the OMM that anchors mitochondria to the cytoskeleton (Fig. 1a). Parkin ubiquitinates Miro, thereby promoting its degradation and inhibiting mitochondrial transport[28]. Thus, blocking mitochondrial transport and enhancing fission could facilitate mitophagy, presumably by providing smaller and immobile mitochondria that can be sequestered by autophagosomes.

Parkin-independent mitophagy. In addition to Parkin, several other ubiquitin E3 ligases, such as Gp78, SMURF1, SIAH1, MUL1 and ARIH1, function in mitophagy regulation[29–33]. Once localized on the mitochondrial surface, they generate ubiquitin chains, triggering the recruitment of autophagy adaptors, including optineurin (OPTN), nuclear dot protein 52 (NDP52) and p62, among others (Fig. 1a). Autophagic components such as unc-51-like autophagy activating kinase 1 (ULK1), double FYVE-domain containing protein 1 (DFCP1) and WD repeat domain, phosphoinositide interacting 1 (WIPI1), are recruited to mediate phagophore biogenesis and autophagosomal membrane expansion[34]. Autophagy adaptor molecules interact directly with autophagosomal light chain

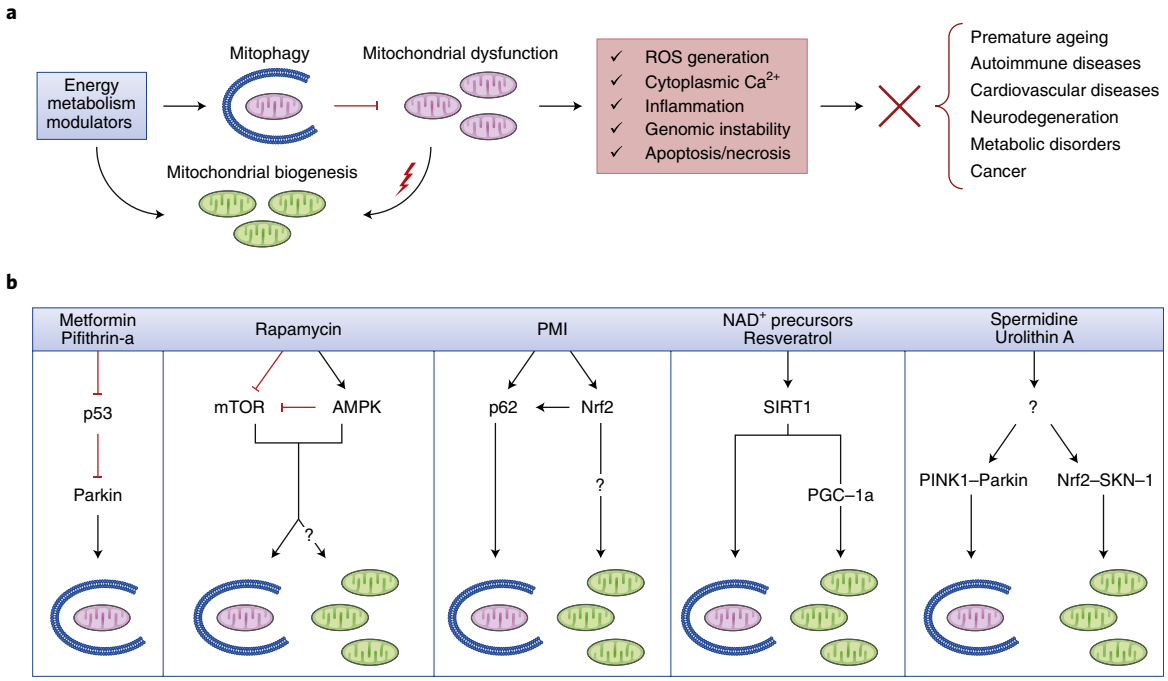

Fig. 3 | Chemical modulators of energy metabolism. a, Administration of chemical compounds, which modulate mitophagy and mitochondrial biogenesis, sustains energy metabolism leading to subsequent cellular and organismal survival. **b**, The molecular mechanisms and mitophagic/biogenic capacities of several energy modulators, such as metformin, pifithrin-a, rapamycin, PMI, NAD^+ precursors, resveratrol, spermidine and urolithin A, are depicted.

