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The Molecular Mechanisms of Mitochondrial Autophagy/ Mitophagy in the Heart

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Abstract

Mitochondrial quality is a crucial determinant of cell viability, and mitochondrial autophagy plays a central role in this control mechanism. Based on studies in yeast, numerous investigations of this process have been conducted and the framework of mammalian mitochondrial autophagy is progressively appearing. However, many enigmas about the molecular mechanisms involved remain unsolved. Furthermore, the pathological significance of mitochondrial autophagy in the heart remains largely unclear. In this review, we discuss the current understanding of mitochondrial autophagy in mammals with reference to that in yeast. Regarding the process in yeast, some points of uncertainty have arisen. We also summarize recent advances in the research of autophagy and mitochondrial autophagy in the heart. This article is a part of a review series on ***Autophagy in Health and Disease***.

Keywords

Mitophagy; autophagy; Pink1; Parkin

Introduction

Mitochondria are evolutionarily preserved organelles found in almost all eukaryotic cells. The primary role of mitochondria in mammalian cells is ATP synthesis, which is achieved primarily through oxidative respiratory phosphorylation. Additionally, mitochondrial proteins also regulate innate immunity, apoptosis, necrosis and autophagy.[1–3] ATP production through the respiratory chain is accompanied by production of reactive oxygen species (ROS) as a byproduct through leakage of electrons from the electron transport chain. Low levels of ROS are directly removed by anti-oxidants within mitochondria or actively stimulate counter-regulatory signaling/transcription pathways in order to maintain the proper redox balance. However, excessive accumulation of ROS during stress damages mitochondrial components, including mitochondrial DNA (mtDNA), protein and lipids, which exacerbates ROS production and mitochondrial dysfunction in a feedforward manner.

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In response to ROS, mitochondria initiate and orchestrate various signaling mechanisms, thereby controlling the growth and death of various cell types [4]. Damaged mtDNA that escapes from lysosomal degradation causes Toll-like receptor 9-mediated inflammation, and myocarditis due to its inflammatogenic unmethylated CpG motifs.[5] Mitochondria-derived excessive ROS eventually promote aging, carcinogenesis, neurodegenerative disorders and cardiovascular diseases.[6, 7]. Since damaged mitochondria induce catastrophic consequences, the quality of mitochondria is essential and regulated by multiple mechanisms, including fission and fusion of mitochondria, degradation of mitochondrial proteins mediated by chaperones (Hsp10, Hsp60 and others), proteinases (Lon, AAA proteases) and proteasomes, and mitochondrial biogenesis [8]. The constant production of ROS necessitates turnover and replacement of mitochondria every 10–25 days, even in the quiescent cell or organ.[9] Increasing lines of evidence suggest that autophagy plays a major role in mediating mitochondrial quality control.[10, 11]

Autophagy is a mechanism of cellular degradation through lysosomes. Autophagy is particularly importnat for cell survival during energy stress, since amino acids and fatty acids recovered through degradation of cellular constituents by autophagy can be recycled to generate ATP. Autophagy is classified into three categories: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA).[12–14] Macroautophagy is characterized by the formation of an autophagosome, a double-membrane structure which engulfs organelles or long-lived proteins and fuses with lysosomes. Microautophagy is defined by the direct incorporation of cytosolic content into a lysosome/vacuole by the invagination of its surface. CMA involves the direct translocation of proteins into lysosomes in an Hsc70-mediated manner. The machinery of macroautophagy is fairly well understood, whereas that of microautophagy has not yet been fully elucidated in mammals. The process of macroautophagy proceeds in multiple sequential steps: initiation and nucleation of isolation membranes, elongation of phagophores and engulfment of organelles or long-lived proteins, docking and fusion of phagophores (autophagosome formation), fusion of autophagosomes with lysosomes, and lysosomal degradation of cargos. Although autophagy has been regarded as a non-selective process, accumulating lines of evidence indicate the existence of selective autophagy, through which damaged organelles such as mitochondria, endoplasmic reticulum, ribosomes, and peroxisomes, are selectively degraded. This selective autophagy is collectively called organelle-specific autophagy. [15, 16] Recent investigations have unveiled defined molecular mechanisms and the functions of organelle-specific autophagy. Among them, mitochondria-specific autophagy, termed mitophagy, has been most intensively investigated. Here, we discuss the current understanding of the molecular mechanisms mediating mitophagy and the role of mitophagy in mediating physiological and pathological functions in the heart.

What is Organelle-Specific Autophagy ?

The existence of a selective autophagic process for organelles was first proposed for the endoplasmic reticulum (ER) in 1973.[17, 18] Since then, molecular mechanisms mediating the organelle-specific autophagy in the ER (ER-phagy/reticulophagy), peroxisomes (pexophagy), ribosomes (ribophagy) and mitochondria (mitophagy) have been investigated in yeast.[19–29] Both macroautophagy and microautophagy play a role in pexophagy, and are

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termed macropexophagy and micropexophagy, respectively.[19–21, 30, 28] Since microautophagy utilizes autophagy-related (Atg) proteins in yeast, it is hard to distinguish the particular contributions of microautophagy and macroautophagy to overall organelle-specific autophagy.[30] Although some studies suggest a contribution of microautophagy to mitophagy, its contribution is largely unknown.[31, 32] Therefore, we hereafter use the term "mitophagy" to reference macro-mitophagy.

Yeast peroxisomes were ideal organelles for the initial studies of selective autophagy.[21] Macropexophagy eliminates redundant or inactive peroxisomes without acceleration of autophagic degradation of other organelles.[21] Although peroxisomes can be degraded non-specifically, the specific process also takes place under certain conditions, and the term "pexophagy" was generally accepted by researchers.[19, 18] Some labeling proteins localized in the peroxisome were later identified, such as Pex14, Atg30 and Atg37, which are essential for macropexophagy.[20, 33, 28] The phosphorylation of Atg30 is essential for its interaction with Atg8 and Atg11, which is followed by macropexophagy.[33, 34, 28]

The term "mitophagy" was first introduced by Lemasters in 2005 to describe the selective autophagy of mitochondria and emphasize the non-random nature of the process in yeast after the identification of Uth1.[9] This term had been widely accepted without enough evidence for its selectivity at the beginning.[18] The molecular basis of mitophagy has since been first investigated in yeast. Most importantly, mitochondrial-targeted proteins, such as Uth1, Aup1p and Atg32, which mark damaged mitochondria and subject them to autophagic removal, have been identified in yeast.[35, 36, 24, 25] Atg32 accumulates in the outer membrane of mitochondria (OMM) in response to starvation or in the post-log phase.[24, 25] Atg32 interacts with Atg8 (Fig. 1).[24, 25] After phosphorylation of Atg32 at Ser 114 and 119 by casein kinase 2 (CK2), the N-terminus of Atg32 binds to the C-terminus of Atg11 (Fig. 1).[26] Subsequently, Atg11, which connects to Dnm1, a mitochondrial fission protein, recruits the fission complex to mitochondria, a step that is essential for mitophagy (Fig. 1).[37]

Although knowledge obtained from yeast has served as a useful resource for elucidating the mechanism of mitophagy in mammalian cells, the metabolic processes during the induction of mitophagy are distinct between yeast and mammalian cells. *Saccharomyces cerevisiae*, a budding yeast, prefers using the glycolytic pathway even when oxygen is abundant.[38] When glucose is exhausted, a "diauxic shift" takes place and yeast start to utilize mitochondrial oxidative respiratory phosphorylation.[38] On the other hand, mammalian cells primarily use mitochondrial oxidative phosphorylation in the presence of oxygen. Thus, induction of mitophagy in yeast and mammalian cells may be distinct in terms of functional significance and mechanism of activation.

In yeast, vacuoles containing mitochondria alone but no other organelles can be observed frequently through electron microscopic analyses. Yeast strains lacking mitophagy still retain bulk autophagy[24], suggesting that mitophagy is clearly separable from general autophagy in yeast. In contrast, the boundary between mitophagy and bulk autophagy of mitochondria appears more obscure in mammalian cells. Thus, when studying mitophagy in mammalian cells, it is crucial to carefully observe whether the autophagosomes contain mitochondria alone or other cargos as well. Due to the fact that many publications in the

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field of cardiovascular medicine do not distinguish mitophagy from general autophagy degrading mitochondria in a non-specific manner, we here use the term mitophagy only when a given work most likely investigates mitochondria-specific autophagy. When the distinction is not clear, we use the term mitochondrial autophagy.

