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Minireview: Selective Degradation of Mitochondria by Mitophagy[*]

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Abstract

Mitochondria are the essential site of aerobic energy production in eukaryotic cells. Reactive oxygen species (ROS) are an inevitable by-product of mitochondria metabolism and can cause mitochondrial DNA mutations and dysfunction. Mitochondrial damage can also be the consequence of disease processes. Therefore, maintaining a healthy population of mitochondria is essential to the well-being of cells. Autophagic delivery to lysosomes is the major degradative pathway in mitochondrial turnover, and we use the term mitophagy to refer to mitochondrial degradation by autophagy. Although long assumed to be a random process, increasing evidence indicates that mitophagy is a selective process. This review provides an overview of the process of mitophagy, the possible role of the mitochondrial permeability transitionin mitophagy and the importance of mitophagy in turnover of dysfunctional mitochondria.

Introduction

Mitochondria are the site of oxidative phosphorylation, which generates ATP coupled to electron transfer from respiratory substrates to oxygen by a series of oxidation-reduction reactions that pump protons across the mitochondrial inner membrane from the matrix space [1]. In mitochondria and submitochondrial particles as well as in intact cells, respiration produces reactive oxygen species (ROS) like H_2O_2 and superoxide anion ($O_2^{\bullet-}$), especially if respiration is inhibited or otherwise disordered [2-5]. ROS derived from mitochondria can promote cytotoxicity and cell death [4;5].

As a major source of ROS production, mitochondria are especially prone to ROS damage. Such damage can induce the mitochondrial permeability transition (MPT) caused by opening of non-specific high conductance permeability transition (PT) pores in the mitochondrial inner membrane (see below). ATP depletion from uncoupling of oxidative phosphorylation then promotes necrotic cell death, whereas release of cytochrome *c* after mitochondrial swelling activates caspases and onset of apoptotic cell death [6;7].

ROS also attack nucleic acids and are thus genotoxic. A lack of histones in mitochondrial DNA (mtDNA) accounts, at least in part, for a 10- to 20-fold higher mutation rate of mtDNA compared to nuclear DNA [8]. Although oxidative stress and various disease processes cause mitochondrial damage and dysfunction, even normal mitochondria will accumulate enough oxidative "hits" over time to become damaged and possibly dangerous to the cell. Such defective mitochondria have the potential for futile ATP hydrolysis, accelerated production of ROS and release of proapoptotic proteins. Timely elimination of aged and dysfunctional mitochondria is essential to protect cells from the harm of disordered mitochondrial metabolism and release of proapoptotic proteins. The mechanism of mitochondrial turnover is predominantly autophagic sequestration and delivery to lysosomes for hydrolytic degradation, a process also called mitophagy. Here, we discuss recent evidence that suggests mitophagy is selective process that can specifically target dysfunctional mitochondria.

General Features of Autophagy

Autophagy is the process by which organelles and bits of cytoplasm are sequestered and subsequently delivered to lysosomes for hydrolytic digestion [9]. Autophagy is ongoing in nucleated cells and is typically activated by fasting and nutrient deprivation. In the liver particularly, glucagon promotes autophagy, whereas insulin negatively regulates it [10;11]. During fasting, autophagy is important for generating amino acids, fueling the tricarboxylic cycle, and maintaining ATP energy production. Autophagy also removes toxic protein aggregates and unneeded organelles. Both insufficient and excess autophagy seem capable of promoting cell injury [12]. Appropriate regulation of autophagy is thus essential for cellular well-being.

During autophagy, an isolation membrane forms a cup-shaped membranous structure called a phagophore or pre-autophagosome that eventually envelopes the autophagic target (Figure 1) [13-15]. The origin of isolation membranes is controversial. One proposed source is ribosome-free regions of the rough endoplasmic reticulum (ER), but others suggest that isolation membranes are novel structures devoid of Golgi and ER markers [14]. As isolation membranes envelop and seal around their targets, double–membrane vesicles called autophagosomes form. These autophagosomes then fuse with lysosomes to form autolysosomes, and their sequestered contents are degraded by lysosomal hydrolases and recycled.

As first characterized in yeast, a machinery of genetically conserved autophagy-related proteins regulate and participate in autophagy [16]. These Atg proteins are grouped into different categories depending on their function, including 1) a pair of novel ubiquitin-like protein conjugating systems, the ATG12 and Atg8 systems, which produce vesicle extension and completion, 2) a Class III phosphatidyl choline-3-kinase (PI3K) complex which functions in vesicle nucleation, and 3) a serine-threonine kinase complex (Atg1, Atg13 and Atg17) involved in autophagic induction in yeast. The mammalian homologue of Atg 1 is recently identified as ULK1 (Unc-51-like kinase) [17].

During sequestration and formation of autophagosomes, an Atg12-Atg5 complex binds to Atg16, which translocates to the isolation membrane and functions as a linker involved in formation and elongation of the phagophore (Figure 1). An E1-like enzyme, Atg7, activates

Atg12, which is transferred Atg10, an E2-like enzyme, and conjugated to Atg5 to form an autophagosomal precursor [16;18].

