Autophagy in health and disease. 5. Mitophagy as a way of life

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Gottlieb RA, Carreira RS. Autophagy in health and disease: 5. Mitophagy as a way of life. Am J Physiol Cell Physiol 299:C203–C210, 2010. First published March 31, 2010; doi:10.1152/ajpcell.00097.2010.—Our understanding of autophagy has expanded greatly in recent years, largely due to the identification of the many genes involved in the process and to the development of better methods to monitor the process, such as green fluorescent protein-LC3 to visualize autophagosomes in vivo. A number of groups have demonstrated a tight connection between autophagy and mitochondrial turnover. Mitochondrial quality control is the process whereby mitochondria undergo successive rounds of fusion and fission with a dynamic exchange of components to segregate functional and damaged elements. Removal of the mitochondrion that contains damaged components is accomplished via autophagy (mitophagy). Mitophagy also serves to eliminate the subset of mitochondria producing the most reactive oxygen species, and episodic removal of mitochondria will reduce the oxidative burden, thus linking the mitochondrial free radical theory of aging with longevity achieved through caloric restriction. Mitophagy must be balanced by mitophagy and mitochondrial biogenesis are coordinately regulated.

Autophagy is also induced by oxidative stress, and amino acid restriction has been shown to trigger production of reactive oxygen species (ROS). Chen et al. (10) showed that the ROS originate from mitochondria, and Elazar identified Atg4 as the component of the autophagy machinery that responds to ROS (90). While this is a global and nonspecific response, we hypothesize that ubiquitin ligases associated with the mitochondrial outer membrane might respond to the local production of ROS, thereby conferring specificity to the process. Autophagy is also induced by failure of the ubiquitin-proteasome system (UPS) (51). Based on the extremely rapid turnover of key rate-limiting components of the autophagy machinery, even transient disruption of UPS-mediated protein degradation may be sufficient to upregulate autophagy (27). The UPS is sensitive to oxidative stress and therefore will amplify the autophagic response to ROS (111).

Interestingly, both Beclin1 and Atg5 contain a Bcl-2-binding domain that resembles the BH3 domain of many of the pro-apoptotic members of the Bcl-2 family. Beclin1 binding by Bcl-2 is reported to negatively regulate autophagy (76), and displacement of Bcl-2 by BH3-only proteins can stimulate autophagy (24, 63). The Bcl-2-binding domain appears to be necessary for autophagy (5). Given the importance of Bcl-2 family members in regulating mitochondrial integrity, it seems reasonable to hypothesize that Bcl-2 proteins might also govern selective mitophagy.

Mitochondria and Their Dark Side

While mitochondria perform essential functions for the cell, notably ATP production via oxidative phosphorylation, heme biosynthesis, and calcium homeostasis, their continued existence within the cell may sometimes seem like a pact with the devil. Damage to the mitochondrial outer membrane leads to release of cytochrome c, triggering caspase activation and apoptosis. More catastrophic stresses can lead to pathologic opening of the mitochondrial permeability transition pore (MPTP), accompanied by transient but massive release of ROS and calcium (4, 112). This can trigger neighboring mitochondria to do the same, culminating in activation of calcium-dependent proteases (calpains) and lipases (cPLA2), as well as ROS-activated iPLA2, which together ensure the necrotic destruction of the cell (64). However, even when mitochondria are functionally normal, 1–2% of the oxygen they consume is
converted to superoxide and then to hydrogen peroxide. Akin to cars that emit smog even when idling, mitochondria produce small amounts of superoxide even when ATP production is minimal. Damaged but still functional mitochondria might release up to tenfold more hydrogen peroxide (30). In a mitochondria-rich, metabolically active organ like the heart, this will impose a substantial oxidative burden over time. Given the mitochondrial half-life of 2 wk and a cardiomyocyte lifespan of many decades, the organism is confronted with a sizable challenge to deal with the cumulative oxidative damage in its longest-lived tissues. Oxidative stress will cause DNA damage, and mitochondrial DNA repair enzymes are less efficient (55). A defective version of mitochondrial DNA polymerase gamma (involved in synthesis and repair) introduces a high frequency of mutations in the mitochondrial genome. A mouse model expressing the mutant polymerase gives rise to a phenotype of accelerated aging (104).