How can we detect mitophagy in mammalian cells?

The simplest and most reliable demonstration of mitochondrial autophagy is through observation of mitochondria engulfed in autophagosomes, using an electron microscope. This approach is laborious but provides critical information, such as the content of the autophagosome and the structure of autophagosomes/autolysosomes. Concomitant use of immunogold antibodies can help identify the cargo content and the origin of the autophagosomes. The use of electron tomography, in which tilt series of data for each section are reconstituted into 3-dimensional images (3D), would be useful to prove the sole presence of mitochondria in autophagosomes, which is prerequisite for documenting the presence of mitophagy separate from general autophagy.[39, 40]

Practically, co-localization of LC3 puncta and mitochondrial proteins is most commonly used to demonstrate the presence of mitochondrial autophagy. LC3 puncta are most conveniently observed with exogenous LC3 fused with fluorescent proteins, such as GFP, mRFP, and mCherry. Time-lapsed imaging in living cells expressing these markers allows continuous observation of the incorporation of mitochondria into autophagosomes with the extension of the isolation membrane.[41] As the fluorescence of GFP disappears under acidic conditions, GFP-LC3 puncta do not label autolysosomes. On the other hand, the fluorescence of mRFP and mCherry is resistant to the acidic environment of lysosomes. Using the distinct properties of GFP and mRFP, mRFP-GFP tandem-fluorescence-tagged LC3 makes it possible to distinguish autophagosomes and autolysosomes. However, this evaluation may overlook the previously reported mitochondrial autophagy mediated by an LC3-independent mechanism.[42–44]

Keima is a coral-derived acid-stable fluorescent protein that emits different-colored signals at acidic and neutral pHs.[45] Mitochondria incorporated into lysosomes are visualized as acidic-colored dots representing fluorescence emitted by mitochondria-targeted Keima.[45] Although this indicator alone cannot prove that translocation of mitochondrial proteins to lysosomes occurs through mitophagy, if the translocation is inhibited by interventions to suppress autophagy or mitophagy, it is possible to show that the lyosomal translocation of mitochondria-targeted Keima is caused by either autophagy or mitophagy.

Although microscopic morphological studies provide solid qualitative data, it is important to also show degradation of mitochondria using biochemical methods. The quantitative analysis of mitochondrial DNA or protein appears indispensable. It should be noted that, in addition to autophagy, the ubiquitin proteasome system (UPS) also mediates degradation of mitochondria.[46] In particular, proteins associated with the OMM undergo degradation through the UPS under certain conditions.[46] In this sense, the molecules in the matrix or inner mitochondrial membrane (IMM), including mtDNA and proteins involved in the electron transport chain may be helpful for the assessment of mitochondrial autophagy. Mito

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Timer, a fluorescent protein targeted to mitochondria, is useful for monitoring the turnover of mitochondrial proteins. Mito Timer transitions from green to red fluorescence over a span of 48 h. This property allows one to evaluate the turnover of mitochondria facilitated by mitophagy.[47] For example, Mito Timer in autophagy-deficient cells with low mitochondrial turnover tends to show more red than green.

The impairment of mitophagy or mitochondrial autophagy is often accompanied by accumulation of dysfunctional mitochondria. In theory, the volume of mitochondria may be quantitated by electron microscopic tomography [48]. However, estimating the total volume of mitochondria in cells using this method is extremely laborious and may not be practical. Accumulation of damaged mitochondria is often accompanied by increases in mtDNA content. This can be evaluated with short-range and long-range qPCR, which allows for calculation of the amount of damaged mtDNA by subtracting intact mtDNA from total mtDNA.[49, 50] It should be noted that the impairment of mitochondrial dynamics caused by genetic deletion of Mfn1 and Mfn2 induces severe depletion of mtDNA through unknown mechanisms leading to mtDNA instability in skeletal muscle. [51] Thus, suppression of mitophagy may not necessarily be accompanied by accumulation of mtDNA.[51] Furthermore, mtDNA has been reported as a poor biomarker of mitochondrial content due to the fact that mitochondrial DNA copy number can vary considerably from animal to animal.[52] Thus, it is recommended that it be used in combination with other biomarkers. For example, accumulation of damaged mitochondria can also be demonstrated by increases in the mitochondrial mass, which can be evaluated by isolating mitochondria and determining their content relative to the starting tissue or cell preparation, or by increases in mitochondrial content, which is estimated by determining the relative ratios of mitochondrial biomarkers, including citrate synthase activity, cardiolipin content and complex I-IV proteins, to cytosolic proteins.[52] Mitochondrial swelling assays are used to show increased sensitivity of the mPTP to Ca^{2+}. The mitochondrial membrane potential is often depolarized, and this can be monitored using JC-1, TMRE or TMRM. Mitochondrial respiration can be evaluated with measurement of the time-dependent oxygen consumption using Clarke electrodes and the activity of the electron transport chain can be monitored with biochemical analyses of freshly prepared mitochondrial fractions.

At present, there is no single experimental method sufficient for the assessment of mitochondrial autophagy, since none of these assays alone can prove the presence of cargo-specific autophagy such as mitophagy. Caution should be exercised when interpreting the results obtained from these assays. If more information regarding how mitophagy is induced becomes available in the future, the mechanism can be traced as an indicator of mitophagy and loss-of-function approaches can be taken to show the specific involvement of mitophagy. Currently, Parkin translocation to mitochondria and Parkin-dependent ubiquitination of mitochondrial proteins (See below) are often used to show activation of mitophagy. However, whether this mechanism represents the major mechanism of mitochondrial autophagy in the presence of a given stress in the heart requires further investigation.

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Molecular mechanisms mediating mitophagy

Most of the studies conducted thus far have focused on the molecular mechanism by which damaged mitochondria are recognized and engulfed by autophagosomes. In the case of mitophagy in yeast, Atg32 labels damaged mitochondria and recruits phagophores by acting as a receptor for Atg8 through its Atg8 family interacting motif (AIM). In the case of mammalian cells, the process appears more complex and there are several ways for damaged mitochondria to be recognized by phagophores. As in yeast, one mechanism utilizes either proteins or lipids on the mitochondrial membrane as receptors for LC3. To date, a mammalian homolog of Atg32 has not been reported. As introduced below, however, Nix/Bnip3L, Bnip3, FUNDC1 and Cardiolipin function as receptors for LC3 (Fig. 2).[53–57] Another mechanism utilizes ubiquitination of mitochondrial proteins followed by interaction with the adaptor proteins connecting ubiquitin with LC3. The representative case is the Pink1-Parkin pathway for the ubiquitination of mitochondrial proteins, with p62 and Nbr1 acting as adaptor proteins (Fig. 3).[58, 59] In addition, CK2, which phosphorylates Atg32 in yeast, has been reported to phosphorylate p62 at Ser 403, and this phosphorylation increases the affinity between its ubiquitin-associated domain (UBA) and polyUb chains on mitochondrial proteins in mammalian cells.[60] However, the involvement of this machinery in mitochondrial autophagy has not yet been validated.[60] The roles of key molecules in mediating mitophagy are discussed below.