LC3 is a mammalian autophagosomal ortholog of yeast Atg8. In mammalian cells, newly synthesized ProLC3 is processed to its cytosolic form, LC3-I. Like Atg12, LC3-I is activated by Atg7, but is instead transferred to Atg3, a second E2-like enzyme, which cleaves 22 amino acids from the C-terminus to form LC3-II. Conjugation with a phospholipid (phosphatidyl ethanolamine in yeast) creates membrane-bound LC3-II [19;20]. LC3-II localizes selectively to forming and newly formed autophagosomes, making LC3-II a useful autophagosomal marker. Some LC3-II becomes entrapped on the inner surfaces of the double membrane autophagosomes. After fusion with lysosomes, this LC3-II is degraded. Surface LC3-II also disappears, most likely by breakdown of the phospholipid conjugate (Figure 1). Recently, a transgenic mouse strain was created that expresses a green fluorescent protein (GFP)-LC3-II fusion protein. In cells and tissues of this mouse, GFP fluorescence selectively identifies the membranes of forming and newly formed autophagosomes [21].

Molecular Control of Autophagy

Phosphoinositide 3-kinases (PI3Ks) phosphorylate phosphatidylinositol at position 3 of the inositol ring and play an important role in the regulation of autophagy [22]. PI3K inhibitors, such as 3-methyladenine, wortmannin, and LY294002, potently block autophagy. However, different classes of PI3K exert opposing effects on autophagy: Class III PI3K promotes sequestration of autophagic vacuoles, whereas class I PI3K inhibits autophagy. Class III PI3K/p150 associates with Beclin1, a mammalian homologue of Atg6 discovered by yeast two hybrid screening for its interaction with Bcl-2. Recruitment of PI3K-Beclin1 complexes together with Atg12-Atg5 is an initial step in autophagosome formation [23]. Mammalian target of rapamycin (mTor) is a kinase downstream of Class I PI3K whose activation suppresses autophagy. Rapamycin, which inhibits mTOR, induces autophagy apparently by activating protein phosphatase 2A (PP2A) [24]. PP2A also dephosphorylates proapoptotic BH3 only Bcl-2 family proteins, such as Bad and Bcl-2, that associate with mitochondria membranes [25] [26]. Thus, a functional relationship between autophagic proteins and mitochondrial proteins may exist.

Heterotrimeric guanine nucleotide-binding proteins are also involved in autophagy. Nonhydrolyzable GTP analogs, such as GTPγS, inhibit autophagy [27;28].

The G_α-interacting protein (GAIP) elicits autophagic sequestration by accelerating GTP hydrolysis and activating $G_{\alpha i3}$. In addition, Rab24, Rab22, and Rab7, small GTP binding proteins that regulate vesicular transport, participate in processing of late autophagosomes [29;30]

Selective Autophagy

Whether autophagy is selective or non-selective has been controversial. Cytosolic enzymes with different half-lifes are sequestered at similar rates during autophagy, and autophagosomes often contain a variety of different cytoplasmic elements, including cytosolic proteins and organelles such as ER, peroxisomes and mitochondria [31;32]. Such findings led to the assumption that autophagy is a non-specific form of lysosomal degradation. However, more recent findings indicate that autophagy can be a selective process. The presence of peroxin 14 on peroxisomes is required for autophagic degradation of peroxisomes in yeast, a process now called pexophagy in recognition of its selectivity [33]. Some pathogens selectively regulate autophagy in mammalian cells for their survival. *Shigella flexneri* produces the protein, IscB, which inhibits the binding of bacterial VirG to Atg5, which would otherwise induce autophagy [34]. In this way, shigella escapes recognition for autophagic sequestration and elimination. In

addition during the postnatal period, glycogen is selectively sequestered into autophagosomes to enhance glycolytic substrate generation after interruption of transplacental nutrition [35] [36]. Autophagosomes formed postnatally contain large amounts of glycogen and rarely contain mitochondria or other organelles [37].

Increasing evidence indicates that autophagy of mitochondria also occurs selectively, and the term mitophagy has been suggested for this selective mitochondrial autophagy [38]. For example in yeast, an outer membrane protein, Uth1p, is required for efficient mitochondrial autophagy, but a corresponding mammalian protein is yet to be identified [39]. Our work, reviewed below, also strongly indicates that autophagy can show selectivity for mitochondria.

Characteristics and Possible Structure of Mitochondrial Permeability Transition Pores

Recent evidence suggests a possible involvement of the MPT in autophagy. In the MPT, opening of PT pores causes mitochondria to become permeable to all solutes up to a molecular mass of about 1500 Da, an event leading to mitochondrial depolarization and activation of the mitochondrial ATPase (ATP synthase operating in reverse) [40-43]. After the MPT, mitochondria undergo large amplitude swelling driven by colloid osmotic forces, which culminates in rupture of the outer membrane and release of proapoptotic mitochondrial intermembrane proteins into the cytosol, including cytochrome *c*, apoptosis inducing factor, Smac/Diablo and others. The immunosuppressant compound, cyclosporin A (CsA), and various of its analogs inhibit the MPT through interaction with cyclophilin D (CypD) [44; 45].

In one model, PT pores are composed of the voltage dependent anion channel (VDAC) in the outer membrane, the adenine nucleotide translocator (ANT) in the inner membrane and CypD in the matrix space (Figure 2A) [45-48]). Other proteins, such as creatine kinase (intermembrane space), hexokinase (outer membrane) and Bax (outer membrane), are also proposed to contribute to the composition of PT pores. However, the MPT still occurs in cells types like hepatocytes that lack creatine kinase and hexokinase and in ANT-deficient mitochondria isolated from conditional double ANT knockout mice [49]. Most recently, CypD knockout mice have been developed, and mitochondria from these mitochondria still display an MPT, but the MPT observed is insensitive to CsA and requires higher concentrations of calcium for induction [50].