**Mitophagy As a General Feature of Autophagy**

Mitochondrial integrity is essential to cellular homeostasis. If the energetic demand is low, excess mitochondria produce small amounts of superoxide even when ATP production is minimal. Damaged but still functional mitochondria might release up to tenfold more hydrogen peroxide (30). In a mitochondria-rich, metabolically active organ like the heart, this will impose a substantial oxidative burden over time. Given the mitochondrial half-life of 2 wk and a cardiomyocyte lifespan of many decades, the organism is confronted with a sizable challenge to deal with the cumulative oxidative damage in its longest-lived tissues. Oxidative stress will cause DNA damage, and mitochondrial DNA repair enzymes are less efficient (55). A defective version of mitochondrial DNA polymerase gamma (involved in synthesis and repair) introduces a high frequency of mutations in the mitochondrial genome. A mouse model expressing the mutant polymerase gives rise to a phenotype of accelerated aging (104).

**Signals for Mitophagy**

In budding yeast, mitophagy requires Atg11, the selective autophagy-specific factor, but not Atg19, the Cvt cargo recep-
Dysfunctional mitochondria are excluded from subsequent decreases in potential and reduced probability of fusion (101). Mitochondria: one subpopulation has increased membrane potential, which is attenuated by treatment with cyclosporin A (45). Twig and colleagues (101) showed that in pancreatic β-cells, fission generates asymmetric daughter mitochondria: one subpopulation has increased membrane potential and high probability of fusion, while the other has decreased potential and reduced probability of fusion (101). Dysfunctional mitochondria are excluded from subsequent rounds of fission and fusion, and eventually they are removed by autophagy. The authors argued against the MPTP as the cause of the depolarization because the depolarization was observed even in the presence of 1 μM cyclosporin A.

In cardiac cells, starvation-induced autophagy has been shown to cause mitochondrial depolarization which is prevented by cyclosporin A (8), indicating the MPTP is involved in starvation-induced mitochondrial depolarization. We have also shown that cyclophilin D, a component of the MPTP, is required for cardiac mitochondrial removal by autophagy induced by starvation (8). Cardiomyocytes from cyclophilin D-deficient mice do not upregulate autophagy when subjected to starvation, in contrast to cardiomyocytes from wild-type mice (8). Cyclophilin D and the MPTP are implicated in the initiation of autophagy and removal of mitochondria in starvation-induced autophagy and may represent a homeostatic mechanism for cellular and mitochondrial quality control.

**Apoptotic Proteins**

Bnip3 and Bnip3-like (Bnip3L), also known as Nix, are proteins with homology to Bcl-2 in the BH3 domain, which induce both cell death and autophagy. Bnip3 and Nix can induce mitophagy by triggering mitochondrial depolarization, which is known to induce mitochondrial removal by autophagy (23, 101). Mitochondrial depolarization has been shown to be both sensitive and insensitive to MPTP inhibitors (80), and therefore there is a debate whether the induction of autophagy by Nix and Bnip3 is through the pore.

Autophagy is induced as a protective response to Bnip3-mediated apoptotic signaling (Fig. 2) (37, 38, 66). Bnip3 is activated following ischemia-reperfusion and is responsible for loss of cardiac myocytes. It functions as a redox sensor where increased oxidative stress induces homodimerization and activation of Bnip3 (54). It has been reported that Bnip3 mediates cell death via opening of the MPTP (53, 86, 102). However, it was shown that mitochondria are present within autophagosomes.
was recently described that in isolated heart mitochondria, Bnip3-mediated mitochondrial permeabilization, swelling, and cytochrome c release are insensitive to cyclosporin A, an inhibitor of the MPTP, and independent of cyclophilin D, an essential component of the MPTP (80). Moreover, Bnip3 causes mitochondrial matrix remodeling and large-amplitude swelling of the inner membrane, which leads to disassembly of OPA1 complexes and release from the mitochondria (80). Given the wide array of consequences of Bnip3 activation, it may induce autophagy by several mechanisms, including via mitochondrial depolarization and opening of the MPTP or through interference with the fusion-fission machinery.