Pink1, Parkin and p62

Pink1- and Parkin-mediated mitochondrial autophagy is currently the most well established mechanism mediating mitophagy in mammalian cells [61]. Both Pink1 and Parkin are linked to the pathogenesis of autosomal recessive juvenile Parkinsonism [62]. Pink1 is a serine/threonine kinase that has a mitochondria-targeting signal in its N-terminus.[63] After import into mitochondria through Translocase of Outer Membrane (TOM) and Translocase of Inner Membrane (TIM) complexes, Pink1 is anchored at the inner mitochondrial IMM.[63] In intact mitochondria, matrix processing peptidase (MPP) and presenilin-associated rhomboid-like (PARL) continuously degrade Pink1.[64, 65] However, in depolarized mitochondria, the import to the IMM is inhibited and Pink1 accumulates at the OMM. Pink1 then forms a 700 kDa complex with TOM and undergoes autophosphorylation at Ser 228 and Ser 402.[66, 67] Pink1 is also accumulated on the OMM in response to an increase in unfolded proteins in mitochondria, thereby playing an essential role in mediating mitophagic removal of polarized mitochondria.[68] Activated Pink1 recruits Parkin, a cytosolic E3 ubiquitin ligase, to damaged mitochondria and promotes their degradation through phosphorylation of multiple substrates. Pink1 phosphorylates Mfn2, which in turn acts as a mitochondrial receptor for Parkin in cardiomyocytes.[69] A defect in Parkin translocation was observed in Mfn2-deficient neurons,[70] suggesting that Mfn2 plays a critical role in recruiting Parkin to mitochondria in multiple cell types. However, it should be noted that the translocation of Parkin to mitochondria can still be observed in mouse embryonic fibroblasts (MEFs) lacking Mfn1 and Mfn2, suggesting the existence of an alternative or compensatory mechanism for recruiting Parkin.[71] Stable expression of Pink1 anchored to the outer membrane can induce mitochondrial accumulation of Parkin, followed by mitochondrial autophagy in a manner independent of membrane potential. [64] Ectopic expression of Pink1 in peroxisomes recruits

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Parkin to peroxisomes, accompanied by ubiquitination and selective autophagic clearance of peroxisomes.[66] These results suggest that Pink1 acts downstream or independently of mitochondrial depolarization. Although initial studies suggested that Pink1 phosphorylates Parkin in its ubiquitin-like domain, thereby stimulating the E3 ligase activity of Parkin,[72, 73] mutations of conserved serine/threonine residues in Parkin do not completely suppress its activity, suggesting that Pink1 has additional substrates through which it activates Parkin. In fact, Pink1 phosphorylates ubiquitin conjugated to mitochondrial proteins at Ser 65,[74–76] which in turn directly activates Parkin and promotes further ubiquitination of mitochondrial proteins.[77] Multiple mitochondrial proteins, including VDAC, Mfn1/2, TOM, Fis1, Miro1/2 and mitochondrial hexokinase, have been identified as substrates of Parkin.[78, 46, 79, 80] It has been shown that Parkin-labeled mitochondria are removed by autophagy bit-by-bit, which occurs where Parkin-labeled mitochondria and endoplasmic reticulum intersect.[81] Polyubiquitination (polyUb) catalyzed by Parkin leads to recruitment of p62, a ubiquitin- and LC3-binding adaptor protein, followed by perinuclear clustering of damaged mitochondria.[59, 82, 83, 78] However, whether p62 is essential for Parkin-mediated mitochondrial autophagy is controversial.[59, 82, 83] Although degradation of the IMM and matrix proteins occurs through mitochondrial autophagy, degradation of OMM proteins can take place also through a proteasome-dependent mechanism mediated by Parkin..[46] .[46] Parkin promotes recruitment of the AAA ATPase VCP/p97, which extracts ubiquitinated proteins for proteasomal degradation, to damaged mitochondria.[84] Parkin and Pink1 are also involved in the removal of damaged mitochondria through a vesicular trafficking pathway in which mitochondria-derived vesicles (MDV) containing mitochondrial proteins are transported to lysosomes for degradation.[85–87] These findings account for the difficulty in distinguishing typical mitochondrial autophagy from other forms of mitochondrial protein degradation based on Pink1-Parkin dependency alone.

Pink1-Parkin-mediated mitophagy is negatively regulated by USP30, a deubiquitinase localized in the OMM, by removing ubiquitin from substrates ubiquitinated by Parkin [88] and Clec16a, a membrane-associated endosomal protein which promotes proteasomal degradation of Parkin through Nrdp1, another E3 ubiquitin ligase. p53 inhibits mitophagy by sequestering Parkin in the cytosol,[89] whereas Bcl-2 family proteins, including Bcl-xL, MCL-1 and Bcl-2, on the OMM, inhibit mitophagy through inhibition of Parkin translocation to depolarized mitochondria.[90]

Besides mitophagy and the MDV pathway, Parkin and Pink1 also affect the quality control of mitochondria through several other mechanisms.[62] For example, Parkin and Pink1 affect mitochondrial motility through Pink1-mediated phosphorylation and Parkin-mediated proteasomal degradation of Miro, an OMM protein involved in mitochondrion trafficking. Stabilization of Pink1 and Parkin activation immobilize damaged mitochondria through degradation of Miro, which in turn facilitates the removal of damaged mitochondria through mitophagy.[91] Parkin positively regulates mitochondrial biogenesis through proteasomal degradation of PARIS, a zinc-finger protein, which suppresses transcription of PGC-1α, a positive regulator of mitochondrial biogenesis.[92] Parkin also regulates fat uptake by controlling the stability of CD36 through ubiquitination in hepatocytes.[93] These studies suggest that Parkin contributes in diverse ways to mitochondrial quality control and metabolism.

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Although many molecules of the autophagic machinery were discovered in yeast, p62 is absent in lower eukaryotes.[94] Recently, Lu *et al* discovered CUET proteins, a new class of ubiquitin-Atg8/LC3 adaptors.[94] The CUET proteins are Cue5 in yeast and Tollip in human.[94] Although it differs in its domain arrangement, Tollip fulfills both the biochemical and genetic criteria for being a functional human homolog of yeast Cue5.[94] These proteins interact with Atg8 (LC3) through AIM (also termed as LC3-interacting region (LIR)) and with ubiquitin through a CUE domain but not through a UBA domain like p62 or Nbr1.[94] Tollip binds to ubiquitin conjugates more tightly than p62, and is more effective at clearing huntington polyQ protein than p62.[94] Tollip appears to specifically target highly aggregation-prone proteins for autophagy but its role in organelle-selective autophagy has not yet been clarified.[94]

Nix/Bnip3L and Bnip3

To date, some mitochondria-labeling proteins, which also bind to LC3 or LC3 homologs through the LIR as a mitochondrial receptor for autophagosomes, have been identified in mammalian cells. Nix/Bnip3L and Bnip3 are included among these OMM proteins.[53–55] They are BH3-only proteins and pro-apoptotic members of the Bcl-2 family.[95–97] The protein level of Nix/Bnip3L is increased in the final stage of erythroid maturation, and genetic ablation of Nix/Bnip3L prevents the loss of mitochondrial membrane potential and sequestration of mitochondria into autophagosomes.[98] Treatment with CCCP, an uncoupling chemical, or BH3 mimetic induces a loss of membrane potential and restores mitochondrial autophagy in Nix/Bnip3L$^{-/-}$ erythroids, suggesting that Nix/Bnip3L-mediated loss of membrane potential is important for mitochondrial autophagy.[98] Nix/Bnip3L regulates Parkin translocation to mitochondria.[99] Mutation of the LIR of Nix/Bnip3L results in a partial reduction of mitochondrial autophagy in reticulocytes.[54]

Kubli *et al* reported that oxidative-stress-induced homodimerization of Bnip3 is observed after ischemia/reperfusion (I/R) in the heart and that this dimerization correlates with its activation.[100] Bnip3 induces extensive fragmentation and autophagy of mitochondria in HL-1 cells, and this phenomenon is independent of mPTP opening or of Bax/Bak.[101, 102] The mitochondrial fragmentation induced by Bnip3 occurs through its direct inhibition of Opa1, a fusion protein.[103] Recent evidence showed that phosphorylation of the LIR in Bnip3 promotes the interaction between Bnip3 and LC3B, thereby inducing mitochondrial autophagy.[104] However, the responsible kinase has not yet been identified. Nix and Bnip3 promote apoptosis of cardiomyocytes through stimulation of OMM permeabilization.[105, 106] Nix also promotes necrosis of cardiomyocytes by stimulating endoplasmic reticulum-mitochondria crosstalk, Ca^{2+} overload and consequent mPTP opening.[107] Bnip3 also stimulates myocardial necrosis in response to doxorubicin treatment by disrupting formation of mitochondrial protein complexes between the key respiratory proteins.[108] Thus, what determines the role of Bnip3 in promoting cell death or survival through mitochondrial autophagy remains to be elucidated in the heart.