3 (LC3) through their LC3-interacting region (LIR) motifs, anchoring Ub-tagged mitochondria into autophagosomes (Fig. 1a). PINK1 ubiquitin kinase activity mediates OPTN and NDP52 recruitment on damaged mitochondria, stimulating mitophagy[34]. The serine/threonine-protein kinase TBK1 (TANK binding kinase 1) modulates the phosphorylation status of OPTN, NDP52 and p62 adaptors and subsequently enhances their binding affinity to Ub chains, promoting mitochondrial removal[5,35–37]. The OPTN–TBK1 complex facilitates a feed-forward mechanism, which reinforces OPTN binding to poly-Ub chains and promotes TBK1 stimulation and OPTN recruitment on defective organelles (Fig. 1a). Questions remain about the role of TBK1 in mitochondrial clearance: What is the initial signal that triggers TBK1 activation following mitochondrial damage? Does TBK1 kinase activity influence the function of proteins other than autophagy adaptors to modulate mitochondrial homeostasis? Does TBK1 promote autophagosomal degradation independently of its kinase activity? Several studies have shown that p62 is dispensable for mitophagy execution[34,38–42]. Therefore, p62 function in mitochondrial elimination is controversial and requires further investigation in cell type- and tissue-specific contexts.

Mitophagy receptors in energy metabolism. In addition to ubiquitin-dependent mitophagy, mitochondrial proteins serve as mitophagy receptors, targeting dysfunctional organelles directly to autophagosomes for degradation (Fig. 1b). Mitophagy receptors interact directly with LC3 and GABARAP autophagosomal membrane proteins through their LIR motifs, mediating mitochondrial elimination[43]. Several functional homologues of mitophagy receptors have been characterized in diverse species. Genetic studies in *Saccharomyces cerevisiae* have identified Atg32 as an essential protein for mitochondrial turnover during respiratory growth[43]. Atg32 localized to the OMM interacts with cargo-specific protein Atg8 and Atg11 adaptor to initiate autophagosome formation[43]. The kinases Hog1 and casein kinase 2 (CK2) regulate Atg32 phosphorylation, enhancing its association with Atg11 and stimulating mitophagy[44]. Atg11 promotes recruitment of mitochondrial fission component Dnm1 (the yeast homologue of human DRP1) to mediate mitochondrial fragmentation[45]. Thus, Atg32 generates a multi-protein complex with Atg8 and Atg11, coordinating mitochondrial dynamics and mitophagy initiation. BCL-2-like protein 13 (BCL2L13) is a functional homologue of Atg32 in vertebrates[46], which contains a LIR motif that enables its binding with LC3 during mitochondrial stress. BCL2L13 mediates mitochondrial fragmentation in DRP1-depleted cells, while inducing mitophagy in a Parkin-independent manner[46]. The anti-apoptotic FK506-binding protein 8 (FKBP8) also mediates Parkin-independent mitophagy. FKBP8 on the OMM interacts by its LIR motif with autophagosomal proteins to initiate mitochondrial degradation[47,48].

