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Receptor-mediated mitophagy in yeast and mammalian systems

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Mitophagy, or mitochondria autophagy, plays a critical role in selective removal of damaged or unwanted mitochondria. Several protein receptors, including Atg32 in yeast, NIX/BNIP3L, BNIP3 and FUNDC1 in mammalian systems, directly act in mitophagy. Atg32 interacts with Atg8 and Atg11 on the surface of mitochondria, promoting core Atg protein assembly for mitophagy. NIX/BNIP3L, BNIP3 and FUNDC1 also have a classic motif to directly bind LC3 (Atg8 homolog in mammals) for activation of mitophagy. Recent studies have shown that receptor-mediated mitophagy is regulated by reversible protein phosphorylation. Casein kinase 2 (CK2) phosphorylates Atg32 and activates mitophagy in yeast. In contrast, in mammalian cells Src kinase and CK2 phosphorylate FUNDC1 to prevent mitophagy. Notably, in response to hypoxia and FCCP treatment, the mitochondrial phosphatase PGAM5 dephosphorylates FUNDC1 to activate mitophagy. Here, we mainly focus on recent advances in our understanding of the molecular mechanisms underlying the activation of receptor-mediated mitophagy and the implications of this catabolic process in health and disease.

***Keywords*:** mitochondria; quality control; mitophagy receptor; protein phosphorylation; mitochondrial stress; selective autophagy

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Introduction

Mitochondria determine both cell life and death [1-4]. Healthy mitochondria function as the powerhouses for energy conversion through the TCA cycle and oxidative phosphorylation. In response to death stimuli, mitochondrial outer membrane becomes permeabilized to release cytochrome c that could bind its cytoplasmic receptor Apaf1 to form the apoptosome and to activate caspase cascades for apoptosis, a form of programmed cell death [5]. Mitochondria are also the major sites for producing superoxide as an inevitable byproduct during oxidative phosphorylation [6-10]. Moreover, mitochondria are the center for iron metabolism and lipid oxidation [9, 11]. Given these key roles, damaged mitochondria could be detrimental to the cell. Accumulation of dysfunctional mitochondria is the characteristic of multiple types of diseases including heart failure, Alzheimer's disease, Parkinson's disease and cancers [12-15].

To maintain the well-being of the cell, eukaryotes have evolved a mechanism to segregate and remove damaged or unwanted mitochondria through mitophagy, an autophagy-dependent process specific to the energy-converting organelles. One of the relevant aspects about mitophagy is its evolutionary conservation. In the budding yeast *Saccharomyces cerevisiae*, transport of mitochondria to the vacuole, a lytic compartment, has been suggested as a possible mechanism by which mitochondrial DNA escapes to the nucleus [16]. Subsequent genetic approaches unambiguously reveal that mitochondria degradation in this unicellular eukaryote fully depends on a set of "core" autophagy-related (Atg) proteins essential for formation of autophagosomes, double membrane-bound vesicles enclosing disposable cargoes [17-20]. In addition to core Atg proteins and other factors required for autophagosome-vacuole fusion and the breakdown of autophagic bodies in the vacuolar lumen, a dozen molecules have

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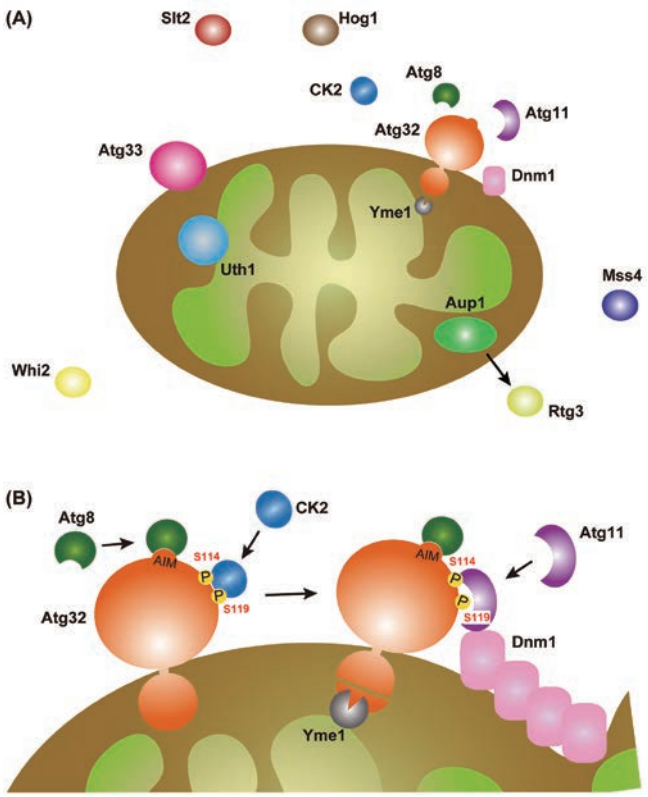