An alternative model of the MPT has been proposed that accounts for these observations (Figure 2B) [51]. This model postulates that PT pores form as a consequence of misfolding of integral membrane proteins caused by ROS, reactive chemicals and other stresses. Becauses misfolding exposes hydrophilic surfaces to the hydrophobic membrane bilayer, the proteins aggregate at these hydrophilic surfaces to enclose channels that conduct all aqueous solutes smaller in size than the channel diameter. Since such permeabilizatoin would be catastrophic to mitochondrial function, chaperones have evolved, including cyclophilin D, that block conductance through these nascent channels. Other chaperones remain to be identified, although indirect evidence suggests that the small heat shock protein, Hsp25/27, and the Rieske iron sulfur protein may be involved [52;53]. When matrix calcium rises to high levels, PT pores open to induce the MPT, an effect mediated by CypD and blocked by CsA. When formation of nascent PT pores from misfolded protein aggregates exceeds the number of chaperones that can regulate and close these pores, an unregulated MPT occurs that is CsA-insensitive and calcium-independent. This change from a regulated to an unregulated PT pore occurs as the time and strength of MPT induction increases. On a molar basis, ANT is the most abundant inner membrane protein and thus is often a target of stresses causing protein misfolding. However, other proteins can also misfold, which explains MPT onset in ANT knockout mice.

Since CypD participates in calcium sensing, the model also explains the greater requirement for calcium for the MPT in CypD deficient mitochondria. Lastly, the model explains why completely exogenous pore-forming peptides like mastoparan and alamethicin induce a CsA-sensitive and calcium-dependent MPT at low concentrations but CsA-insensitive and calcium-independent mitochondrial swelling at higher concentrations [51;54]. At low concentration, chaperones recognize the pore-forming peptides as misfolded protein aggregates and block their conductance, but as the chaperone supply becomes exhausted conductance can no longer be blocked, and mitochondrial swelling, depolarization and uncoupling ensue.

Mitophagy Induced by Nutrient Deprivation

A role of the MPT in mitophagy is implicated in cultured hepatocytes during nutrient deprivation. Autophagic stimulation of rat hepatocytes by serum deprivation and glucagon (a hormone released to the liver during fasting) increases the rate of spontaneous depolarization of mitochondria by 5-fold to about 1% of mitochondria per hour (Figure 3) [55]. These depolarized mitochondria move into acidic vacuoles, which also increase in number after nutrient deprivation. The acidic structures containing mitochondrial remnants are autophagosomes and autolysosomes, and serial imaging reveals an average mitochondrial digestion time of about 7 min after autophagic sequestration [56]. CsA, the MPT blocker, suppresses both mitochondrial depolarization during nutrient deprivation and the proliferation of autophagosomes and autolysosomes. Tacrolimus, an immunosuppressant that does not block the MPT, does not block autophagosomal proliferation, whereas NIM811, a CsA analog and MPT inhibitor that is not immunosuppressive, does block [55;56].

Time-lapse confocal imaging of hepatocytes isolated from transgenic mice expressing GFP fused with LC3 [21] allows direct visualization of the progression of nutrient deprivation-induced mitophagy. In nutritionally replete culture medium, GFP-LC3 fluorescence is diffuse except for small (0.2-0.3 μm) dotted structures distributed randomly throughout the cytoplasm (Figure 4). After imposition of nutrient deprivation, such green dotted structures appear in close association with mitochondria and then form crescent structures surrounding mitochondria. These phagophores or pre-autophagosomes go on to sequester completely the individual mitochondria (Figure 4). Sequestration occurs in about 6 min from the first appearance of dots. In some instances, only a portion of an individual mitochondria becomes sequestered, which indicates that mitochondrial fission occurs coordinately with autophagosome formation. Partial mitochondrial sequestration can occur from both the ends and middle parts of mitochondria [57].

After ring closure, mitochondria depolarize in about 10 min, as revealed by the release of the potential-indicating fluorophore, tetramethylrhodamine methylester (TMRM) (Figure 4). As mitochondria inside autophagosomes depolarize, acidification occurs as revealed by uptake of LysoTracker Red (LTR). After vesicle acidification, GFP-LC3 fluorescence is released or degraded. Typically, fusion with lysosomal precursors occurs at about this time, leading to formation of autolysosomes. Preliminary data indicates that CsA does not block sequestration and formation of GFP-LC3-labeled autophagosomes during nutrient deprivation-induced mitophagy. Rather, CsA may block mitochondrial depolarization and acidification occurring after sequestration. Taken together, these results show that LC3-containing membranes sequester polarized mitochondria during nutrient deprivation-induced mitophagy. Mitochondrial depolarization apparently linked to the MPT then follows completion of autophagosome formation. With acidification and fusion with lysosomal precursors the autophagosomes become autolysosomes [55-57].