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FUNDC1

FUNDC1 is a mitochondrial outer membrane protein that integrates with LC3 through its LIR under hypoxic conditions.[56] During starvation, mitochondrial clearance does not depend on FUNDC1, suggesting that it is specific for hypoxia-induced mitochondrial autophagy.[56] FUNDC1 is expressed under normoxia; however, the interaction between FUNDC1 and LC3 is stabilized under hypoxic conditions, which is attributable to dephosphorylation at Tyr 18 during hypoxia. FUNDC1 also acts as a mitochondrial receptor for ULK1.[109] ULK1 translocates to depolarized mitochondria and phosphorylates FUNDC1 at Ser 17, thereby promoting mitochondrial autophagy.[109] Mitochondrially localized PGAM5 phosphatase directly dephosphorylates FUNDC1 at Ser 13 upon hypoxia or FCCP treatment.[110] This dephosphorylation of FUNDC1 promotes its interaction with LC3 and mitochondrial autophagy, whereas CK2 phosphorylates the same residue of FUNDC1, thereby negatively regulating mitochondrial autophagy.[110] CK2 phosphorylates Atg32 and promotes mitophagy in yeast. However, the effect of phosphorylation of FUNDC1 is opposite to that of phosphorylation of Atg32.

Cardiolipin

Cardiolipin, a phospholipid of the IMM, has been reported to translocate to the OMM in response to mitochondrial injury and interact directly with LC3 in primary cortical neurons and SH-SY5H cells.[57] The inhibition of cardiolipin synthase or of phospholipid scramblase-3 reduces the delivery of mitochondria to autophagosomes.[57] Mutation of the residues predicted to be cardiolipin-interaction sites in LC3 inhibits mitochondrial autophagy.[57] Given that oxidized cardiolipin promotes the release of pro-apoptotic factors into the cytosol, Chu *et al* speculated that externalized cardiolipin undergoes oxidation and accelerates apoptosis in the event of unsuccessful clearance of damaged mitochondria.[57]

Overexpression of acyl-CoA:monolysocardiolipin acyltransferase (ALCAT1), an enzyme that catalyzes pathological remodeling of cardiolipin, stimulates oxidative stress and clearance of mitochondria via autophagy in H9c2 cells. ALCAT knock-out mice show upregulation of Pink1, and both oxidative stress and mitochondrial dysfunction in response to L-tyroxine-induced stress are attenuated in these mice. Whether cardiolipin peroxidation directly affects mitochondrial autophagy or mitochondrial autophagy is regulated secondarily by mitochondrial dysfunction/oxidation remains to be elucidated [111].

General Control of Amino Acid Synthesis 5-like 1 (GCN5L1)

Increasing lines of evidence suggest that lysine acetylation controls many cellular functions, including autophagy, in response to metabolic stress.[112] Acetyl-CoA, an intermediate product of metabolic pathways whose acetyl moiety is transferred for protein acetylation, is a negative regulator autophagy.[113] During starvation, the level of Acetyl-CoA is thought to be diminished. In addition, GCN5L1, a component of the mitochondrial acetyltransferase machinery, is downregulated, whereas Sirt3, a mitochondrial deacetylase, is activated in HepG2 cells.[114] Downregulation of GCN5L1 diminishes acetylation of mitochondrial proteins and stimulates mitochondrial autophagy, which, in turn, slows down respiration and induces stress-resilience in cells.[114] This mitochondrial autophagy is both Atg5- and p62-

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dependent but Parkin-independent, and thus, whether or not autophagy regulated by GCN5L1 is mitochondria-selective is currently unknown. One mechanism through which the reduced protein acetylation induces mitochondrial autophagy is stimulation of transcription factor EB (TFEB), a master regulator of lysosomal biogenesis. Interestingly, PGC-1α, a key regulator of mitochondrial biogenesis, is upregulated in parallel, so that mitochondrial turnover is stimulated without diminishing the total number of mitochondria [115]. Whether such a mechanism also exists in cardiomyocytes, and, if so, when it is activated and to what extent it is involved in the clearance of damaged mitochondria in the heart remains to be elucidated. In theory, mitophagy and mitochondrial biogenesis should be coupled. If so, investigating the underlying mechanisms, particularly the role of TFEB in regulating PGC-1α/β, NRF1/2 and Tfam, in cardiomyocytes is of great interest.

Mitochondrial Fission and Fusion Proteins

Mitochondria are highly dynamic organelles and their morphology changes continuously through fusion and fission.[116] The fusion of mitochondria is regulated by Mfn1 and Mfn2 localized on the OMM and Opa1 on the IMM, whereas fission is regulated by Drp1, Fis1 and Miff.[117] Although Dnm1, a yeast homolog of mammalian Drp1, plays an important role in mediating mitophagy in yeast,[118] whether fission is prerequisite for mitochondrial autophagy and, if so, how it is coupled to autophagy are not fully understood in mammalian cells.[11, 41] Twig *et al* reported that fission events often generate uneven daughter mitochondria and that the one with reduced membrane potential has a reduced probability to re-fuse in INS1 cells.[11] The daughter mitochondria that are unable to re-fuse are characterized by decreased expression levels of Opa1. Inhibition of fission through a dominant negative form of Drp1 or knockdown of Fis1, a factor that is involved in recruiting Drp1 to mitochondria, inhibits mitochondrial autophagy and causes accumulation of dysfunctional mitochondria.[11] This study suggests that mitochondrial fission is important in order to segregate dysfunctional mitochondrial for their removal through autophagy. MEF cells deficient in Opa1 or Mfn1/2 exhibit an increase in fragmented mitochondria and mitochondrial autophagy during starvation, whereas downregulation of Drp1 induces unopposed fusion of mitochondria and prevents mitochondrial autophagy.[119] Parkin promotes mitochondrial fission through ubiquitination and degradation of Mfn1 and Mfn2, which, in turn, leads to increases in mitophagy.[120] Mild and transient oxidative stress induces mitophagy, but not non-selective autophagy, in a Drp1-dependent manner in HeLa cells,[121] and overexpression of Fis1 stimulates mitochondrial fragmentation and mitophagy in MEF cells.[122] These results are all consistent with the notion that mitochondrial autophagy couples to mitochondrial fission. However, it is possible that interventions used to alter mitochondrial fission and fusion could also directly or indirectly affect mitochondrial autophagy independently of the fission and fusion.[123] For example, Drp1 affects general autophagy through its interaction with Bcl-xL in cardiomyocytes.[124] Even if mitochondrial fission directly affects autophagy, the molecular mechanisms by which fission mediates mitochondrial autophagy remain to be elucidated.

Genetic manipulation of proteins involved in mitochondrial fission/fusion induces both morphological changes and functional impairment in mitochondria in cardiomyocytes. Mitochondrial fragmentation and autophagy induced by overexpression of Bnip3 were both

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inhibited in the presence of dominant negative Drp1 in adult ventricular cardiomyocytes.[125] Python mutant mice, which have a single mutation in exon 11 of the *Drp1* gene, exhibit elongated mitochondria, suggesting impairment of mitochondrial fission.[126] These mice show an approximately 50% reduction in ATP level in the heart and develop dilated cardiomyopathy.[126] Although basal cardiac function is impaired in cardiac specific Drp1 KO mice, a consensus has not been reached as to whether or not removal of dysfunctional mitochondria by autophagy is reduced in these mice. Ikeda *et al* reported that genetic deletion of Drp1 suppresses both general autophagy and mitochondrial autophagy in the mouse heart *in vivo*.[124] Kageyama *et al* reported that Drp1 is required for Parkin-independent mitochondrial autophagy in the heart and that Drp1 and Parkin act synergistically to promote mitochondrial homeostasis in the brain.[48] On the other hand, Song *et al* showed that Drp1 ablation increases mitophagy and causes generalized loss of mitochondria.[127]

