Trends in Molecular Medicine

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Review

Mitochondria–Lysosome Crosstalk: From Physiology to Neurodegeneration

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Cellular function requires coordination between different organelles and metabolic cues. Mitochondria and lysosomes are essential for cellular metabolism as major contributors of chemical energy and building blocks. It is therefore pivotal for cellular function to coordinate the metabolic roles of mitochondria and lysosomes. However, these organelles do more than metabolism, given their function as fundamental signaling platforms in the cell that regulate many key processes such as autophagy, proliferation, and cell death. Mechanisms of crosstalk between mitochondria and lysosomes are discussed, both under physiological conditions and in diseases that affect these organelles.

Highlights

The functions of mitochondria and lysosomes are coordinated with cellular metabolism and signaling.

AMPK and mTORC1 mediate mitochondria–lysosome crosstalk.

Acute and chronic mitochondrial stress has opposite effects on lysosomal biogenesis.

Lysosomal storage diseases trigger repression of mitochondrial biogenesis.

Mitochondria–lysosome crosstalk is impaired in neurodegenerative diseases.

Principles of Cellular Coordination

Life is an elaborate system of chemical reactions which require organization. This chemistry is often compartmentalized by phases or physical barriers. Cellular organelles such as mitochondria, peroxisomes, lysosomes, and others present extreme examples of compartmentalization by containing a specific set of chemical reactions within the limits of their membranes. These organelles carry out different functions and together contribute to the survival of the cell, and eventually to its growth and division. The compartmentalization provided by organelles presents, however, a challenge to the cell: the organelles must be coordinated to ensure that the whole cell is tuned.

Multicellular organisms have many different cell types, but they all have key common aspects such as the use of ATP and thioester bonds as energy currency, as well as the ability to survive different stresses (e.g., ischemia-induced hypoxia, fever-related heat shock). Cells cope with stress by triggering the appropriate responder, which triggers a carefully executed plan for adaptation and survival. One of the major stresses met by almost all cells and organisms is the availability of nutrients and energy, or lack thereof.

Metabolism is a key component of stress responses because it allows redirection of fuels to generate energy (catabolism) or for conversion into building blocks (anabolism). Both branches of metabolism rely heavily on mitochondria and lysosomes, not only as providers of energy and building blocks but also as regulators of metabolic activity across the cell. Therefore, it is pivotal that mitochondria and lysosomes are effectively coordinated. The purpose of this review is to discuss recent advances in understanding the communication between mitochondria and lysosomes, how this crosstalk contributes to physiological cell function, and how perturbations in mitochondria–lysosome crosstalk are involved in pathology. Although the outcomes are conserved between higher and lower Eukarya, the underlying mechanisms differ significantly. We therefore focus on mammalian mitochondria–lysosome crosstalk.

Organelle Signaling

To coordinate the function of different organelles, several aspects need to be controlled: how and when the organelle is built (biogenesis), where it is positioned, which functions it performs, and how it is removed. Reciprocally, the organelle must inform the cell of its status and needs, and the cell needs to react accordingly. The kinetics of any of these aspects is pivotal because the cell may need to respond to a particular stress within seconds or minutes (e.g., hypoxia, nutrient depletion, exercise, activation of immune cells) or may need to remain under stress conditions for a long time (e.g., infection, genetic defects in an organelle).

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Box 1. Mitochondrial and Lysosomal Biogenesis

The transcriptional programs of organelle biogenesis comprise coordinated transcription of the complete set of genes necessary to make a functional new organelle. These are complex programs that involve ~600 genes for lysosomes [98] and ~1000–1500 genes for mitochondria [99]. Specific transcription factors that have *cis*-elements in the promoters or enhancers of these genes drive their coordinated expression.

Multiple transcription factors promote mitochondrial biogenesis, of which the nuclear respiratory factor 1 (NRF1), peroxisome proliferator-activated receptor α (PPAR-α), PPAR-β/δ, PPAR-γ, estrogen-related receptor α (ERRα), ERRγ, Myc, and nuclear respiratory factor 2 (known as GA-binding protein transcription factor, composed of α and β subunits, GABPA and GABPB; NRF2 should be avoided because this is often used as a reference to an unrelated transcription factor, nuclear factor erythroid 2-like 2, NFE2L2, which coordinates antioxidant responses) are pivotal examples. Many other transcription factors have also been implicated in the expression of some mitochondrial genes, but it is less clear whether they can coordinate the entire program of mitochondrial biogenesis, such as SP1, MEF2, CREB, FoxO, YY-1, and E2F1, among others. The transcription factors promoting mitochondrial biogenesis often work in association with the transcriptional coactivators PGC-1α or PGC-1β. A comprehensive discussion of the mechanisms of mitochondrial biogenesis is outside of the scope of this article and has been summarized elsewhere [100]. Notably, although many transcription factors are known to activate mitochondrial biogenesis, two transcription factors were recently found to repress biogenesis. KLF2 and ETV1 are able to silence the expression of nucleus-encoded mitochondrial genes, and their overexpression results in impairment of the mitochondrial respiratory chain [42].