Nix is required for programmed mitophagy during reticulocyte maturation (89, 91, 110). The maturation process includes elimination of membrane-bound organelles, such as mitochondria (31, 52). Mitochondria are cleared from reticulocytes through an autophagophagy-related process with the major difference that the contents of the autophagic vacuole are not recycled but are eliminated by exocytosis (31, 40). Novak et al. (73) showed that in murine reticulocytes, Nix functions as a selective autophagy receptor that binds to LC3/GABARAP proteins. Autophagy machinery is still functional in Nix-deficient reticulocytes, but mitochondria are not engulfed by the autophagosome. The authors speculate that Nix-dependent recruitment of autophagosome-associated LC3/GABARAP proteins to mitochondria might mediate membrane tethering and/or hemifusion of mitochondria with autophagosomes (73). This could be the reason why mitochondria align to autophagosomes but are not engulfed in Nix-deficient reticulocytes (73). Nix-deficient mice exhibit defective erythroid development, mild-to-moderate anemia, reticulocytosis, and an increase in splenic erythropoiesis (21, 89, 91). Interestingly, knockout of autophagy-specific genes (Ulk1, Atg5, and Atg7) does not prevent mitophagy during reticulocyte maturation, suggesting that there is an alternative pathway for mitochondrial removal (56, 65, 110).

**Mitochondrial Turnover and Role of Bouts of Autophagy**

Mitochondrial biogenesis consists of the growth and division of preexisting mitochondria. According to the endosymbiotic theory, mitochondria descend from a proteobacteria endosymbiont that became established in a eukaryotic host cell. Given their bacterial origin, mitochondria have their own genome, and mitochondrial proteins are encoded by both nuclear and mitochondrial genomes (103). Mitochondrial biogenesis requires the coordination of several distinct processes: 1) inner and outer mitochondrial membrane synthesis; 2) synthesis of mitochondrial proteins; 3) synthesis and import of proteins encoded by the nuclear genome; 4) replication of mitochondrial DNA; and 5) mitochondrial fusion and fission.

Mitochondrial turnover comprises mitophagy followed by biogenesis. Mitochondria were proposed to turn over as a unit, since inner membrane proteins such as cytochrome aa3, b, and c, the inner membrane lipid cardiolipin, and mitochondrial DNA in the matrix all have the same half-life (29, 81, 82). The mitochondrial half-life values found in the literature are very disparate. Reports from the early 1970s suggest that under normal conditions, mitochondria of nonproliferating tissues (e.g., brain, heart, kidney, and liver) turn over with a half-life of 10–25 days (69, 77), while others suggest 5–6 days for rat heart and liver (82). The methods used to determine mitochondrial turnover, usually electron microscopy and radioactively-labeled components (81), present several problems. Electron microscopy, which only shows a snapshot of a section of the cell, also fails to provide information about mitochondria viability—mitochondria might have normal shape, but not be functional and therefore will be targeted for removal (100). The rate of decay of radioactively labeled components of the mitochondria is used as an indicator of mitochondrial destruction (81). However, some of the isotopic precursors used in such experiments can be reutilized for resynthesis of macromolecules, and the decay rates will vary depending on whether the isotope is reutilized or not (81). Given the technical limitations, new methods to determine mitochondrial turnover are warranted; progress on this question awaits the development of better approaches. At present, the rate of mitochondrial turnover is not definitively known.