Figure 1 (A) Mitophagy-related proteins in yeast. Aup1 is a protein phosphatase in the mitochondrial IMS that may be linked to Rtg3, a transcription factor responsible for signaling from mitochondria to the nucleus [83, 84]. Uth1 is a mitochondrial inner membrane protein of unknown function [26, 85]. Atg33 is a mitochondrial outer membrane protein of unknown function [18, 26]. Whi2 is a stress response protein in the Ras-PKA signaling pathway [41, 86]. Mss4 is a phosphatidylinositol-4-phosphate 5-kinase [87]. Slt2 and Hog1 are MAP kinases acting in the Wsc1-Pkc1-Bck1-Mkk1/2-Slt2 and Ssk1-Pbs2-Hog1 signaling cascades, respectively [21, 34]. **(B)** Atg32 and its interacting partners. See text for details.

been reported as important for mitophagy in yeast (see Figure 1A and 1B). Among those, Atg32 is the key molecule that has been identified through two independent genome-wide screens for non-essential gene deletion mutants defective in degradation of mitochondria [19, 20]. Importantly, Atg32 serves as a protein receptor for recruiting Atg8, a conserved ubiquitin-like protein essential for all autophagy-related processes that is conjugated to the phospholipid phosphatidylethanolamine (PE) and localized to autophagosomes, and Atg11, a scaffold protein acting as a platform for core Atg protein assembly during selective autophagy-related processes [19, 21, 22].

In mammalian cells, previous studies have suggested that both NIX/BNIP3L and BNIP3 have a putative LC3-interacting region (LIR) that interacts with LC3, an Atg8 homolog, and defines NIX/BNIP3L as a mitophagy receptor. Another important mitophagy receptor is FUNDC1 containing a typical LIR motif Y(18)xxL [23]. In this review, recent progress toward understanding the regulation of receptor-mediated mitophagy will be focused and described.

Mitophagy in yeast

Mitophagy in yeast absolutely relies on Atg32, a single-pass membrane protein of 59 kDa localized on the outer membrane of mitochondria with its N and C termini facing the cytosol and mitochondrial intermembrane space (IMS), respectively [19, 20]. When cells are grown in non-fermentable medium, a condition in which mitochondrial respiration is indispensable for viability, Atg32 is strongly induced and accumulates on the surface of mitochondria [19]. Suppression of Atg32 expression reduces mitophagy efficiency [19]. Conversely, overexpression of Atg32 has the opposite effect [24]. Thus, it seems likely that Atg32 is a rate-limiting factor to control the amount of mitochondria to be degraded. Yeast cells lacking Atg32 exhibit no obvious defects in general autophagy and the cytoplasm-to-vacuole targeting (Cvt) pathway, an autophagy-related process for selective transport of several proteins to the vacuole [19, 20], supporting the idea that Atg32 is a *bona fide* mitophagy-specific factor. Although mitochondria autophagy is an evolutionarily conserved process, Atg32 homologs have so far been identified only in yeast species.