The coordinated expression of lysosomal genes (nucleus-encoded genes which encode lysosomal proteins) is ensured by the microphthalmia transcription factors TFEB, MITF, TFE3, and TFEC [101]. The coactivator ACSS2 cooperates with TFEB to promote lysosomal biogenesis [102]. Other transcription factors can also induce the expression of lysosomal genes, such as CREB, FoxO, PPARα, and E2F1 [103]. Several autophagy-related genes are under the same regulatory circuits as lysosomal genes. As in the case of mitochondrial biogenesis, there are negative regulators of the transcriptional program of lysosomal/autophagy biogenesis, such as the farnesoid X receptor (FXR) and the bromodomain protein BRD4 [103].

Interestingly, several transcription factors can influence both mitochondrial and lysosomal/autophagy biogenesis, such as CREB, FoxO, and E2F1. Although it remains unclear whether these transcription factors can trigger both programs simultaneously, there is evidence that TFEB and TFE3 can also promote the transcriptional program of mitochondrial biogenesis in cell lines [101] and *in vivo* [104], possibly involving induction of the coactivator PGC1α, a major coordinator of mitochondrial (and peroxisomal) biogenesis [105]. Therefore, TFE3 may represent a mechanism that coordinates the biogenesis of mitochondria and lysosomes (and possibly of peroxisomes).

Organelle biogenesis is often used both in reference to the transcriptional programs that trigger the expression of the complete repertoire of an organelle proteome, as well as to the mechanisms leading to the import and assembly of those proteins into macromolecular complexes in the organelle. In this review, organelle biogenesis refers to the transcriptional programs. The key aspects of mitochondrial and lysosomal biogenesis are described in Box 1, but it is important to note that several transcription factors seem to be able to trigger both mitochondrial and lysosomal biogenesis.

The positioning of the organelles is an important but often overlooked issue. There are several populations of lysosomes in the cell, and their positions relative to the plasma membrane and the nucleus are important to their specific functions [1]. Mitochondria are, under normal conditions, spread around the cell in a relatively uniform manner, but rapidly relocate to the perinuclear region at the onset of different stresses [2,3]. Importantly, the relative distribution of organelles must also to be considered given the importance of physical organelle interactions (e.g., contact sites) for some organelle functions (e.g., mitochondrial fission) and for many aspects of cellular function such as Ca^{2+} homeostasis, cholesterol trafficking, and phospholipid synthesis [4]. The role of the contact sites is discussed later in this article.

Mitochondria and lysosomes constantly inform the rest of the cell on their functional status by using multiple strategies. These are often referred to as 'retrograde' signaling because they originate in the organelles and eventually affect the nucleus, as opposed to 'anterograde' signaling in which the

nuclear gene expression machinery modulates the function of the organelle [5]. For example, lysosomes can retain calcium (Ca^{2+}), iron, cholesterol, or sphingomyelin, and each of these can be sensed by the cell [6]. Mitochondria can relay stress to the rest of the cell by increasing/decreasing their Ca^{2+} uptake, slowing down protein import, releasing metabolites, reactive oxygen species (ROS), and vesicles, as well as by exporting peptides (such as the case of ClpP- and HAF-1-dependent peptide export in *C. elegans* under mitochondrial proteotoxic stress and MOTS-c export in mammals) or mitochondrial DNA (mtDNA) [7,8].

As with any other signaling paradigm, organelle retrograde signaling depends on key properties of each signal: trigger, intensity, frequency, and duration. The duration of the signal is a fundamental aspect of signaling because the responses to acute and chronic organelle stress may be remarkably different, at least for mitochondria [9]. The major determinants of the duration of a signal are its trigger and its termination. Although on some occasions cessation of the stress trigger (e.g., normoxia after hypoxic conditions) is sufficient to remove the stress signal, the termination of an organelle stress signal can often only be achieved by removal of the perturbed organelle.