The role of mitochondrial fusion in regulating autophagy and cardiac function appears more complex. Although homozygous mutation of Opa1 is embryonic lethal,[128] heterozygous $Opa1^{+/-}$ mice exhibit late onset of cardiomyopathy, with a reduced mtDNA copy number and mitochondrial dysfunction.[128] Whether mitochondrial autophagy is enhanced in this model is unknown. Downregulation of Mfn1 increased the number of small, spherical mitochondria,[129] whereas downregulation of Mfn2 increased pleiomorphic and enlarged mitochondria in cardiomyocytes.[130] In both cases, the cardiomyocytes were protected against stress, although another report showed that downregulation of Mfn2 induces mitochondrial dysfunction.[69] Combined ablation of Mfn1 and Mfn2 in the adult heart induces mitochondrial fragmentation and respiratory dysfunction, resulting in dilated cardiomyopathy.[131, 132] Downregulation of Mfn2 inhibits Parkin-mediated mitophagy, whereas unopposed fission caused by either single or combined downregulation of Mfn1 and Mfn2 may positively affect non-selective autophagy.[133] Thus, the overall effects of Mfn1/2 downregulation upon mitochondrial clearance by autophagy is unclear. Furthermore, Bhandari *et al* demonstrated that suppression of mitochondrial fusion rescues the cardiomyopathy induced by Parkin deficiency in *Drosophila*.[134] These studies indicate that mitochondrial fusion contributes to the cardiac dysfunction of Parkin deficiency by promoting contamination of the healthy mitochondrial pool with damaged mitochondria, independently of mitophagy.

Atypical forms of mitochondrial clearance

Macroautophagy is separated into several specific steps, including induction, recognition and selection of cytoplasmic substrates, formation of the autophagosome around substrates, autophagosome-lysosome fusion, degradation of the autolysosomal contents, and release of the degradation products into the cytoplasm. The canonical, or conventional, autophagic pathway consists of evolutionarily conserved signaling molecules encoded by Atgs, including Atg4, Atg5, Beclin1 (Atg6), Atg7, Atg12, and Atg16, that govern these steps. On the other hand, increasing lines of evidence suggest that non-canonical autophagic pathways may also be present. Nishida *et al* revealed that $Atg5^{-/-}Atg7^{-/-}$ double-knockout cells are still able to form autophagosomes and degrade autophagic substrates inside autolysosomes in response to certain stimuli.[42] During this process of Atg5/Atg7-independent autophagy,

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termed "alternative autophagy," lipidation of LC3 does not occur. Instead, Rab9, a small GTPase involved in membrane trafficking and fusion between the trans-Golgi network (TGN) and late endosomes, plays a critical role in generating autophagosomes in the alternative autophagic pathway by promoting fusion of the phagophore with vesicles derived from the TGN and late endosomes. Recent evidence suggests that ULK1-dependent, Atg5-independent macroautophagy is the dominant process for removing mitochondria from reticulocytes in the final stage of erythrocyte maturation.[44] Oxidative stress induces a vesicular transport pathway that selectively removes mitochondrial proteins for delivery to the lysosomes in COS7 cells. This mechanism does not require mitochondrial depolarization and is independent of Atg5 and LC3, indicating that it is distinct from either autophagy or selective mitophagy.[85] However, the functional significance of alternative autophagy has not yet been demonstrated in the heart. Although conventional macroautophagy is attenuated in Drp1 knockout (KO) mice, Parkin-mediated mitophagy may be stimulated.[127] Thus, it is possible that multiple mechanisms may mediate degradation of damaged mitochondria.

What is the role of mitophagy or mitochondrial autophagy in the heart at baseline?

Conditional cardiac specific *atg5* KO mice, which serve as a loss-of-function model for general autophagy, develop cardiac dysfunction, suggesting that general autophagy plays an essential role in maintaining the physiological function of the adult heart at baseline.[135] These mice exhibit accumulation of misfolded proteins and damaged organelles, including mitochondria, consistent with the notion that protein/organelle quality control mediated by autophagy is essential for the heart even under resting conditions. Interestingly, *beclin1*+/− mice, another loss-of-function model of autophagy, do not exhibit any significant baseline cardiac phenotype, despite the fact that stress-induced autophagy is significantly inhibited.[136] The difference in baseline cardiac phenotype in these mice may be caused by a difference in the level or the timing of autophagy suppression. Atg5 and Beclin1 (Atg6) may regulate qualitatively different types of autophagy.[42] It is also possible that the development of compensatory mechanisms may vary depending upon which Atg is downregulated. Alternatively, the difference may be related to the fact that autophagy related genes also mediate functions other than autophagy in cells. These issues represent the difficulty and limitations of elucidating the functional significance of mitochondrial autophagy or mitophagy in the heart using a single genetically altered mouse model.

The aforementioned studies suggest the importance of general autophagy in the maintenance of protein/organelle quality control and cardiac function at baseline. However, whether or not mitochondrial autophagy or mitophagy is critically involved in this process remains unknown. Although Parkin and Pink1 regulate multiple cellular functions besides mitophagy,[62] their direct involvement in mitophagy means that loss-of-function studies of Parkin and Pink1 can provide valuable information regarding the functional involvement of Pink1-Parkin-mediated mitophagy in the heart. In fact, increasing lines of evidence suggest that Pink1-Parkin-mediated autophagy participates in mitochondrial quality control and the maintenance of cardiac function in the heart at baseline. Cardiomyocytes in which Bnip3 is overexpressed are able to eliminate damaged mitochondria through autophagy via a Parkin-

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dependent mechanism, indicating that Parkin-mediated mitochondrial autophagy exists in cardiomyocytes.[125] Parkin KO mice exhibit normal cardiac function at baseline, but their cardiomyocytes exhibit morphologically disorganized mitochondria with or without mitochondrial dysfunction.[137, 138] Interestingly, clearance of mitochondria by autophagy appears to be maintained at baseline in these mice. This may be due to a low rate of mitochondrial degradation by autophagy at baseline, compensation for the lack of Parkin E3 ligase activity by other E3 ligases [134], such as tumor necrosis factor-receptor-associated factor 2, [139] or compensation by Parkin-independent mechanisms of autophagy, such as general autophagy. Song *et al* suggested that the lack of Parkin-mediated mitophagy in heart specific Mfn2 KO mice is compensated for by activation of non-selective autophagy in cardiomyocytes,[133] thereby allowing cardiomyocytes to maintain mitochondrial quality. This suggests that both mitophagy and general autophagy cooperatively participate in the removal of mitochondria in cardiomyocytes. Currently, molecular mechanisms by which mitochondria are degraded by Mfn2-Parkin-independent autophagy are unknown. However, it would be interesting to clarify whether these two forms of autophagy degrade the same targets or qualitatively or quantitatively different populations of mitochondria, as well as to assess the relative contributions of mitophagy and general autophagy to overall mitochondrial quality control in cardiomyocytes. It should be noted that Parkin deficiency induces significant contractile dysfunction in the heart in *Drosophila*, suggesting that such cross talk may not exist in lower organisms. [134] Aging-induced upregulation of p53 sequestrates Parkin in the cytosol, thereby inhibiting mitophagy in mice. Since p53-deficient mice exhibit less of a decline in mitochondrial integrity and cardiac reserve during aging, it is possible that endogenous Parkin may play an important role in mediating mitophagy and that Parkin-mediated mitophagy declines with aging, in part due to p53.[89]

Whether or not endogenous Pink1 plays an essential role in mediating mitophagy in the heart remains to be demonstrated. Since Pink1-mediated phosphorylation of Mfn2 leads to recruitment of Parkin and induction of mitophagy,[69] it is expected that downregulation of Pink1 inhibits mitophagy. Consistently, mouse hearts lacking Mfn2 develop cardiac dysfunction with age,[69] suggesting that mitophagy regulated by the Pink1-Mfn2 pathway plays an important role in maintaining cardiac function at baseline. Another study showed, however, that downregulation of Mfn2 exerts protective effects in the heart in response to cell death stimuli and ischemia/reperfusion.[130] Thus, further investigation is required to clarify the role of Mfn2 in mediating mitophagy and mitochondrial quality control in the heart. Genetic deletion of Pink1 induces more severe cardiac effects than that of Parkin,[140] despite the fact that Pink1 and Parkin are thought to work together to mediate mitophagy. This may be due to the fact that Pink1 has additional functions besides Parkin-mediated autophagy, including phosphorylation of TNF receptor-associated protein 1 (TRAP1), to protect against cell death.[141]