**Mitochondrial Fusion, Fission, Autophagy, and Biogenesis**

Another limitation of the techniques referred in the previous section is that they do not reflect mitochondrial dynamics. Mitochondria are under constant cycles of fission and fusion; if subjected to stress they may be removed by autophagy, and biogenesis might increase to meet energetic demand (42). The removal of mitochondria under stress conditions can serve to supply the cell with amino acids, rid the cells of damaged mitochondria that are deleterious, or prevent cell death (3). A drastic depletion of mitochondria—a purge—might be useful to eliminate mtDNA copies that contain enough mutations to interfere with function. Such a bottleneck has been proposed to explain the observation that of all the mtDNA copies present in the germ-cell precursor, only a fraction will be amplified to generate the ~10^6 mtDNA copies present in the mature oocyte. Studies with the polymerase gamma mutator mouse show that mutations that do not result in amino acid changes are tolerated, while mutations causing amino acid changes are strongly underrepresented in the protein-coding genes, providing incontrovertible evidence that mtDNA is subject to strong purifying selection in the maternal germ line, and hints at a “fitness test” of the mitochondria that express proteins encoded by the mitochondrial genome. This purifying selection of functional mtDNA genomes will decrease the frequency of transmission of mutated genomes to the offspring (93). While this has been clearly demonstrated in the oocyte, it is unknown whether such a purge might take place in somatic cells to maintain fitness of the mitochondrial genome. We recently showed that in cell culture, an episode of starvation results in significant depletion of mitochondria (8). Whether this process is followed by mitochondrial repopulation with the most functional mtDNA genomes and improved mitochondrial function remains to be determined. However, episodic fasting and refeeding may result in myocardial dysfunction (78); excessive mitochondrial purging may be maladaptive.

The removal of mitochondria usually needs to be compensated by mitochondrial biogenesis or it will become detrimental (11). For example, in the liver during subacute sepsis, mitochondrial function is impaired and mitochondria are removed by autophagy. In this model, clearance of mitochondria is followed by mitochondrial replenishment (16). Mitochondrial biogenesis in the presence of defective mitochondrial function is also observed in type 1 diabetes (92). In hearts of...
OVE26 mice, a chronic model of type 1 diabetes, there is upregulation of mitochondrial proteins, increase in mitochondrial area and number, and mitochondrial DNA (92). Despite the higher number of mitochondria, their function is impaired. Similar results are observed in insulin-resistant mice (22). It is possible that increased biogenesis is a compensatory mechanism for defective mitochondrial function. Biogenesis in the absence of balanced mitophagy to remove defective mitochondria may be maladaptive. Further studies are warranted to clarify this.

In most situations, increased mitochondrial biogenesis is beneficial, such as in caloric restriction and aging, exercise, amyotrophic lateral sclerosis (ALS) treatment, neonatal cardiomyocytes response to LPS, and renal proximal tubular cells subjected to oxidative stress. In skeletal muscles, caloric restriction enhances mitochondrial protein turnover by enhancing mitochondrial degradation via autophagy and by stimulating mitochondrial biogenesis. Caloric restriction activates SIRT1, which activates autophagy, via deacetylating autophagy proteins (57), and mitochondrial biogenesis, via activation of PGC-1α (12, 35, 62). The net result is that caloric restriction ensures good mitochondrial function with aging by promoting mitochondrial turnover. A similar mechanism is observed with exercise (60, 61, 68, 105). Skeletal muscle biopsies of humans performing high-intensity interval training showed an increase in SIRT1, nuclear PGC-1α, and mitochondrial transcription factor A, which lead to an increase in skeletal muscle mitochondria and improved exercise performance (60, 61). Biopsies performed in older men show that even with aging, exercise enhances mitochondrial respiratory chain activity and mitochondrial DNA, which is likely related to increases in mitochondrial biogenesis (68). ALS is characterized by the presence of intracellular aggregates in the motor neurons, mitochondrial dysfunction, and deficiency of autophagy (88).