Atg32 domain features

The key mitophagy protein Atg32 consists of three major modules, an N-terminal 43 kDa cytosolic domain, a predicted single-helical transmembrane (TM) domain and a C-terminal 13 kDa mitochondrial IMS domain [19]. The TM domain functions in targeting to mitochondria and insertion into the outer membrane [19, 21]. The cytosolic domain contains two consensus motifs critical for interaction with Atg8 and Atg11 [19, 21, 22] (see below for details). Strikingly, a variant of this module anchored to peroxisomes can promote peroxisome autophagy (pexophagy) [22], suggesting that the Atg32 cytosolic domain is necessary and sufficient for recruiting autophagic machineries. The IMS domain, which is dispensable for mitophagy [21, 22], seems to be processed by Yme1, a mitochondrial inner membrane AAA (ATPases associated with diverse cellular activities) protease facing the IMS [25]. The role of Yme1 in mitophagy is, however, controversial [16, 25, 26]. Nevertheless, Yme1-depen-

dent processing has been proposed to regulate Atg32-Atg11 interaction [25].

Atg32 induction

Although how yeast cells trigger mitophagy is not fully understood, oxidative stress is likely to be a signal to induce Atg32 expression. Supporting this idea, the Atg32 protein level drastically increases in cells during respiratory growth (10-20 fold higher than that in cells during fermentable growth) [19]. In addition, the antioxidant *N*-acetylcysteine (NAC) can suppress Atg32 induction and mitophagy, possibly by increasing the glutathione pool [19, 27]. Since glutathione is a major component that maintains cellular redox homeostasis, one scenario could be that an increase in mitochondrial and/or cytosolic oxidative stress results in a shift of cellular redox balance to oxidized state, which in turn activates redox-sensing transcription factors responsible for Atg32 expression.

It has been thought that a shift from respiration to starvation, or prolonged respiratory growth is needed to induce Atg32 and mitochondria degradation. Surprisingly, a recent study reveals that mitophagy can also be promoted independently of mitochondrial respiration [24]. When cells grown in fermentable medium are challenged with nitrogen starvation, bulk autophagy is rapidly activated. Under the same conditions, mitophagy also occurs without strong Atg32 induction, but much later than bulk autophagy [24]. Strikingly, respiration-deficient mutants are competent for this starvation-induced mitophagy [24], suggesting that oxidative stress is not a general prerequisite for degradation of mitochondria.

Atg32-Atg8 and -Atg11 interactions

The Atg32 43 kDa cytosolic domain contains a motif called Atg8-family interacting motif (AIM, equivalent to LIR) crucial for binding to Atg8 [19, 22]. Mutations in Trp86 and Ile89 of the W/YXXI/L/V consensus sequence weaken Atg32 binding to Atg8, resulting in a partial mitophagy defect [19]. Crystal structure of the Atg8-Atg32 AIM peptide complex reveals an interaction mode very similar to those of the Atg8-Atg19 (Cvt receptor) and Atg8-Atg3 (E2 enzyme for Atg8 lipidation) complexes in yeast, and the LC3-p62 (selective autophagy adaptor), LC3-NIX (mitophagy receptor in erythrocyte maturation) and LC3-Atg4B (cysteine protease for Atg8 lipidation) complexes in mammalian cells [22, 28-32]. Atg32 binds both soluble and PE-conjugated Atg8 independently of other core Atg proteins [22]. The AIM is not absolutely required for mitophagy [19, 21, 22], suggesting that Atg32-Atg8 interaction serves as an auxiliary to facilitate formation of autophagosomes surrounding mitochondria. Interestingly, in the methylotrophic yeast *Pichia pastoris*, a threonine residue near the AIM of PpAtg32 seems to be a potential phosphorylation site that contributes to Atg8 binding and mitophagy [33].