The role of mitophagy or mitochondrial autophagy in protection against cardiac stress

The protective effect of mitophagy and mitochondrial autophagy in the heart generally appears more prominently during stress. In response to myocardial infarction (MI),

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activation of autophagy is observed most prominently in the border zone during the sub-acute phase (1 week after MI), returning to normal during the chronic phase (3 weeks after MI).[142] The autophagic vacuoles in the border zone are large and contain organelles, including degraded mitochondria. In contrast, autophagy with normal-sized autophagosomes is upregulated in the remote zone during the chronic phase.[142] Enhancing autophagy with rapamycin 2 weeks after coronary ligation ameliorates cardiac dysfunction and maladaptive remodeling, whereas inhibition of autophagy with bafilomycin A1 worsens them.[142] These observations imply the existence of multiple forms of autophagy and suggest that some of them may play a role in mediating the clearance of mitochondria in a phase-dependent fashion after MI. A study using *beclin1* +/− mice also showed that suppression of autophagy is detrimental during the chronic phase of MI. Hoshino *et al* reported that autophagy peaks during the acute phase of MI (several hours after coronary ligation) in the border zone, as evaluated with GFP-LC3 transgenic mice.[50] In this model, mitochondrial autophagy, as indicated by the presence of autophagosomes containing mitochondria, also takes place during the same period.[50] MI induced by permanent coronary artery ligation upregulates p53 and TP53-induced glycolysis and apoptosis regulator (TIGAR) in mice. Genetic deletion of p53 or TIGAR in mice stimulates mitochondrial autophagy and inhibits accumulation of damaged mitochondria and apoptosis, an effect which is abolished by chloroquine treatment. The authors suggest that the beneficial effects of the p53 or TIGAR downregulation may be mediated by mitophagy.[50] These results are consistent with the negative regulation of mitophagy by cytosolic p53.[89] Translocation of Parkin to mitochondria and ubiquitination of mitochondrial proteins occur during the acute phase of MI, suggesting that Parkin-mediated mitophagy is stimulated.[137] Parkin-deficient mice exhibit a decrease in mitochondrial autophagy, accumulation of dysfunctional mitochondria and a reduced survival rate after MI.[137] Taken together, these results suggest that either mitophagy or mitochondrial autophagy is stimulated in the heart during the chronic phase of cardiac remodeling and that they are protective for the heart.

During myocardial reperfusion after a period of myocardial ischemia, mitochondria generate ROS, initiating a feedforward mechanism of oxidative stress, mitochondrial injury and cell death.[143] Selective elimination of damaged mitochondria by mitophagy or mitochondrial autophagy is predicted to protect cardiomyocytes during reperfusion. In fact, loss of Pink1 has been reported to increase the infarct size after I/R, although this study did not examine mitochondrial autophagy in the heart.[144] Ischemic preconditioning (IPC) induces the translocation of Parkin to mitochondria and the IPC-mediated cardioprotective effect is abolished in Parkin KO mice.[145] The translocation of p62 to mitochondria is also impaired in these mice, suggesting that Parkin and p62 act together to induce mitochondrial autophagy in response to IPC.[145] Whether the cardioprotective effect of IPC requires mitophagy rather than the mitophagy-independent functions of Parkin remains to be elucidated. If mitochondrial autophagy does mediate the effect of IPC, investigating how pre-activated autophagy protects the heart against I/R may allow identification of novel modalities of cardioprotection. Myocardial I/R injury is exacerbated in cardiac specific *Drp1* +/− mice, in which mitochondrial fission and autophagy are both inhibited.[124] Thus, suppression of mitophagy and mitochondrial autophagy appears to be detrimental during I/R. Since translocation of Drp1 from the cytosol to mitochondria is stimulated by I/R,

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enhancement of the quality control mechanism appears to be essential for minimizing myocardial injury during reperfusion. On the other hand, it has been shown previously that Beclin1 haploinsufficiency suppresses autophagy and inhibits I/R injury.[136] Why haploinsufficiency of Drp1 and Beclin1 exhibit directionally opposite effects upon I/R injury is currently unknown. However, one possibility is that Drp1 and Beclin1 may affect general autophagy and mitochondrial autophagy to different extents. Another, less attractive, possibility is that Drp1 and Beclin1 differentially affect cellular functions other than autophagy. Further investigation is required to address this issue.

There are fewer publications addressing mitochondrial autophagy during pressure overload. However, the protein level of Pink1 is known to be decreased after TAC. Since Pink1-deficient mice develop age-dependent hypertrophy and cardiac dysfunction accompanied by mitochondrial dysfunction even without pressure overload,[140] downregulation of Pink1 and consequent suppression of mitochondrial autophagy may be detrimental during pressure overload as well.

Perspective

In summary, the recent progress regarding molecular mechanisms of mitophagy, including how damaged mitochondria are recognized and how they are engulfed by autophagosomes, has greatly improved our understanding of quality control mechanisms in mitochondria. However, our knowledge regarding mitophagy in the heart and the cardiomyocytes therein is very limited. First of all, whether mitochondria are degraded by mitophagy or general autophagy requires more studies, using more sensitive and accurate methods to monitor lysosomal degradation of mitochondrial proteins,[45] together with electron microscopic observation to determine whether autophagosomes contain mitochondria alone or other materials as well. Establishing more reliable methods to monitor mitochondrial autophagy would dramatically advance our understanding of the molecular signaling mechanisms of mitophagy/mitochondrial autophagy. In particular, whether or not the Pink1/Parkin pathway is involved in mitochondrial autophagy/mitophagy in cardiomyocytes in response to relevant stresses *in vivo* urgently requires more investigation. If the Pink1/Parkin pathway does play an important role in mitophagy/mitochondrial autophagy in cardiomyocytes during relevant stresses, then more investigation is required regarding interactions between the Pink1/Parkin pathway, the receptor-mediated mechanisms, including those mediated by Bnip3, Nix, FUNDC and cardiolipin, and the protein kinases, such as ULK1/2, AMPK, mTOR and CK2. In addition, we cannot exclude the presence of novel mechanisms. For example, it is possible that novel functional homologs of yeast Atg32 and Atg11 may exist. Unbiased approaches are therefore essential to clarify how damaged mitochondria are recognized by autophagy.[146]

Increasing lines of evidence suggest that autophagic degradation of mitochondria can be observed even when Parkin, Mfn2, or Drp1, molecules previously shown to mediate mitochondrial autophagy in the heart, are downregulated.[138, 133, 127] Thus, mitochondria may be degraded through multiple mechanisms.[8] If mitochondria are degraded by both mitophagy and general autophagy, identifying an intervention that could conveniently distinguish between these mechanisms would be helpful, since general autophagy and

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mitophagy may have distinct functional significance. If mitochondria are also degraded by the alternative autophagy pathway identified by Nishida *et al*, some other convenient method would be required to monitor these autophagosomes since they are LC3-independent.[42] When one mechanism of mitochondrial degradation is inhibited, another mechanism may be activated to compensate for its loss.[147, 127] If this is shown to be the case, elucidating the interactions between the multiple mechanisms of autophagy may be of great interest.

Although it remains controversial as to whether uncontrolled autophagy can kill cells, a growing line of evidence suggests that autophagy may be detrimental under some conditions.[148, 149] One can speculate that excessive elimination of mitochondria may be detrimental for cardiomyocytes, but whether this can be caused by excessive mitochondrial autophagy or mitophagy, and, if so, what would induce such a condition remain unknown.

Increasing lines of evidence suggest that autophagy is intimately involved in the survival and death of cardiomyocytes. It is very likely that the beneficial effects of autophagy are partly mediated through elimination of damaged mitochondria and consequent prevention of mitochondrial dysfunction, oxidative stress, and cell death. In order to introduce modification of autophagy and mitophagy into the treatment of cardiovascular disease, further investigations are needed to better understand when autophagy is activated or inhibited and how it affects the function of mitochondria in response to a wide variety of cardiovascular stress conditions.