Mitophagy after Photodamage

Autophagic processes have long been proposed to remove damaged and dysfunctional mitochondria. Direct experimental confirmation of a role of autophagy in removing damaged mitochondria comes in experiments in which selected mitochondria inside living hepatocytes are subjected to laser-induced photodamage [58]. When portions of GFP-LC3-expressing hepatocyes cells containing 5 to 10 mitochondria are exposed to a pulse of 488-nm light from an argon laser, mitochondrial depolarization occurs in a light dose-dependent fashion, as documented by release of TMRM (Figure 5). At lower doses of light, mitochondria depolarize transiently but subsequently recover TMRM fluorescence within a few minutes. After a greater exposure, mitochondrial depolarization becomes irreversible. After irreversible but not reversible photodamage, green GFP-LC3 fluorescence begins to envelop and completely encircle depolarized mitochondria after about 30 min (Figure 5). This photodamage-induced mitophagy is not dependent on TMRM as a photosensitizer, since photodamage also induces mitophagy in the absence of TMRM. After formation of these autophagosomes, or more specifically mitophagosomes, acidification occurs, as shown by uptake of LTR.

Unlike autophagy induced by nutrient deprivation, photodamage-induced mitophagy is not blocked by PI3K inhibition with 3-methyladenine (10 mM) or wortmannin (100 nM) [58]. Rather, PI3K inhibition appears to augment GFP-LC3 association with photodamaged mitochondria. These findings suggests that light activates mitophagy downstream of PI3K signaling. The results again suggest an involvement of the MPT in autophagy, since previous work shows that photodynamic damage to mitocondria is likely mediated by MPT onset [59; 60]. However, photostimulation of mitophagy appears to bypass the classical upstream PI3K signaling pathways involved in nutrient deprivation-induced autophagy (Figure 1).

Mitophagy and Cell Death

Controversy exists as to whether autophagy promotes or prevents cell death [12;61;62]. If autophagy removes damaged mitochondria that would otherwise activate caspases and apoptosis, then autophagy should be protective. In agreement, disruption of autophagic processing and/or lysosomal function promotes caspase-dependent cell death [62;62;63]. However, excessive and dysregulated autophagy may promote cell death, since enzymes leaking from lysosomes/autolysosomes, such as cathepsins and other hydrolases, can initiate mitochondrial permeabilization, caspase activation and apoptosis, and in certain instances deletion of autophagy genes decreases apoptosis [63]. Indeed, autophagy is often a prominent feature of programmed cell death to the extent that autophagic cell death has been proposed as a distinct mode of cell death [62;64].

The MPT provides a common pathway leading to mitophagy, apoptosis and necrosis (Figure 6). With low intensity stresses, limited MPT onset may only increase mitophagy to rid cells of damaged mitochondria as a repair mechanism. With increasing stress, mitophagy may no longer contain proapoptotic factors being released from mitochondria undergoing the MPT, in which case apoptosis begins to occur. Additionally, an overburdened autophagic apparatus may release lysosomal enzymes and possibly other factors to promote cell death signaling. Lastly when extreme stress causes MPT onset in virtually all cellular mitochondria, ATP levels plummet. Because of bioenergetic failure, neither autophagy nor apoptosis can progress, and only necrotic cell death ensues. The progression from mitophagic repair to apoptosis and then to necrosis after increasing MPT onset has been termed necrapoptosis (Figure 6). Interventions that modulate the extent of MPT onset after stresses therefore affect the relative amount of autophagy, apoptosis and necrosis that follows [6;65].

Mitophagy in Aging

Aging seems to affect mitochondria particularly. Because of mitochondrial ROS generation, protein damage occurs in mitochondria, and mutations of mtDNA accumulate. mtDNA is more susceptible to oxidative damage than nuclear DNA since histones are not present in mitochondria to protect mtDNA and because DNA repair mechanisms in mitochondria are less robust than in the nucleus [8;66]. Moreover, virtually all mtDNA is transcriptionally active compared to 2 or 3% of nuclear DNA, which also makes mtDNA relatively more vulnerable to damage. mtDNA mutations lead to synthesis of abnormal mitochondrial proteins or block synthesis altogether, which further exacerbates mitochondrial dysfunction. Thus in postmitotic cells of aged organisms, morphological abnormalities of mitochondria are often observed, including swelling, loss of cristae, and destruction of inner membranes [67-69]. Moreover, ATP production and respiration in mitochondria from aged animals are lower than in mitochondria from young animals.

Mitochondria of non-proliferating tissues like heart, brain, liver, and kidney constantly turnover with a half-life of 10 to 25 days. Mitochondrial biogenesis occurs by fission of pre-existing mitochondria analogously to bacterial division, but loss of mitochondria during turnover is primarily due to mitophagy [70;71]. Such mitophagy may important to the elimination of dysfunctional mitochondria and mutated mtDNA. Certain mtDNA mutations may decrease recognition signals for mitophagy and therefore accumulate with age [38;72]. For example, a mtDNA mutation causing respiratory defects leading to decreased ROS generation might make a mitochondrion less likely to be targeted for mitophagy, which would promote retention and amplification of the mutation.

Many studies show that mtDNA mutations accumulate with age at an accelerating rate, a phenomenon that may represent an age-related diminishment of autophagic activity [73]. Indeed, formation and processing of autophagosomes diminish with aging [69;74]. Another link between mitophagy and aging is Uth1p. Deletion of Uth1p leads to a selective defect in mitophagy and decreased longevity in yeast during nutrient deprivation [39;75-77]. Uth1p also confers resistance to superoxide- and H_2O_2-induced injuries [39]. Caloric restriction is well known to increase longevity in rodents and other animals [73;78]. Such caloric restriction is an inducer of autophagy, and increased longevity might thus be due, at least in part, to enhanced removal of oxidatively damaged mitochondria and their mutated mtDNA. These hypotheses and speculations relating mitophagy and aging need further investigation.