The OMM proteins NIX (NIP3-like protein X), BNIP3 (BCL2 interacting protein 3) and FUNDC1 (FUN14 domain- containing protein 1) are mitophagy receptors that fine-tune mitochondrial populations in response to various stimuli (Fig. 2). NIX has a critical role in programmed mitophagy during differentiation[49–52]. NIX-deficient cells accumulate mitochondria, leading to increased apoptosis and developmental defects[50–53]. LIR motif phosphorylation enhances NIX association with LC3 under stress conditions[54]. Although the signalling cascade of NIX-mediated mitophagy is not yet determined, Rheb small GTPase may be involved, as its mitochondrial localization and physical interaction with NIX regulates mitochondrial removal and maintenance of energy metabolism[55]. Similar to NIX, BNIP3 serves as a mitophagy receptor, mediating mitophagy. BNIP3 interferes with mitochondrial dynamics, promoting fission of damaged organelles through OPA1 disassembly and release, and DRP1 recruitment to the mitochondrial surface (Fig. 1b)[56,57]. BNIP3 mediates PINK1 stabilization by inhibiting its proteolytic cleavage[58]. Both NIX and BNIP3 sustain mitochondrial homeostasis through regulation of Parkin recruitment, suggesting crosstalk between mitophagy receptors and the PINK1–Parkin pathway[38,59]. NIX ubiquitination is affected by Parkin enzymatic activity, which promotes its recognition by autophagy adaptors, enhancing mitophagy[60]. Genetic and biochemical studies in *C. elegans* revealed that DCT-1, the homologue of mammalian BNIP3 and NIX, mediates