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Abbreviations

AIM	Atg8 family interacting motif
AMPK	AMP (adenosine monophosphate)-activated protein kinase
ATP	adenosine triphosphate
Bak	Bcl-2-antagonist/killer 1
Bax	Bcl-2-associated X protein
Bcl-2	b-cell leukemia/lymphoma 2 protein
Bnip3	BCL2/adenovirus E1B 19kDa interacting protein 3
CCCP	Carbonyl cyanide m-chlorophenyl hydrazine
CK2	casein kinase 2
CMA	chaperone-mediated autophagy

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Cue5	Cue5p
Dnm1	dynamin-related GTPase DNM1
Drp1	dynamin 1-like
FCCP	Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone
Fis1	fission 1
Foxo1	forkhead box O1
FUNDC1	FUN14 domain containing 1
GFP	green fluorescence protein
HDAC	histone deacetylase
Hsc70	70kDa Heat-Shock Cognate Protein
IMM	inner mitochondrial membrane
IPC	ischemic preconditioning
I/R	ischemia/reperfusion
Lamp-2	lysosomal-associated membrane protein 2
LC3	microtubule associated protein 1 light chain 3
LIR	LC3-interacting region
MARF	Mitochondrial assembly regulatory factor
MDV	Mitochondria-derived vesicles
Mfn	mitofusin
MI	myocardial infarction
Miro1/2	ras homolog family member T1/2
mPTP	mitochondrial permeability transition pore
mtDNA	mitochondrial deoxyribonucleic acid
Nbr1	neighbor of BRCA1 gene 1
Nix/Bnip3L	NIP3-like protein X
OMM	outer mitochondrial membrane
Opa1	optic atrophy 1
Parkin	Parkinson juvenile disease protein 2
Pex14	Peroxisomal membrane anchor protein PEX14
PGAM5	phosphoglycerate mutase family member 5
PGC-1	peroxisome proliferator-activated receptor-gamma coactivator-1
Pink1	PTEN-inducible putative kinase 1

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polyQ	poly-glutamine
p53	tumor protein p53
p62	sequestosome 1
qRCR	quantitative polymerase chain reaction
RFP	red fluorescent protein
RNAi	ribonucleic acid interference
ROS	reactive oxygen species
Ser	serine
Sirt3	sirtuin 3
TFEB	transcription factor EB
TIGAR	chromosome 12 open reading frame 5
Tollip	toll interacting protein
Tyr	tyrosine
UBA	ubiquitin-associated domains
ULK1	unc-51 like autophagy activating kinase 1
UPS	ubiquitin proteasome system
Uth1	SUN family protein UTH1
VDAC	voltage-dependent anion channel
VCP/p97	valosin containing protein

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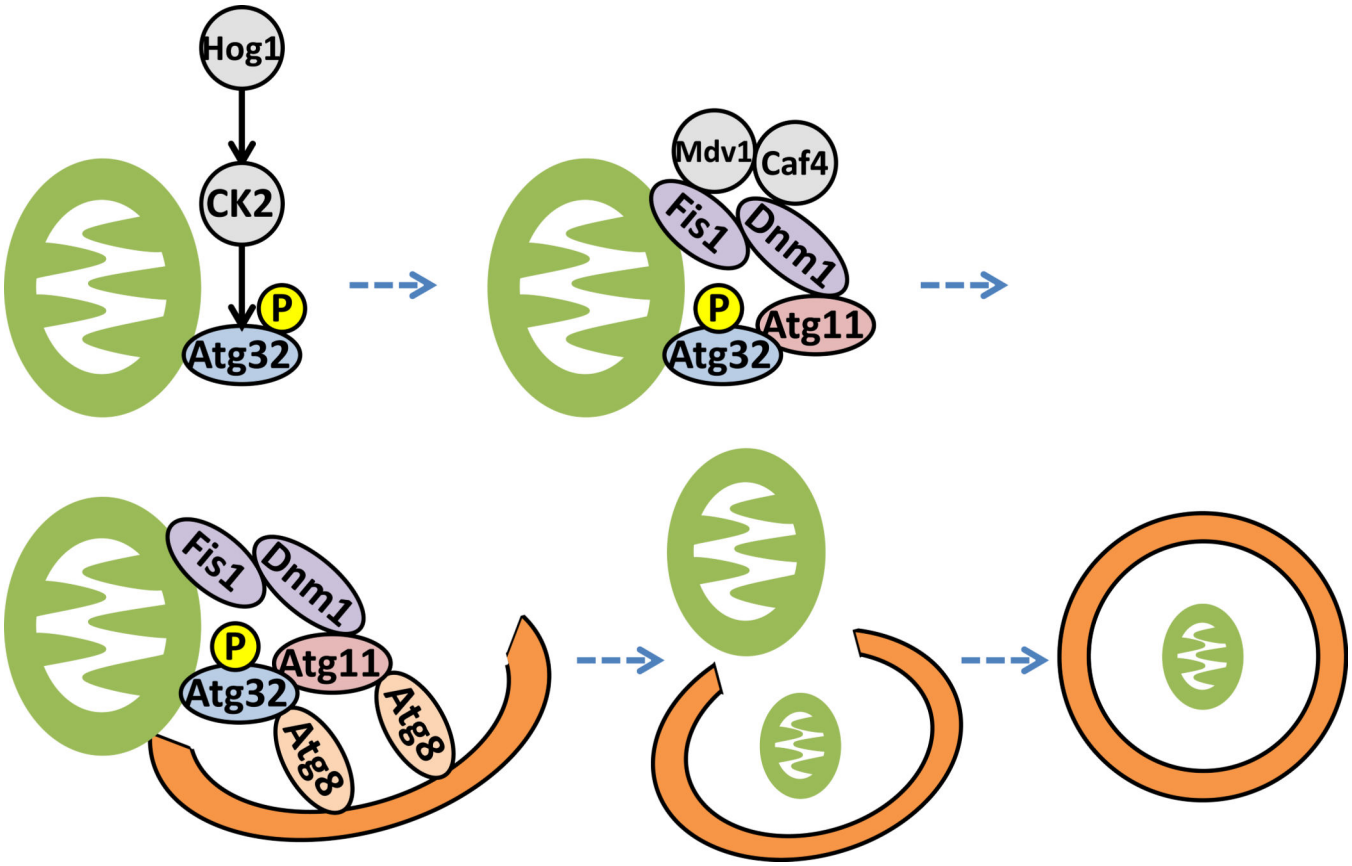

Fig. 1.

The mechanism of mitophagy in yeast. CK2 directly phosphorylates Atg32 at Ser 114 and Ser 119 when cells are cultured in lactate medium and then shifted to nitrogen starvation medium supplemented with glucose. This phosphorylation, especially phosphorylation at Ser 114, is critically important for the Atg32-Atg11 interaction, which is required for the delivery of mitochondria to the phagophore assembly site (PAS). Atg11 recruits the mitochondrial fission complex, which consists of Dnm1, Fis1, Mdv1 and Caf4. Subsequently, mitochondrial fission and mitophagy take place. Mitochondrial division is important for mitophagy in yeast.