Atg32 also has I/VLS, a consensus motif important for binding to Atg11 [21]. An alanine substitution in Ser114 of the I/VLS motif affects Atg32 phosphorylation and strongly impairs mitophagy [21]. PpAtg32 Ser159 is the corresponding amino acid residue in its I/VLS motif that appears to function in a manner similar to that of Atg32 Ser114 [33]. The Atg32 I/VLS motif-containing region physically associates with the Atg11 C-terminal coiled-coil domain that is also crucial for Atg19 binding [21, 22]. Atg32 interacts with Atg11 at the early stage of mitophagy, and even in the absence of Atg8 and other core Atg proteins [22]. Hence, it seems likely that Atg32-Atg11 interaction is an initial event distinct from autophagosome formation. Mitophagy absolutely requires Atg11, whereas the Cvt pathway in cells lacking Atg11 can still partially operate in a manner dependent on Atg17, another scaffold protein crucial for bulk autophagy [19]. The reason for this different Atg11 dependency remains uncertain. In conclusion, the primary function of Atg32 is to recruit Atg11 to the surface of mitochondria and localize the site of core Atg protein assembly for mitophagy.

Protein kinases and Atg32 phosphorylation

Recent studies demonstrate that two mitogen-activated protein kinase (MAPK) signal transduction pathways, Wsc1-Pkc1-Bck1-Mkk1/2-Slt2 and Ssk1-Pbs2-Hog1, are important for mitophagy in yeast [21, 34]. In contrast, Slt2 and Hog1, MAP kinases known to be involved in cell wall integrity signaling and hyperosmotic stress response, respectively, are dispensable for bulk autophagy and the Cvt pathway [21, 34]. Notably, Atg32 is phosphorylated under mitophagy conditions [21, 22]. This posttranslational modification depends, at least in part, on Hog1, but not Slt2, although Atg32 is not a direct substrate for Hog1 [21]. Whether and how signals specific for mitophagy are transduced into these pathways remains to be investigated. Atg1, a serine/threonine protein kinase essential for all autophagy-related processes, also plays a direct or indirect role in Atg32 phosphorylation [22], although the underlying molecular mechanism is currently unknown.

In addition to the induction of its protein synthesis, phosphorylation is a critical posttranslational modification for Atg32 to become active for mitophagy. In particular, Ser114 and Ser119 are key amino acid residues for Atg32 phosphorylation by casein kinase-2 (CK2), an extremely multitasking protein kinase conserved during evolution [35]. CK2-dependent phosphorylation of Atg32

stabilizes Atg32-Atg11 interaction, which then leads to core Atg protein assembly, subsequent autophagosome formation and ultimately mitochondria degradation [35]. CK2 is not important for bulk autophagy, pexophagy and the Cvt pathway [35, 36], suggesting its specific role in mitophagy. CK2 in yeast also phosphorylates protein import factors in the outer membrane of mitochondria, thereby positively regulating the biogenesis of this organelle [36]. In mammalian cells, p62 is a target for CK2: its specific phosphorylation promotes selective autophagic turnover of polyubiquitinated cargo proteins [37]. How CK2-dependent phosphorylation of Atg32 is regulated upon mitophagy is not clear. Moreover, whether there is a direct link between CK2 and the HOG signaling pathway remains to be explored.

Atg32-Atg8 interaction may also be regulated through phosphorylation. In *P. pastoris*, PpAtg32 contains a potential phosphorylation site near its AIM, Thr119, that is required for full mitophagy activities in this methylotrophic yeast [33]. Whether phosphorylation regulates Atg32-Atg8 interaction in *S. cerevisiae* has not yet been clarified.

Mitochondrial fission and mitophagy

It is quite conceivable that fragmented mitochondria would be easier targets for mitophagy than tubular mitochondria, since the size of autophagosomes containing mitochondria in yeast mitophagy under prolonged respiratory growth is limited to 200-300 nm in diameter [19]. In addition, autophagosome formation *per se* is unlikely to mediate mitochondrial fragmentation. Consistent with this idea, studies in mammalian cells demonstrate that fragmentation is a critical step for mitochondria to be efficiently sequestered into autophagosomes [38-40].

Recently, it has been reported that Atg11 interacts with Dnm1, a dynamin-related GTPase required for mitochondrial fission in yeast [41]. A single mutation, E728R or D729R, in the Dnm1 C-terminal GTPase effector domain does not affect mitochondrial shape, but impairs Atg11 binding and partially suppresses mitophagy [41]. It remains uncertain if Dnm1 contributes to stabilizing Atg32-Atg11 interaction, and/or assists in any other events during degradation of mitochondria. Whether Dnm1 foci associated with the Atg32-Atg11 complex are indeed active fission sites to generate small mitochondrial fragments is also an intriguing issue for future studies. Nonetheless, there may be other factor(s) and mechanism(s) mediating mitophagy-specific mitochondrial fission, as loss of Dnm1 does not completely block degradation of mitochondria.