The removal of damaged organelles is pivotal for the maintenance of healthy organellar function. There are therefore strategies in place to recognize and eliminate the damaged organelles, typically by selective autophagy of mitochondria (mitophagy) or lysosomes (lysophagy). The manner in which the defective organelles are recognized differs between mitochondria and lysosomes. The major 'red flag' for mitochondria seems to be their depolarization, which by diverse mechanisms results in ubiquitination of the outer membrane, recruitment of mitophagy receptors, and delivery of mitochondria-containing autophagosomes to the lysosome for degradation [10]. For lysosomes, the main event leading to their demise seems to be membrane rupture, which results in the exposure of parts of the lysosomal glycocalyx that are recognized by the galectin family of proteins, which then trigger the selective autophagy of the lysosomes that are ruptured [11]. The detailed mechanisms of mitophagy and lysophagy are comprehensively discussed in recent reviews.

Impact of Defective Mitochondria on Lysosomes

The interdependence of mitochondria and lysosomes is underscored by many lines of evidence obtained from cells with mitochondrial malfunction. For example, mouse embryonic fibroblasts lacking AIFM1 (apoptosis-inducible factor), that is required for respiratory chain function, OPA1, that is required for mitochondrial fusion, or PINK1, that is involved in quality control of the respiratory chain and mitophagy, show lysosomal impairment [12]. This is evidenced by enlargement of lysosomal (LAMP1-positive) vesicles, which become nonacidic and lose their hydrolytic activity [12]. The detrimental effect of mitochondrial dysfunction on lysosomes is constant across different cell types *in vitro* (e.g., clonal cell lines) and *in vivo* (e.g., activation of effector T cells, Huntington's disease heart) [13–16]. Importantly, this effect does not seem to be related to decreased ATP availability [12–14].

As mentioned above, defective lysosomes are sensed by the rest of the cell. A common response in lysosomal storage diseases is the increase in lysosomal biogenesis. A similar paradigm is observed when mitochondrial dysfunction impairs lysosomal function: TFEB (transcription factor EB) and other microphthalmia family transcription factors are activated to promote an increase in lysosomal biogenesis [13,17–19]. Interestingly, however, activation of lysosomal biogenesis occurs in acute mitochondrial stress, but not in chronic mitochondrial stress [17]. This was observed in pharmacologic models of mitochondrial respiratory chain inhibition in cultured cells, in which TFEB activity was high in the first hours of respiratory chain inhibition, but eventually returned to basal levels, or is actively inhibited when the inhibition persisted over 24 h [17]. However, in T cells lacking the mitochondrial transcription factor A (TFAM) protein, that is essential for the maintenance, transcription, and replication of mtDNA, lysosomal biogenesis was upregulated [13]. Nevertheless, it remains to be determined whether this response is TFEB-dependent, and what its functional role is, given that the lysosomes in *Tfam*$^{-/-}$ T cells were still dysfunctional [13]. Furthermore, cells with chronic defects in the respiratory chain are not able to trigger lysosomal biogenesis even when using classic lysosomal biogenesis-stimulating conditions such as amino acid starvation or mTORC1 inhibition [14].

Considering the biogenesis and function of lysosomes during acute and chronic mitochondrial stress, a general picture emerges in which acute mitochondrial stress gears the cells to activate autophagy and lysosomal biogenesis, whereas under chronic stress lysosomal biogenesis is shut down and autophagy is inhibited. This can be interpreted that, under acute mitochondrial stress, the cell triggers programs that ultimately result in the elimination of the defective mitochondria, first by stimulating autophagosome formation, which promotes mitophagy, and then by ensuring that the capacity for autophagosome degradation is increased by building more lysosomes. However, should this program occur indefinitely, the cell would eventually run out of mitochondria, which would constitute a major threat to the life of most cell types. Thus, after a period in which the 'clean-up' mode is on, the cell prefers to shut down the degradation of mitochondria by inhibiting both lysosomal function and autophagosome formation – it is better to live with defective mitochondria, that are unable to efficiently carry out respiration and oxidative phosphorylation, than with no mitochondria, which would irreversibly impair the synthesis of phospholipids, heme, and Fe–S clusters.