A clinical study demonstrated that lithium slows the progression of ALS in patients by activating autophagy, thereby leading to the removal of defective mitochondria and protein aggregates, and by stimulating mitochondrial biogenesis (25, 26). In brain and heart, hypoxic preconditioning was shown to stimulate mitochondrial biogenesis via increase of PGC-1α, nuclear respiratory factor-1, and mitochondrial transcription factor A (34, 67). In neonatal rat cardiomyocytes the protective response to LPS consists of the rapid removal of damaged organelles by autophagy and replacement of dysfunctional mitochondria by mitochondrial biogenesis. Hickson-Bick et al. (41) reported that LPS treatment in neonatal cardiomyocytes increases the transcription of mitochondrial transcription factor A, nuclear accumulation of redox-sensitive nuclear respiratory factor 1, and expression of PGC-1. The dose of LPS used did not induce apoptosis or necrosis in these cells. However, when autophagy was blocked, ROS production was increased and apoptosis was induced, showing that autophagy plays a protective role in LPS-induced injury (109). In renal proximal tubular cells subjected to oxidative stress, there is a decrease in mitochondrial function and an increase in mitophagy (83). In these cells, following injury, there is an increase in PGC-1α, which induces mitochondrial biogenesis (83). The authors observed upregulation of PGC-1α from day 1 to 3 following injury, which was consistent with the initiation of the recovery of mitochondrial function. At day 4, PGC-1α levels returned to basal values concomitant with the restoration of mitochondrial function (83). This illustrates a negative feedback mechanism that prevents excessive mitochondrial formation once mitochondrial function is restored. It also represents a fine balance between mitophagy and mitochondrial biogenesis, where a decrease in mitochondria number would lead to energetic deficiency, but also an excess of mitochondria could be deleterious, by increasing the formation of ROS and leading to oxidative stress. Autophagy and biogenesis work as intertwined mechanisms that assure mitochondrial quality and cellular homeostasis.

What Happens When Mitochondrial Turnover Is Inadequate?

Proliferating cells can remove “biological wastes” by cell division; however, cardiac myocytes cannot because they are terminally differentiated and maintain cellular homeostasis only by the activation of degrading pathways, namely, macroautophagy, microautophagy, and chaperone-mediated autophagy (97). Thus, the accumulation of defective mitochondria and lysosomes in aged myocytes is a reflection of inefficient autophagy (99). Senescent myocytes are characterized by the existence of giant mitochondria originating from oxidative damage followed by inefficient mitochondrial DNA repair and autophagy (9, 14, 95, 98). Terman and Brunk (96) suggested that autophagic engulfment of large mitochondria requires more energy and, consequently, is less efficient (96). In an ideal situation of mitochondrial turnover, mitochondrial recycling should provide for removal of damaged mitochondria and their replacement by normal, replicating mitochondria (6, 95). However, this is not always the case and the accumulation of defective mitochondria in postmitotic cells is frequently observed (6). de Grey (17, 18) suggested that the lower respiratory rate of defective mitochondria would be accompanied by less oxidative damage than that caused by normal mitochondria, which might make them less prone to autophagy. Nekhaeva and collaborators (71) showed that in humans, mitochondrial fission can be increased by certain mutations in the mitochondrial DNA, resulting in the replacement of normal mitochondria by mutated ones which are less susceptible to oxidative damage and potentially less vulnerable to autophagy. With increasing age, lysosomal activity decreases and autophagy is inefficient due to the accumulation of lipofuscin, a brown granular pigment that consists of cross-linked lipids and proteins produced during lysosomal digestion (48, 97). In the aging heart, the gradual inhibition of autophagy is at least in part caused by the intralysosomal accumulation of lipofuscin (96). The impairment or suppression of autophagy plays a critical role in the development of aging-related disorders in the heart. Therapeutic approaches to increase autophagy and mitophagy may prove to be cardioprotective.

Life in the Balance, Longevity the Goal

Self-eating, recycling, cash-for-your-clunkers:
Trade up to the mitochondrial equivalent Prius.
The road to rejuvenation is paved with destruction,
For clearing the rubble precedes reconstruction.
But remember that life’s circular dance
Depends on opposite forces in balance:
Excess destruction, too much biogenesis,
Brings heart failure, cancer, or neurodegeneris.
REFERENCES


Themes

C210  MITOPHAGY AS A WAY OF LIFE