Conclusion

In normal physiology, cells utilize autophagy to get rid themselves of damaged, dysfunctional and superfluous cytoplasmic components to maintain cellular homeostasis and adjust to changing physiological demands. In this respect, mitochondrial degradation by autophagy (mitophagy) may play an essential role in maintaining mitochondrial functional and genetic integrity. However, there is a need for a better understanding of the regulatory pathways that control mitophagy and the specific signals and markers that target individual mitochondria for autophagic degradation. Such information will likely lead to new insights into the aging phenomena and the pathogenesis of different diseases.

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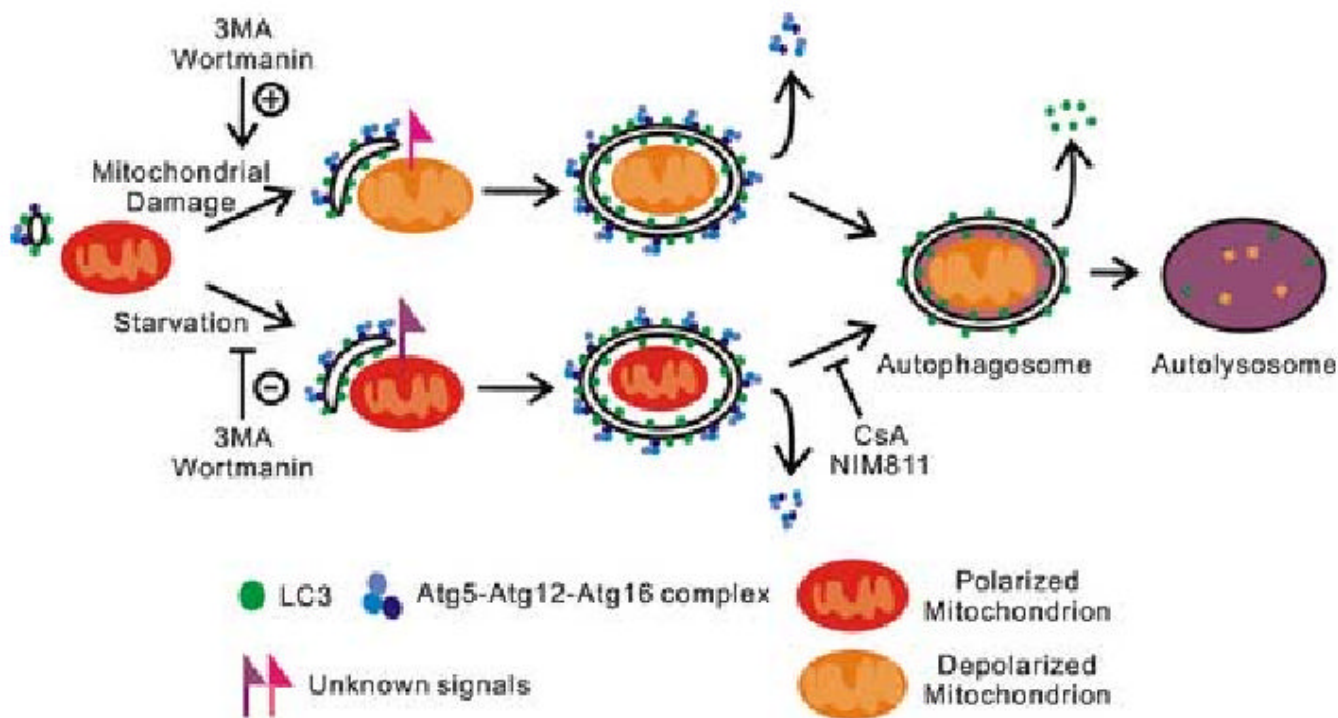
Figure 1. Scheme of Mitophagy
Atg12-Atg5-Atg16 and LC3 complexes localize to isolation membranes. In nutrient
deprivation (starvation), isolation membranes target individual mitochondria by unknown
signals in a process inhibited by the PI3K inhibitors, 3-methyladenine (3MA) and wortmannin.
Isolation membranes completely envelop individual mitochondria to form double membrane
vesicles (autophagosomes). After this sequestration, mitochondria depolarize in a CsA and
NIM811 sensitive fashion, and Atg12-Atg5/Atg16 complexes are released from the
autophagosomal surface. Autophagosomes then acidify and fuse with lysosomal vesicles to
form autolysosomes. Lysosomal hydrolases digest the inner autophagosomal membrane and
degrade LC3 trapped inside autophagosomes. Remaining LC3 on the surface of
autophagosomes is released. After mitochondrial damage, mitochondria first depolarize and
then are recognized and sequestered by isolation membranes recognizing unknown markers
on the damaged mitochondria. 3MA and wortmannin do not inhibit this process but actually
seem to augment it. In both pathways, sequestered mitochondria are completely digested and
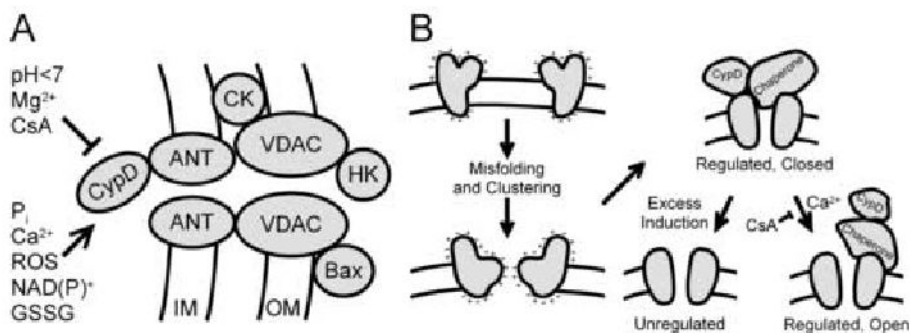their molecular components recycled to the cytoplasm.