Physiological significance of mitophagy

Although cells lacking Atg32 exhibit no obvious defects in respiratory growth [19, 20], mitophagy seems to become important under stress conditions. In particular, mitochondrial DNA deletion frequently occurs in the *atg32*-null mutant during prolonged nitrogen starvation [42]. This phenotype is due to oxidative stress derived from excess reactive oxygen species (ROS), which can be suppressed by NAC treatment [42]. Similarly, the elevated ROS levels in mitophagy-deficient cells under caloric restrictions cause mitochondrial dysfunctions such as reduced respiration, lowered membrane potential and aberrant morphologies, ultimately leading to shortened chronological life span [43].

A closely related study in the fission yeast *Schizosaccharomyces pombe* reveals that transport of mitochondria to the vacuole is drastically promoted in proteasome-deficient cells at G0 phase (quiescent state) [44]. Under the same conditions, ROS accumulate in mitochondria and the nucleus [44]. Disruption of the *ATG8* gene causes a strong increase in the ROS levels and loss of the mutant viability [44], suggesting a critical role of autophagy-dependent mitochondria degradation in cell homeostasis. Strikingly, NAC treatment prevents ROS accumulation and restores cell survival [44]. It should be noted that mitochondria degradation is neither facilitated in vegetatively growing proteasome-deficient mutants, nor in wild-type cells at G0 phase [44]. Hence, both autophagy and the proteasome may synergistically contribute to mitochondrial quality control in the quiescent state.

In conclusion, mitophagy in yeast serves as one of the multilayered systems for the management of mitochondrial fitness. When non-dividing cells are exposed to severe stresses, mitophagy becomes essential for the maintenance of healthy mitochondria.

Mitophagy receptors in mammalian systems

Like Atg32 in yeast, mitophagy receptors in mammalian systems are localized at the outer membrane of mitochondria and have the classic tetrapeptide sequence W/F/YxxL/I that mediates the interaction with LC3 for selective autophagy. Currently, there are two types of mitophagy receptors identified in mammalian cells. One family includes NIX/BNIP3L and BNIP3 [45], and the other mitophagy receptor is FUNDC1 [23].

NIX/BNIP3L and BNIP3

BNIP3 was first identified as a Bcl-2 interacting molecule, and NIX/BNIP3L was identified based on its homology (56% identity) to BNIP3 [46, 47]. NIX/BNIP3L and BNIP3 are localized to mitochondria and the ER, and are involved in regulating apoptotic death or pro-

grammed necrosis by affecting mitochondrial respiration or ROS production [48, 49]. Studies from Ivan Dikic and colleagues showed that NIX/BNIP3L has a putative LIR (equivalent to AIM in yeast) that interacts with LC3 and thus defines NIX/BNIP3L as a mitophagy receptor [30, 50]. BNIP3 also contains a typical LIR motif and functions in mitophagy [51, 52]. Mutations in the conserved LIR motif of these proteins abolish their interactions with LC3/GABARAP. Genetic ablation analysis revealed that NIX/BNIP3L functions in autophagic degradation of mitochondria in reticulocytes, a process essential for red blood cell maturation [53]. NIX/BNIP3L is highly induced at the late stage of erythrocyte maturation and anchored to the surface of mitochondria. It should be noted that mitochondrial clearance in reticulocytes also occurs, at least to some extent, independently of NIX and some core Atg proteins [54, 55].