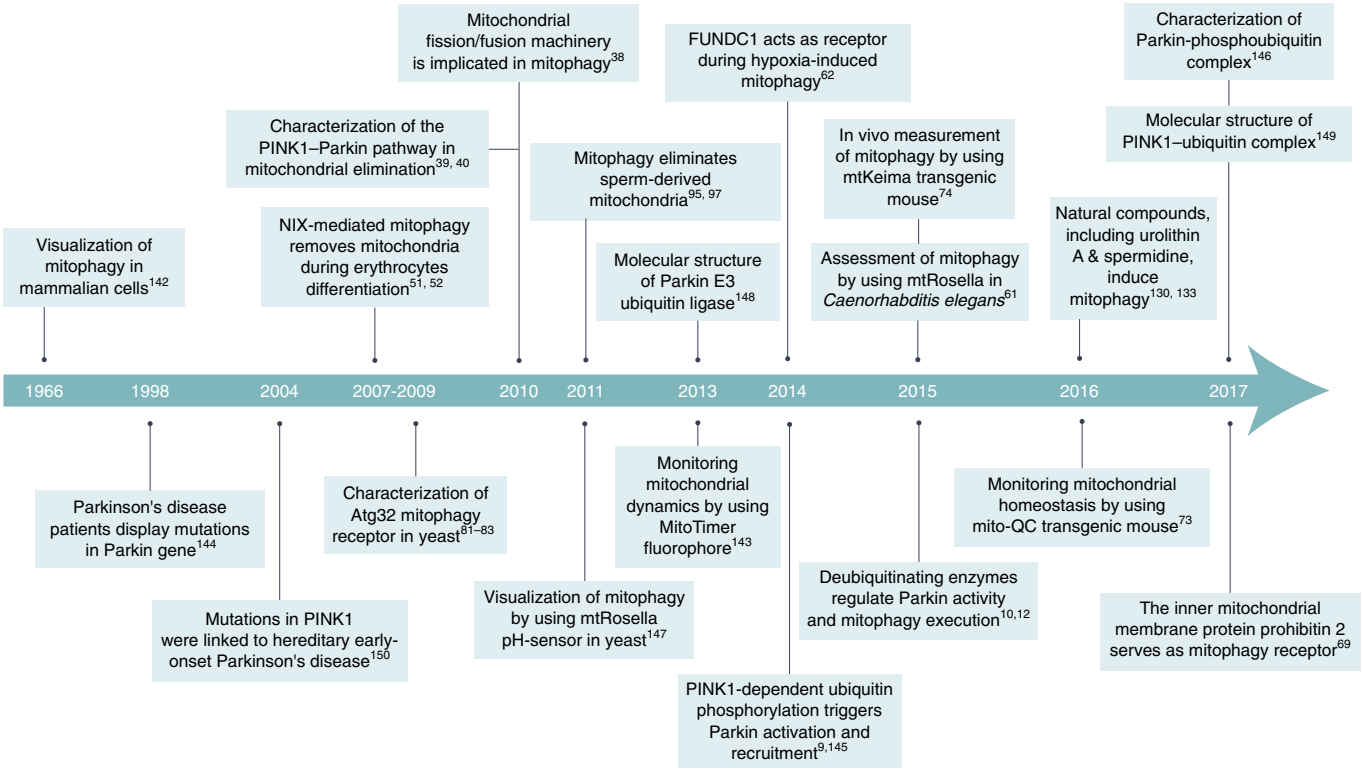

Fig. 4 | Milestones in mitophagy research. A selection of seminal contributions in mitophagy-related research are depicted in chronological order[10,12,38–40,51,52,61,62,73,74,81–83,95,97,130,133,142–150]. However, including all important discoveries was not possible due to space limitations.

mitophagy and preserves cellular and organismal homeostasis during ageing[61]. DCT-1 is also ubiquitinated by PDR-1 (the nematode Parkin homologue) in a PINK1-dependent manner following mitophagy-inducing conditions.

FUNDC1 is a conserved mitophagy receptor that promotes mitochondrial clearance during hypoxia (Fig. 1b)[62]. The Sc and CK2 kinases phosphorylate FUNDC1 LIR motif to suppress its activity under non-stress conditions[63]. FUNDC1 interacts with both fission and fusion machinery components, regulating mitochondrial dynamics. Mitochondrial phosphatase PGAM5 dephosphorylates FUNDC1, thereby disrupting its physical association with OPA1 and inhibiting mitochondrial fusion under hypoxic conditions[64]. In turn, FUNDC1 translocates to ER–mitochondrial contact sites, mediating DRP1 recruitment and mitochondrial fragmentation[65]. Thus, FUNDC1 coordinates mitochondrial morphology and mitophagy under stress (Fig. 1b). ULK1 could also phosphorylate FUNDC1, resulting in mitophagy stimulation[66]. FUNDC1 may serve as an ULK1 adaptor; their interaction promotes ULK1 relocation on mitochondria, allowing de novo phagophore biogenesis. In addition to FUNDC1, mitophagy during hypoxia is also regulated by NIX and BNIP3. The hypoxia response regulator HIF1 (hypoxia inducible factor 1), upregulates BNIP3 and NIX expression to promote mitophagy during hypoxic-like conditions[67,68]. Although crosstalk between FUNDC1, BNIP3 and NIX has not been fully characterized, their coordinated action ensures the efficiency of mitochondrial quality control and energy homeostasis.

Prohibitins (PHB) are IMM proteins that regulate mitochondrial metabolism and physiology[69]. PHB2 has been identified as a putative mitophagy receptor mediating Parkin-dependent mitochondrial removal during energetic stress[69]. Mitochondrial membrane depolarization and increased proteasomal activity results in OMM disruption, triggering PHB2 cytoplasmic exposure and subsequent association with LC3 (Fig. 2). A multi-protein complex composed of PHB2, LC3 and p62 regulates phagophore generation near dysfunctional mitochondria, promoting their elimination and preserving cellular homeostasis[70]. Cardiolipin is a phospholipid, primarily synthesized and distributed along the IMM. Like PHB2, cardiolipin is externalized to the OMM during mitochondrial damage[71]. The redistribution of cardiolipin and its interaction with LC3 initiates a signalling cascade that promotes engulfment of damaged mitochondria by autophagosomes (Fig. 1b)[71,72].

Taken together, the diverse repertoire of receptor and adaptor molecules highlights the existence of compensatory mechanisms that regulate mitochondrial number in response to environmental and/or intracellular signals. The complex interplay between mitophagy pathways ensures energy metabolism and tissue homeostasis. Thus, maintenance of mitochondrial function, through a fine-tuned mitochondrial quality control system, is critical for cellular and organismal survival.