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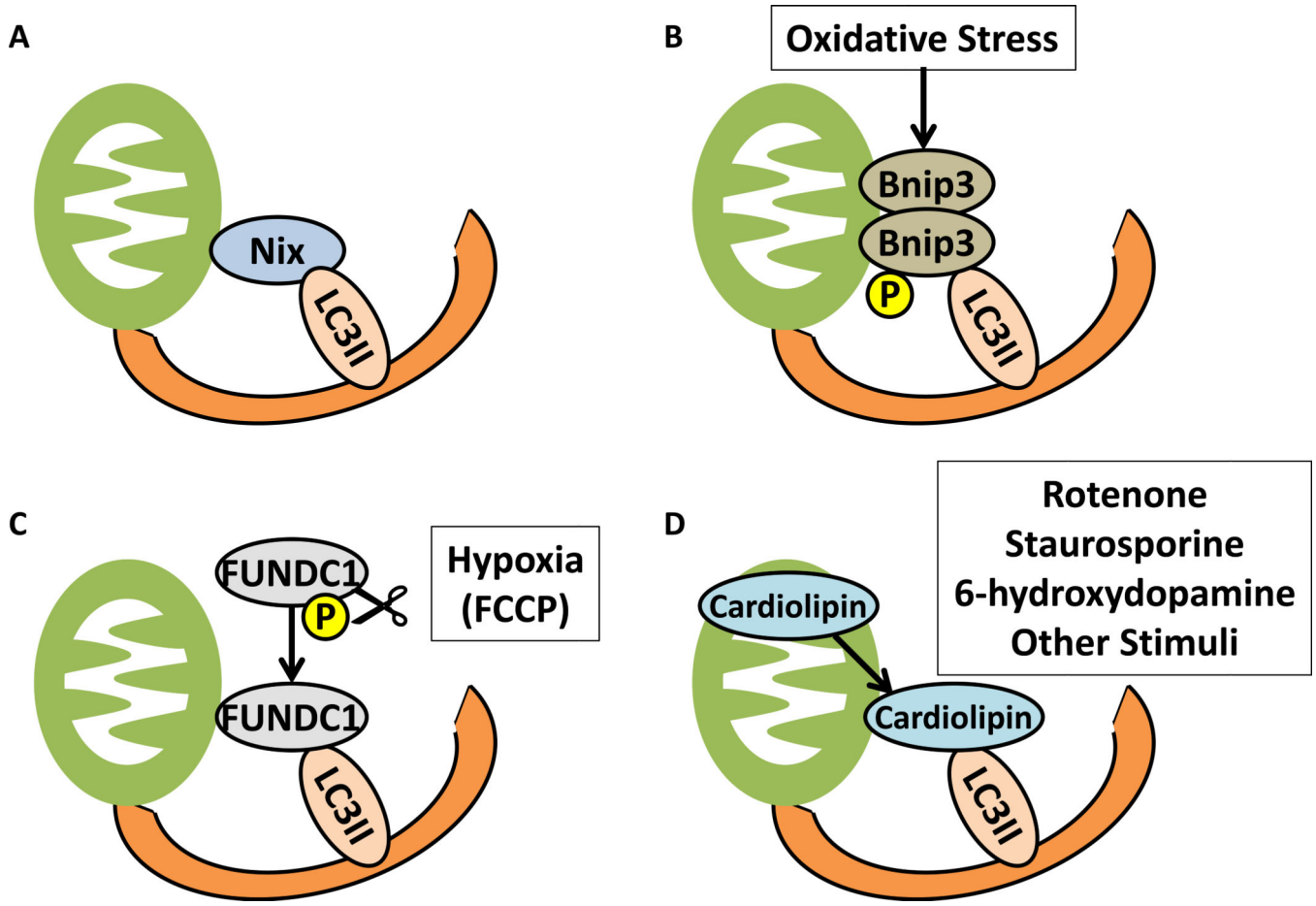

Fig. 2.

LC3-interacting molecules act as receptors for autophagosomes on mitochondria. (A, B) Nix and Bnip3, BH3-only proteins, interact with LC3 through the LIR and regulate mitochondrial autophagy. Oxidative stress induces homodimerization and activation of Bnip3. Phosphorylation of the LIR in Bnip3 promotes the association between Bnip3 and LC3. (C) Under hypoxia, dephosphorylation of FUNDC1 at Ser 13 or Tyr 18 stabilizes the interaction between FUNDC1 and LC3. (D) Cardiolipin, a phospholipid of the IMM, translocates to the mitochondrial surface in response to mitochondrial damage. The externalized cardiolipin binds to LC3.

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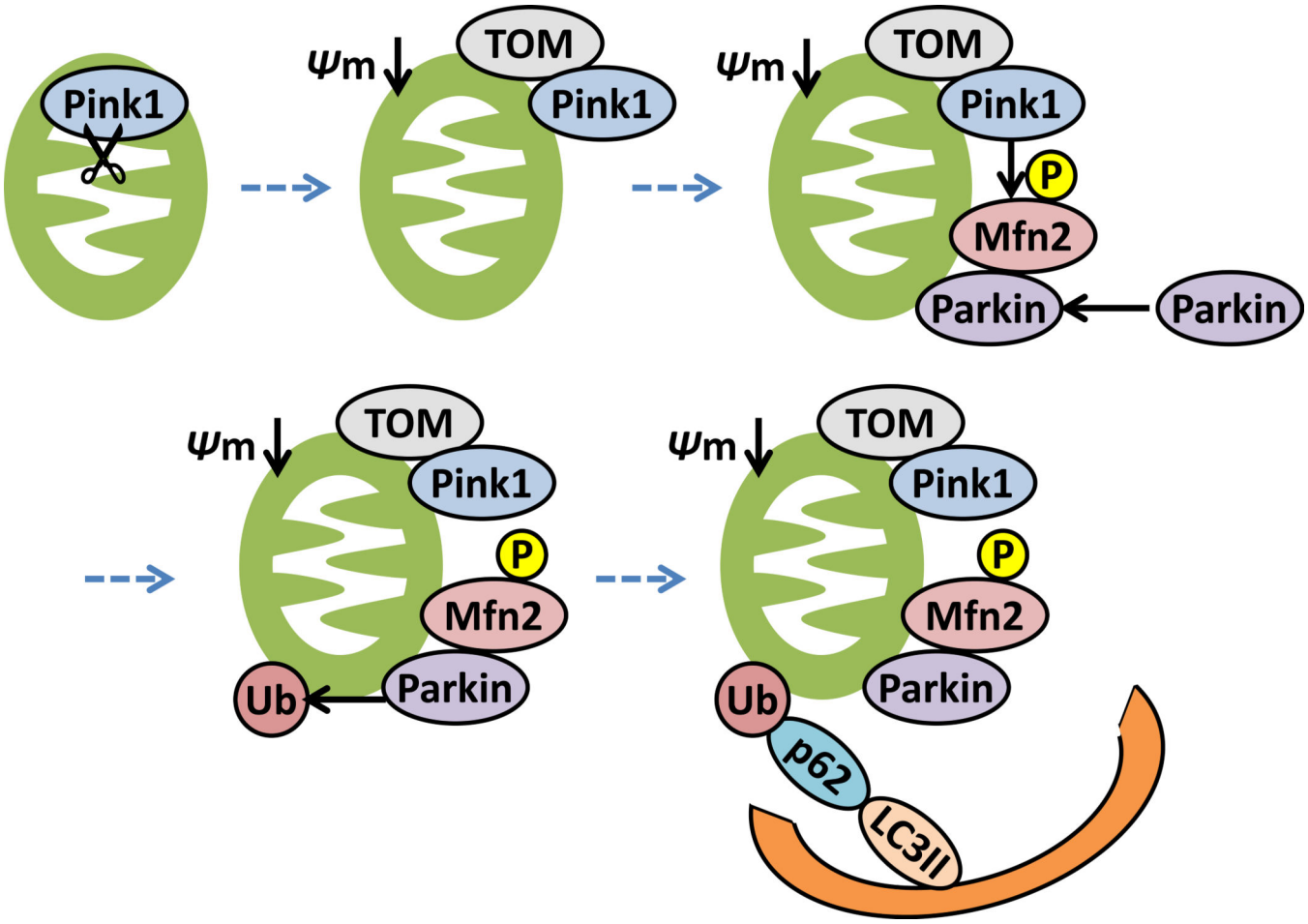

Fig. 3.

The mechanism of mitochondrial autophagy mediated by Pink1-Parkin in mammalian cells. Mitochondrial proteases and peptidases continuously degrade Pink1 in intact mitochondria. However, Pink1 is not imported to the inner membrane and is not cleaved in depolarized mitochondria. Pink1 then accumulates at the outer membrane and recruits Parkin. Mfn2 phosphorylated by Pink1 acts as a receptor for Parkin on mitochondria. Parkin ubiquitinates multiple proteins of the outer membrane. These ubiquitinated proteins are recognized by p62, a ubiquitin- and LC3-binding adaptor protein, followed by mitochondrial autophagy.