NIX/BNIP3L and BNIP3 are also involved in hypoxia-induced mitophagy. One would expect that these mitophagy receptors are induced by hypoxia. Indeed, NIX/BNIP3L and BNIP3 are transcriptionally regulated through HIF or FOXO3 [48, 56]. In addition, it has been shown that phosphorylation of BNIP3 at Ser17 and Ser24 promotes its binding to LC3-B and GATE-16, and facilitates subsequent mitophagy [52]. Kinases and phosphatases for BNIP3 are yet to be discovered. Recently, NIX/BNIP3L has been suggested to interact with Rheb, a small GTPase of the Ras superfamily, to promote mitophagy in cells highly active for oxidative phosphorylation [57].

FUNDC1 and its posttranslational modifications

FUNDC1 is a mitochondrial outer membrane protein with three TM domains [23]. The N-terminal region (aa 1-50 of FUNDC1) is exposed to the cytosol and it contains a typical LIR, Y(18)xxL [23]. The conserved Y18 and L21 are essential for the interaction between FUNDC1 and LC3 [23]. Mutations in Y18 and L21, or deletion of the LIR abolish FUNDC1 interaction with LC3 and its function to mediate mitophagy [23]. Although FUNDC1 is involved in hypoxia-induced mitophagy, the mRNA level of FUNDC1 decreases under hypoxic conditions and its protein levels were significantly reduced due to mitophagy [23]. The exact mechanism by which hypoxia suppresses FUNDC1 expression was not yet clear. Promoter analysis does not reveal any conserved HIF-1 recognition site (unpublished observation).

The activity of FUNDC1-mediated mitophagy is regulated by reversible phosphorylation at the posttranslational level. We have found that dephosphorylation activates FUNDC1-mediated mitophagy in mammalian systems [23]. Both Ser13 and Try18 of FUNDC1 are dephosphorylated in response to hypoxic stress and the loss of mitochondrial membrane potential [58]. Specifically, we found that under normal physiological conditions Tyr18 of FUNDC1 is phosphorylated by Src kinase and Ser13 is phosphorylated by CK2 [58]. These two kinases are highly abundant and constitutively active, ensuring the inhibition of mitophagy under unstressed conditions. Inactivation of either Src kinase or CK2 alone by knockdown approach or by pharmacological inhibitors was not sufficient to activate mitophagy, while inhibition of both kinases strongly activates mitophagy [58]. Furthermore, we have identified that the mitochondria-localized phosphatase PGAM5 is responsible for dephosphorylation of FUNDC1 at Ser13 [58]. Dephosphorylated FUNDC1 has a significantly higher affinity to LC3 and thus this results in an increased interaction between FUNDC1 and LC3, leading to selective autophagosome incorporation and subsequent autophagic removal of the affected mitochondria [23, 58] (see Figure 2).

Mitochondrial segregation and mitophagy

It is well documented that mitochondrial fission or fragmentation precedes mitophagy. Mitochondrial fission could be part of the sorting mechanism that distinguishes the mitochondria to be engulfed by mitophagy. It was found that the fragmented mitochondria either fuse back to the network when their membrane potential is sustained, or undergo mitophagy when the membrane potential is dissipated [40]. This sorting mechanism is well illustrated in PINK1/Parkin-mediated mitophagy. Loss of mitochondrial membrane potential will stabilize PINK1 at the outer membrane that is able to recruit Parkin onto the surface of mitochondria [59, 60]. Parkin as a potent E3 ligase then ubiquitinates mitofusin 1 and mitofusin 2 [61], two key mitochondrial fusion mediators, and a number of other mitochondrial proteins [62, 63]. Proteasome-dependent degradation of ubiquitinated mitofusins leads to mitochondrial fragmentation and subsequent mitophagy. Conversely, excessive fusion, such as in the cases of overexpression of dominant-negative Drp1 or wild-type OPA1, retards autophagic degradation of mitochondria in mammalian cells [64, 65]. It should, however, be noted that mitochondrial fragmentation without membrane potential dissipation does not induce Parkin-dependent mitochondrial turnover, suggesting that mitochondrial fission is necessary but not sufficient for mitophagy [66]. Parkin-labeled mitochondria are bit-by-bit engulfed by autophagic membranes one at a time [67]. Using a light-activation scheme to impair long mitochondrial tubules, it was shown that sites undergoing bit-by-bit mitophagy display preferential ubiquitination [67], and are situated where Parkin-labeled mitochondri-