Figure 2. Models of the Permeability Transition Pore
In one model (**A**), the PT pore is composed of ANT from the inner membrane (IM), CypD from the matrix, VDAC from the outer membrane (OM) and other proteins, including hexokinase (HK), creatine kinase (CK) and Bax, a proapoptotic Bcl2 family member. Ca^{2+}, inorganic phosphate (Pi), ROS and oxidized pyridine nucleotides $NAD(P)^+$ and glutathione (GSSG) promote PT pore opening, whereas CsA, Mg^{2+} and pH less than 7 inhibit opening. In an alternative model (**B**), PT pores form from misfolding and aggregation of damaged mitochondrial membrane proteins at hydrophilic surfaces facing the hydrophobic membrane bilayer. CypD and other chaperones bind to the nascent PT pores and block conductance of solutes through the aqueous channels formed by the protein clusters. High Ca^{2+} opens these regulated channels acting through CypD, an effect blocked by CsA. As misfolded protein clusters exceeds the number of chaperones available to regulate them, constitutively open unregulated channels form that are not inhibited by CsA.
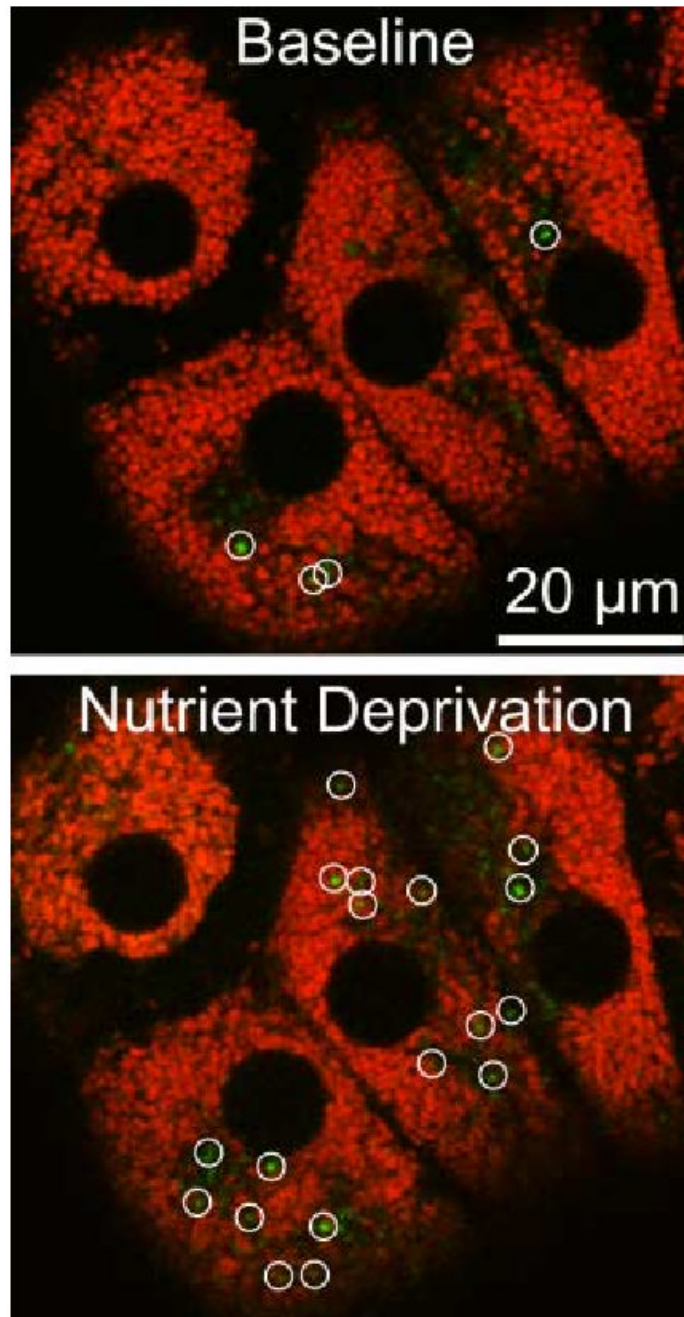
Figure 3. Mitochondrial Depolarization in Hepatocytes after Nutrient Deprivation
Cultured rat hepatocytes were loaded with MitoTracker Green and TMRM and imaged by confocal microscopy before (**Baseline**) and 60 min after (**Nutrient Deprivation**) changing from complete growth medium to a modified Krebs-Ringer buffer containing glucagon. Green-fluorescing structures (circles) in the overlay images are newly depolarized mitochondria.
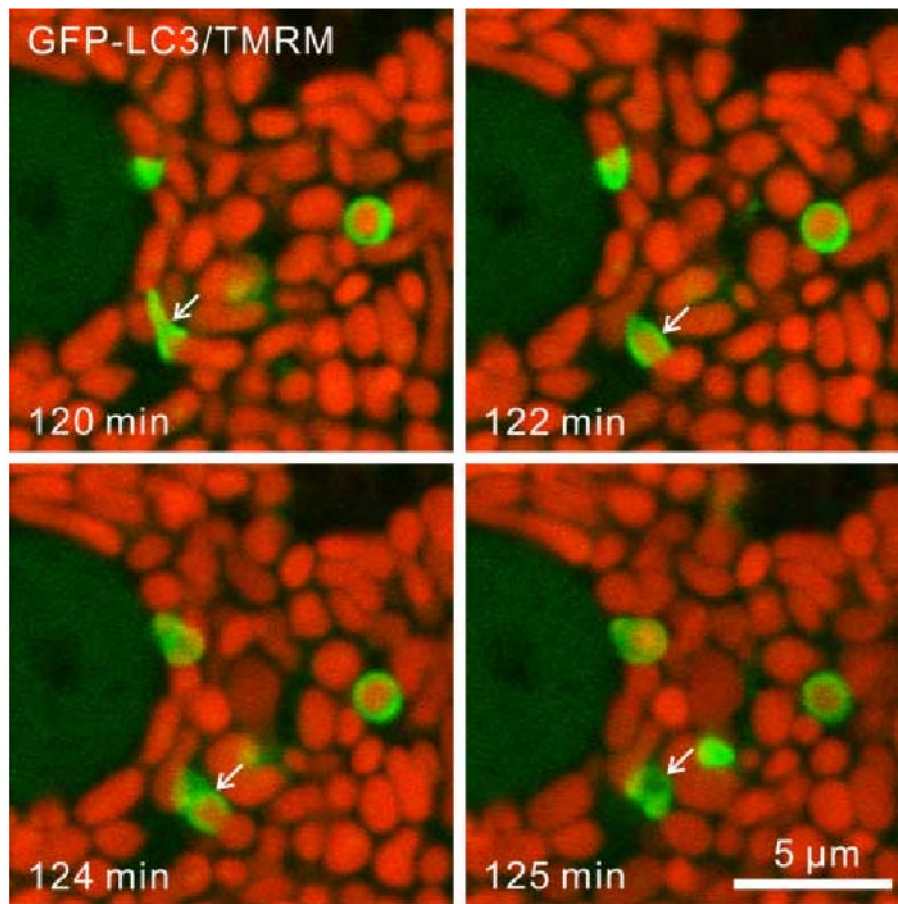
Figure 4. Mitophagy During Nutrient Deprivation in Hepatocytes from Transgenic GFP-LC3 Mice
Hepatocytes were loaded with TMRM to monitor mitochondrial membrane potential and subjected to nutrient deprivation in modified Krebs-Ringer buffer containing glucagon. In the time-lapse confocal images, note green-fluorescing GFP-LC3 enveloping and then sequestering a red-fluorescing mitochondrion (arrows). After sequestration, the mitochondrion loses its red fluorescence, indicating depolarization.
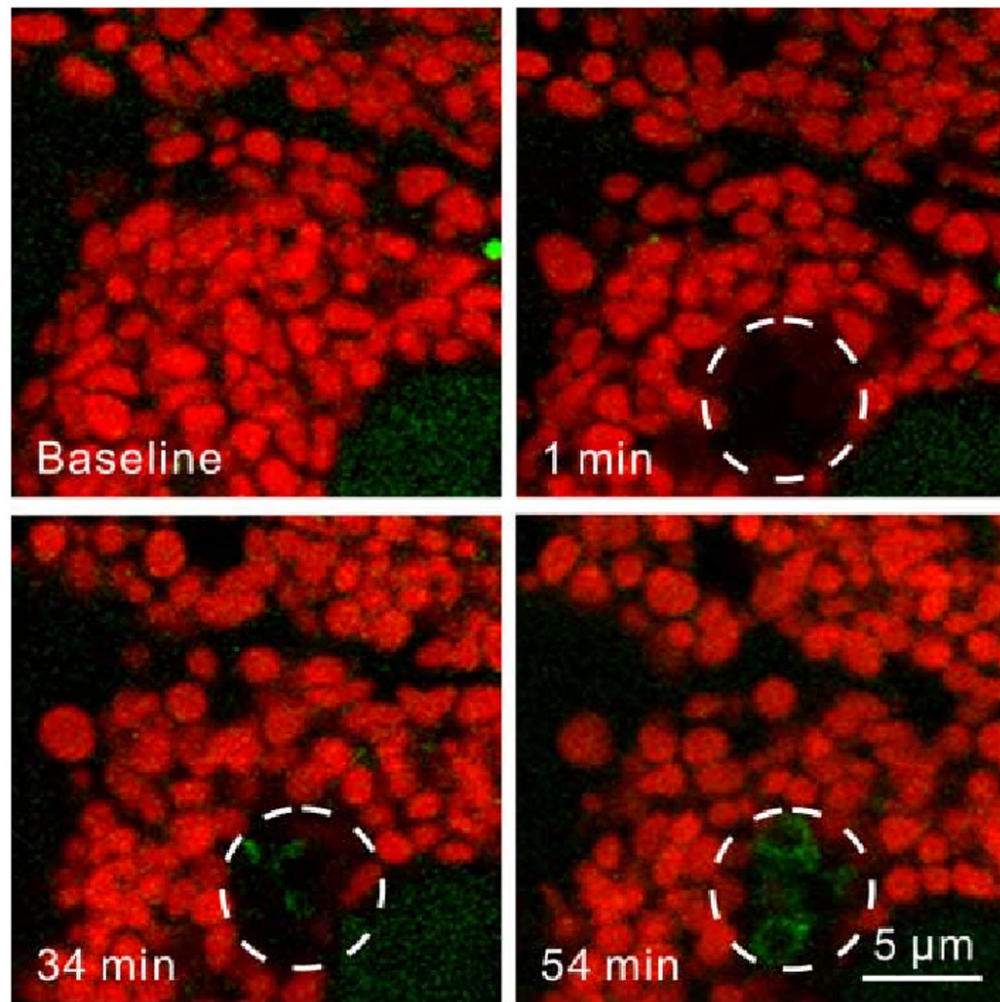
Figure 5. Photodamage-induced Mitophagy