Physiological roles of mitophagy

Depending on the physiological context, mitophagy is classified as basal, stress-induced or programmed. Basal mitophagy refers to continuous mitochondrial housekeeping that ensures the recycling of old and damaged organelles[73,74]. Extracellular stress signals affect mitochondrial physiology and may induce acute mitochondrial clearance, by stress-induced mitophagy[6]. 'Programmed mitophagy' is activated in different cell types during development (Fig. 2)[50–52].

Basal mitophagy. Until recently, mitophagy was mostly studied in vitro in cell lines overexpressing key components of the pathway following artificial induction by chemical compounds. Information on steady-state mitophagy levels, referred to as basal mitophagy, is thus limited (Fig. 2a). The study of basal mitophagy was enabled by the construction of two transgenic mouse models expressing mitochondria-targeted pH-sensitive fluorescent reporters[73,74]. Both transgenics showed that most cell types undergo basal mitophagy

during routine mitochondrial maintenance[73,74]. The extent varies between tissues and different cell types within the same tissue, suggesting cell autonomous regulation. Specifically, the thymus and spleen exhibit low levels of basal mitophagy in contrast to the heart, skeletal muscle, nervous system, hepatic and renal tissue[74]. Surprisingly, basal mitophagy is PINK1-independent in metabolically active tissues in contrast to chemically induced mitophagy, which is blocked in a *PINK1*$^{-/-}$ background[75]. Studies in *Drosophila* and mammalian cells demonstrate the dispensable role of the PINK1–Parkin pathway in basal mitophagy[76,77]. Several mitophagy regulatory factors do not display ubiquitous expression patterns across tissues, indicating tissue-specific mitophagy regulation, which warrants further investigation[78,79].

Stress-induced mitophagy. Mitophagy is potently induced during stress and facilitates mitochondrial quality control to mediate metabolic adjustment to an external challenge (Fig. 2b). Mitochondrial uncouplers induce the PINK1–Parkin pathway[80]. Alternatively, starvation or hypoxia mainly induce receptor-mediated mitophagy mechanisms[62,81]. In yeast, mitophagy is either induced or blocked following nitrogen starvation depending on the supplied carbon source. Non-fermentable carbon sources inhibit nitrogen-induced mitophagy whilst fermentable carbon sources promote mitophagy under the same conditions[82]. These findings suggest that mitophagy is regulated separately from bulk autophagy, which is invariably induced during starvation. As mentioned above, prolonged respiratory growth has been suggested to induce mitophagy in an Atg32-dependent manner[83]. Subsequently, mitophagy can be induced by nitrogen starvation even without respiration, and is temporarily distinct from bulk autophagy[84]. Further analysis is needed to unravel the role of mitophagy during starvation. It is tempting to speculate that following nutrient deprivation mitophagy participates in mitochondrial network regeneration to cope with the imposed stress. In this case, mitophagy would remove existing mitochondria poised to function in the presence of nutrients and mitochondrial biogenesis would supply new organelles, tailored to function under stress. This hypothesis is in accordance with the reported coordination between mitophagy and mitochondrial biogenesis[61]. Hypoxia leads to mitophagy-dependent reduction of mitochondrial mass affecting transcriptional regulation and post-translational modifications of mitophagy receptors[62,64,85]. This event may adjust mitochondrial load under hypoxia. Although a receptor-mediated mitophagic program is mainly implicated in hypoxia-induced mitochondrial clearance[62,66,86], putative crosstalk with the PINK1–Parkin pathway has also been suggested[58,62,66,86,87]. In mammalian cells, starvation- and hypoxia-induced mitophagy are partly independent of conventional macroautophagy and largely dependent on RAB9A and RAB9B, which are essential for alternative autophagy[88]. Several recent studies revealed communication between the endosomal pathway and mitochondrial elimination[89–92], highlighting the complexity of mitophagy pathways under different physiological conditions.