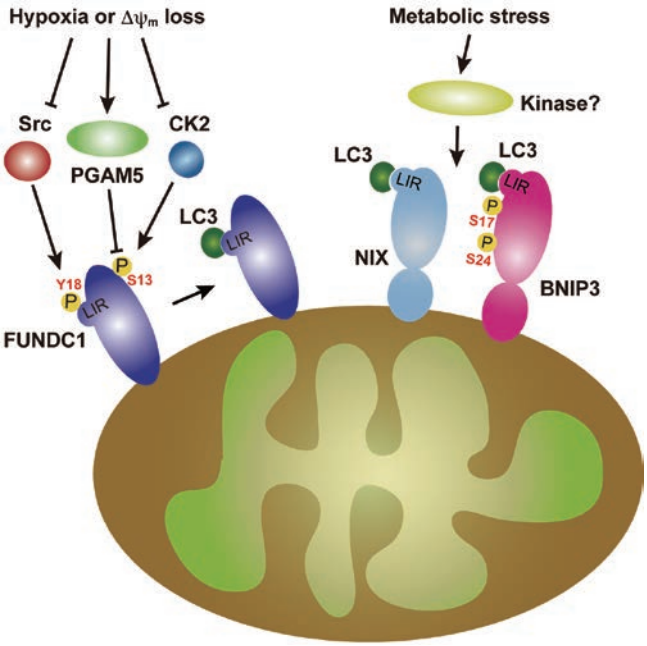

Figure 2 Mitophagy receptors in mammalian systems. FUNDC1, NIX/BNIP3L and BNIP3 interact with LC3 through LIRs in their N-terminal region. The interaction between FUNDC1 and LC3 is regulated by reversible phosphorylation. In normal conditions, FUNDC1 is phosphorylated at Try18 and Ser13 by Src kinase and CK2, respectively. Hypoxia and FCCP promote FUNDC1 dephosphorylation by inactivating Src kinase and CK2, and by activating PGAM5. Dephosphorylated FUNDC1 has a significantly higher affinity to LC3. The interaction between BNIP3 and LC3 is positively regulated by phosphorylation of Ser17 and Ser24 in BNIP3. The kinase for BNIP3 phosphorylation remains unknown.

al tubules and the endoplasmic reticulum intersect.

The exact contribution of mitochondrial fission in receptor-mediated mitophagy is a subject that is of substantial interest. Overexpression of FUNDC1 induces massive mitochondrial fragmentation prior to mitophagy and knockdown of FUNDC1 enhances mitochondrial fusion, suggesting that mitochondrial fission is required for mitophagy [23]. Interestingly, deletion of the LIR motif of FUNDC1 almost completely abolished its mitophagy activity, while the mutant protein still strongly induced mitochondrial fragmentation, suggesting that FUNDC1-mediated mitochondrial fission and degradation can be uncoupled [23].

Early studies have shown that PGAM5 recruits the mitochondrial fission factor Drp1 and activates its GTPase activity by dephosphorylating Ser637 of Drp1 [68]. Since FUNDC1 is also a substrate of PGAM5, it is possible that mitochondrial fission and mitophagy can be highly orchestrated by PGAM5. The molecular details of this orchestration for mitochondrial fission and mitophagy warrant further investigations.

Signaling toward receptor-mediated mitophagy

Current advances in the field have shown that mitophagy activity is highly sensitive to a wide array of cellular and mitochondrial cues. Indeed, perturbation of the cellular bioenergetic status, changes of oxygen tension, loss of mitochondrial membrane potential, an increase in cellular ROS [69] (either derived from the cytosol or mitochondria), a disturbance of Ca^{2+} signaling, defects in mitochondrial protein import or export [70], mtDNA damages [71], and perturbation of the mitochondrial protein quality control system or accumulation of protein aggregates in mitochondria can all activate mitophagy [72].