GFP-LC3 transgenic hepatocytes were loaded with TMRM (**Baseline**), and 5 to 10
mitochondria were exposed to a pulse of 488-nm laser light at full power (circles).
Mitochondria lost red TMRM within a minute, indicating mitochondrial depolarization. After
30 min, green GFP-LC3 fluorescence localized to the region where mitochondria were
damaged. After 55 min, the photodamaged mitochondria were sequestered into GFP-LC3-
labeled autophagosomes, which indicated that the damaged mitochondria were selectively
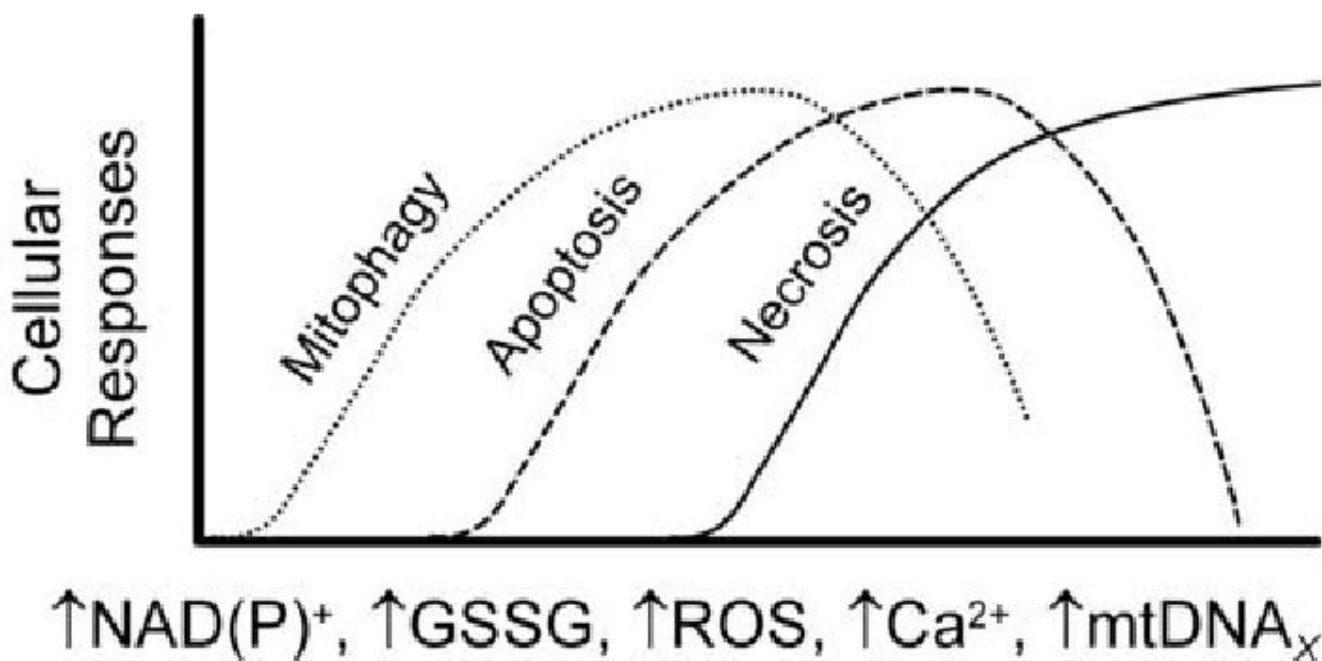targeted for mitophagy.

Figure 6. Progression of Mitophagy, Apoptosis and Necrosis
Inducers of the MPT include $NAD(P)^+$, GSSG, ROS, Ca^{2+}, and mutations of mtDNA
($mtDNA_X$). As the MPT involves an increasing proportion of mitochondria, cellular responses
progress from mitophagy, a repair mechanism, to apoptosis driven by release of cytochrome
c and other proapoptotic factors from mitochondria, and finally to necrosis due to ATP
depletion. As ATP becomes depleted, mitophagy and apoptosis become inhibited.