Programmed mitophagy. Several cell types undergo mitochondrial clearance during development (Fig. 2c). During erythrocyte differentiation, mitochondrial removal depends on NIX-mediated mitophagy in an ATG5-independent manner[51,52,93,94]. Additionally, mitophagy regulates the degradation of sperm-derived mitochondria from fertilized oocytes in nematodes, flies and early mouse embryos, preventing paternal mtDNA inheritance[95–97]. Mitophagy functions in cardiomyocyte maturation and adult heart cells, under enhanced metabolic demands, preferentially use fatty acids for ATP production in mitochondria[98]. During cardiomyocyte maturation, Parkin–MFN2–PINK1-mediated mitophagy removes fetal mitochondria that primarily use glucose for ATP production. Thus, the mitochondrial network is reformed, both functionally and morphologically, to meet the contractile demands of the adult heart[98].

Adjusting cellular mitochondrial load facilitates the transition from oxidative phosphorylation (OXPHOS) to glycolysis and vice versa. Tissue hypoxia triggers HIF1 stabilization leading to enhancement of NIX-dependent mitophagy concomitant with a switch from OXPHOS to glycolysis, during differentiation of retinal ganglion cells (RGCs)[50]. Similarly, mitophagy-driven glycolytic shift occurs during macrophage polarization towards the proinflammatory glycolytic M1 phenotype[50]. Additionally, both the stem cell identity of embryonic stem cells and their ability to differentiate depend on their metabolic status[99]. Pluripotent stem cells are highly glycolytic and contain few small spherical mitochondria. During pluripotent stem cell generation by somatic cell reprogramming, mitochondrial mass initially increases and subsequently decreases via NIX-dependent and mitochondrial membrane potential-independent mitophagy[100]. PINK1 deficiency results in impaired reprogramming of embryonic stem cells attenuating their metabolic features[99]. Conversely, during cellular differentiation, mitochondria become tubular in shape, their mass increases and their metabolism switches to OXPHOS[101]. Mitophagy is thus emerging as a critical regulator of metabolic rewiring, thereby regulating stem cell fate and somatic reprogramming[100,102].

Mitophagy in pathophysiology

Mitophagy impairment is associated with ageing and a plethora of pathological conditions, such as neurodegenerative diseases, myopathies, metabolic disorders, inflammation and cancer[1] (Fig. 3a). Below we discuss implications of mitophagy in the pathology of tissues with high metabolic demands, which are more sensitive to conditions disrupting mitochondrial quality control[1].

The human heart is one of the most energy consuming organs of our body. Mitochondrial function is intimately linked to cardiac function and the progression of age-related cardiomyopathies[1]. PINK1 protein levels are significantly reduced in end-state heart failure[103]. Cardiomyocytes of *PINK1*$^{-/-}$ mice have increased mitochondrial content with altered morphology and elevated ROS levels, and exhibit pathological cardiac hypertrophy and ventricular dysfunction[103], suggesting that mitophagy impairment contributes to heart pathology. Mitophagy is reduced both in aged mouse hearts and following doxorubicin-induced cardiotoxicity[104]. Moreover, mitophagy regulates platelet activation, conferring protection from ischemia/reperfusion (I/R)-induced heart injury[105,106]. These data underline the emerging role of mitophagy in normal cardiac function and protection after I/R injury.