Whether receptor-mediated mitophagy is involved in some or all of these above mentioned mitochondrial or cellular perturbations to activate mitophagy remains to be examined. It would also be interesting to investigate if these distinct damage or stress signals converge at the mitochondrial receptor level for cross talk with the autophagy machinery. Given the critical role of reversible phosphorylation in the regulation of mitophagy, we propose that distinct kinases such as Src kinase and CK2 or phosphatases such as PGAM5 function as the sentinel for mitochondrial stress signals responsible for initiation of mitophagy. Perturbation of mitochondrial physiology is likely to either activate or inactivate kinases or phosphatases, modulating the interation between mitophagy receptors and the core autophagy-related proteins, thereby leading to activation or prevention of mitophagy. Indeed, both FCCP and hypoxia inactivate Src kinase as well as CK2, and activate PGAM5 to promote FUNDC1-mediated mitophagy in mammalian cells [23, 58].

Early studies have clearly shown that ROS can activate mitophagy in mammals [73]. The antioxidant NAC prevents mitophagy induction in yeast [27, 74], suggesting that the mitochondrial redox status or the ROS production level is one of the common factors contributing to the regulation of mitophagy. Acute ROS production could lead to the opening of the mitochondrial permeability transition pore and loss of mitochondrial membrane potential, which ultimately activates PINK1/Parkin-mediated mitophagy. Indeed, ROS production induced by a mitochondria-targeted photosensitizer resulted in a loss of membrane potential and subsequent activation of Parkin-dependent mitophagy [75]. One could speculate that slight, but neither acute nor severe, increases in ROS levels may be sufficient to affect kinases or phosphatases to activate receptor-mediated mi-

tophagy. In yeast, the MAPK Hog1 pathway, which can be modulated by oxidative stress, is known to regulate CK2- and Atg32-dependent processes in mitophagy [76]. We also found that hypoxia can inactivate Src kinase to activate FUNDC1-mediated mitophagy in mammalian cells [23]. NAC can also prevent hypoxia-induced mitophagy, suggesting that ROS are involved (unpublished observations). Interestingly, it has been shown that ROS and the mitophagy receptor NIX/BNIP3L promote the induction and initiation of mitophagy by enhancing the translocation of Parkin onto damaged mitochondria [77], suggesting that PINK1/Parkin-mediated mitophagy and the receptor-mediated pathways could be interdependent.

Perspectives

Receptor-mediated mitophagy is a highly conserved mechanism to selectively remove unwanted or damaged mitochondria. It plays a critical role in controlling mitochondrial quality and quantity in response to the energy needs and other mitochondrial and cellular cues. Mitophagy receptors are highly regulated by reversible phosphorylation by distinct kinases such as CK2 and Src kinase, which can be counterbalanced by phosphatases such as PGAM5 in mammalian cells. Regulation of mitophagy could be more complex and additional players are likely to be discovered as the field is attracting greater attention. For example, cardiolipin can also bind to LC3 to mediate mitophagy [78]. Selective mitophagy induced by 6-OHDA, rotenone and staurosporine in neuronal cells involves the externalization of cardiolipin to the outer mitochondrial membrane, which acts as a signal for subsequent mitophagy [78]. Moreover, iron chelation can induce mitophagy in a BNIP3- and Parkin-independent manner [79]. Although the PINK1/Parkin-mediated pathway has been extensively explored in *Drosophila* [80], whether specific receptors also regulate mitophagy in this fly model remains uncertain. Notably, paternal mitochondria are eliminated via fertilization-triggered autophagy in *Caenorhabditis elegans* embryos [81, 82], yet the underlying molecular mechanisms are not understood. Future studies will reveal how receptor-mediated mitophagy coordinates with sorting mechanisms for selective removal of unwanted or damaged mitochondria to regulate mitochondrial quality and quantity.

Defects in mitophagy are proposed to cause accumulation of dysfunctional mitochondria, which is widely observed in aging-related diseases. However, this idea has not been rigorously tested, due to the lack of suitable model systems and reliable quantitative measurements of mitophagy *in vivo*. Utilization of appropriate model systems for extensive molecular studies will pave new avenues for treatments of diseases involving dysfunctional mitochondria.

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