Age-dependent decline of mitochondrial function is accompanied by mitophagy impairment, as shown by in vivo studies in vertebrate and invertebrate model organisms[61,74]. The latter raise the intriguing possibility that defective mitophagy triggers age-related accrual of dysfunctional mitochondria. Post-mitotic cells, like neurons, which need to survive throughout the lifetime of an organism, are particularly sensitive to mitochondrial dysfunction. Although critical in vivo data are lacking, mitophagy perturbations may be causally related to development of neurodegenerative diseases. Proteotoxic stress similar to that experienced by expression of mutant Huntingtin results in reduced mitophagy levels in vivo[74]. In hippocampal neurons of a mouse model for Alzheimer disease (AD) expressing mutant amyloid precursor protein (APP), PINK1 levels are diminished while mitochondrial number and oxidative stress are increased[107]. Consistently, pharmacological or genetic stimulation of mitophagy protects against development of AD-related phenotypes[108]. Mutations in *PARK2* and *PARK6*, the human genes encoding Parkin and PINK1 respectively, are linked with familial forms of Parkinson's disease (PD). In flies, mutations in PINK1 and Parkin homologues cause mitochondrial dysfunction associated with PD phenotypes, such as loss of dopaminergic neurons, muscle degeneration and decreased lifespan[109–111]. In mice, PINK1 and Parkin knockout failed to reproduce the PD pathology despite causing

pronounced mitochondrial dysfunction[112]. An emerging idea is that PINK1 and Parkin may also have mitophagy-independent roles in mitochondrial maintenance and impairment of other components may need to be combined with the PINK1–Parkin defect to trigger development of the PD phenotype. Indeed, when Parkin-depleted animals are crossed with mutator mice (homozygous for a DNA polymerase γ deficiency leading to accumulation of mitochondrial DNA mutations[113]), progeny develop age-related loss of TH^+ and DAT^+ neurons and associated motor symptoms that are rescued by L-DOPA treatment, mimicking PD phenotypes[114]. Although mitophagy disruption was not specifically assessed, the PINK1–Parkin pathway was enhanced in dopaminergic neurons, presumably because this cell type is under more mitochondrial stress than others[114]. Therefore, systemic impairment of PINK1–Parkin affects primarily dopaminergic neurons following additional stress.

Studies in patient-derived fibroblasts re-differentiated to TH^+ dopaminergic neurons revealed a pathway parallel to PINK1–Parkin that regulates mitophagy in both familial and sporadic cases of PD (ref. [115]). This pathway is associated with the most common mutation linked with PD pathogenesis, the G2019S mutation in the kinase domain of leucine rich repeat kinase 2 (LRRK2). LRRK2 regulates the immobilization of dysfunctional mitochondria. Partial knockdown of Miro rescues the effect of G2019S mutation. Although Miro is marked for proteasomal degradation by the PINK1–Parkin pathway[116], Parkin overexpression cannot rescue the delayed Miro degradation. These data highlight the existence of non-redundant molecular pathways regulating neuronal mitophagy, and emphasize the complexity of mitophagy-related defects leading to associated pathology[115,117].

PINK1–Parkin-pathway-deficiency has been suggested to contribute to neurodegeneration by regulating autoimmune responses through a mitochondrial antigen presentation pathway that relies on MDVs (ref. [118]). PINK1–Parkin-depletion enhanced mitochondrial antigen presentation on MHC-class I molecules of immune cells. Dopaminergic neurons express MHC-class I complexes that could explain their vulnerability to mitochondrial damage[118]. Moreover, defective neuronal mitochondria could be extruded from neuronal cells and degraded in adjacent astrocytes, as in retinal ganglion cell axonal mitochondria[119]. This mechanism suggests cell non-autonomous effects of mitophagy impairment in brain tissue with possible implications in neurodegeneration.

Hyperactivation of mitophagy can also result in pathologic conditions. Recently, the AAA-ATPase ATAD3 has been shown to facilitate PINK1 mitochondrial import, thereby suppressing mitophagy. ATAD3-depleted mice displayed decreased bone-marrow cellularity, erythroid anaemia and B cell lymphopenia, phenotypes that were largely rescued by PINK1 depletion[120]. NIX and BNIP3 overexpression cause apoptotic cell death of cardiomyocytes whilst BNIP3 deficiency protects from apoptosis following myocardial infarction and I/R injury[49,121,122]. Although it was hypothesized that the latter was mediated by the pro-apoptotic function of NIX and BNIP3, their currently appreciated function in mitophagy indicates a putative role for enhanced mitophagy in cardiomyocyte apoptosis.

Mitophagy modulation as therapeutic intervention

Impaired mitophagy is emerging as a common denominator among various pathological conditions. Thus, interventions targeting mitophagy may possess therapeutic potential (Fig. 3a). Pharmacological screens to identify chemical agents to manipulate and restore the efficient elimination of dysfunctional organelles are underway, and several synthetic and natural chemical compounds have been shown to modulate mitophagy[1,123].

General autophagy-inducing drugs, such as rapamycin and metformin, reportedly attenuate AMPK (AMP-activated protein kinase) and mTOR (mammalian target of rapamycin) activity, preserving energy metabolism possibly through mitophagy and mitochondrial biogenesis stimulation[124,125]. Rapamycin supplementation exerts beneficial effects against mitochondrial dysfunction by sustaining energy homeostasis and stress resistance in murine and human cells[126,127]. Metformin administration induces mitophagy by promoting Parkin activity through p53 downregulation[128]. Cytoplasmic p53 interacts directly with Parkin, suppressing mitochondrial removal. Genetic- and pifithrin-α-mediated inhibition of p53 promotes Parkin-dependent mitophagy, diminishing mitochondrial defects and protecting against glucose tolerance and heart failure (Fig. 3b)[104,129].

Naturally occurring compounds, including resveratrol, spermidine, urolithin A and antibiotics, have been shown to maintain mitochondrial integrity through mitophagy induction (Fig. 3b). Supplementation of these compounds results in mitophagy-mediated cytoprotective and anti-ageing effects through energy metabolism restoration in several model organisms, such as yeast, flies, nematodes and mice[74,130–134]. However, the molecular mechanisms of their action require further investigation. Resveratrol and urolithin A also promote mitochondrial biogenesis (Fig. 3b). Similar to synthetic NAD^+ precursor molecules, resveratrol activates the sirtuin 1 (SIRT1)–PGC-1α axis triggering organelle biosynthesis[135–137]. Urolithin A treatment may enhance mitochondrial biogenesis, possibly through SKN-1 (the mammalian homologue of nuclear factor E2-related factor 2, Nrf2) activation in nematodes[133]. SKN-1 coordinates both mitochondrial removal and biogenesis following oxidative stress[61]. PMI (p62–SQSTM1-mediated mitophagy inducer), a synthetic chemical substance, triggers p62-mediated mitophagy by stabilizing Nrf2 in mammalian cells[138]. Both p62 and NDP52 autophagy adaptors are transcriptionally regulated by Nrf2[139,140]. Nrf2 regulates both mitophagy and mitochondrial biogenesis, preserving mitochondrial metabolism[141]. The mitochondrial biogenic capacities of PMI and urolithin A require further investigation.

Proper mitochondrial function and organismal homeostasis necessitate tight coordination between mitochondrial biogenesis and degradation[1]. Identification of compounds with both biogenic and mitophagic activities holds promise for the development of therapeutic interventions with impact on human physiology and mitochondria-related diseases.

Concluding remarks

Major milestones in the mitophagy field have been achieved in the last decade (Fig. 4). Nevertheless, important questions remain regarding the in vivo role of mitophagy components, the spatiotemporal regulation of mitophagy within distinct physiological and pathological contexts and the complex interplay between different mitophagy pathways. Combining in vivo mitophagy imaging systems with disease animal models could help to unravel disease aetiology and progression and contribute to translational research. Chemical-induced mitophagy stimulation should be further evaluated in vivo in different cell types and tissues. Identifying mitophagy modulators may lead to therapeutic intervention strategies targeting mitochondrial-associated pathologies and provide critical insights with broad relevance to human health and quality of life.

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Competing interests

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