The mechanisms that underlie the effects of mitochondrial deficiency on lysosomal biogenesis and function have only recently been uncovered. Lysosomal biogenesis triggered by acute mitochondrial stress requires the function of the microphthalmia transcription factors, particularly TFEB and MITF, although exactly which ones are necessary is likely to be dependent on the cell type [13,17–19]. The activation of TFEB signaling and lysosomal biogenesis is dependent on AMPK (AMP-dependent protein kinase) activation, both under mitochondrial stress in cultured cells [17] as well as under physiological conditions *in vivo* [20]. AMPK is a sensor of the energy charge and other stress signals [21], and is the grand regulator of metabolism that activates key catabolic pathways while inhibiting anabolism. It functions almost as the counterbalance to mTORC1, the kinase that coordinates most anabolic pathways. The interplay between AMPK and mTORC1, and a brief introduction to their roles in the regulation of metabolism and cell function, is presented in Box 2. The biology of AMPK and mTORC1 has been studied in great detail (as recently reviewed [21,22]). TFEB-mediated lysosomal biogenesis requires AMPK, further supporting the role of this kinase in the autophagic process. AMPK promotes the formation of autophagosomes via phosphorylation of ULK1 and ULK2 [23], and stimulates autophagic flux by enhancing the number of lysosomal particles, thus regulating the entire autophagic flux. Therefore, activation of AMPK by acute mitochondrial stress results in increased autophagic flux. It is easy to imagine that persistently high AMPK signaling would result in excessive autophagy and possibly in complete removal of mitochondria in the case of chronic mitochondrial defects. This agrees with observations that persistently high AMPK signaling results in cell death *in vivo* [24–26]. However, mitochondria remain present in the cell even with chronic mitochondrial defects. The underlying reason may be that, under persistent mitochondrial stress, AMPK signaling is shut down. This was observed in clonal cells with chronic respiratory chain deficiency as well as in the brain of a mouse lacking a subunit of complex I [14].

In addition to autophagy, acute activation of AMPK, for example, due to glucose starvation or energy stress, favors autophagy and also mitochondrial fission, eventually promoting mitophagy [27]; however, when autophagy is triggered by amino acid starvation or by inhibition of mTORC1, the mitochondrial network is maintained in a hyperfused state by repression of mitochondrial fission mediated by activation of protein kinase A (PKA) [28,29] or MTFP1 (mTORC1-regulated protein mitochondrial fission process 1) [30]. This shows how different circumstances elicit cellular programs to protect the integrity of the mitochondrial network.

Given that AMPK regulates not only autophagosome formation and mitochondrial dynamics but also lysosomal biogenesis, it is important to define whether repression of AMPK signaling in chronic mitochondrial defects has additional consequences. AMPK is also required for basal lysosomal function. AMPK signaling has two main consequences for lysosomal activity: assembly and activation of the lysosomal vATPase [31], and activation of the enzyme PIKfyve [14], which is associated with the lysosomal membrane, where it generates the signaling molecule phosphatidylinositol 3,5-bisphosphate [PI(3,5)P_2]. When AMPK signaling is low, decreased levels of PI(3,5)P_2 result in lower function of several lysosomal proteins, particularly the channel MCOLN1 (mucolipin-1; also known as TRPML1). MCOLN1 is involved in the release of Ca^{2+} (and possibly of other divalent metals) from the lysosomal

Box 2. AMPK and mTORC

AMPK functions as a trimer of one catalytic α subunit, one scaffold β subunit, and one regulatory γ subunit. Mammals have two different α subunits, α1 and α2 (encoded by *PRKAA1* and *PRKAA2*, respectively), two β subunits (β1, *PRKAB1*; β2, *PRKAB2*) and three γ subunits (γ1, *PRKAG1*; γ2, *PRKAG2*; γ3, *PRKAG3*) [106]. The regulation of AMPK occurs by allosteric modulation, post-translational modifications, proteolysis, subcellular localization, and protein–protein interactions. The best-defined mechanisms of AMPK activation are phosphorylation at T172 of the α subunit and by AMP and/or ADP binding to the γ subunit (which is competitively inhibited by ATP). Phosphorylation of the AMPK α subunit at T172 is regulated both by kinases and phosphatases. The first described activator of AMPK, the kinase STK11 (usually known as LKB1), primarily regulates α2-containing complexes [107], whereas activation of AMPK by Ca^{2+}/calmodulin-dependent kinase 2 (CaMKK2) mostly seems to affect α1-containing complexes [108].

Termination of AMPK signaling is less well characterized. The phosphatases PP2A, PP2C, and PPM1E can readily dephosphorylate T172-P under energy-replete conditions, but binding of AMP or ADP to the γ subunit precludes access of the phosphatases to the α subunit, thus resulting in increased T172 phosphorylation [109]. Ubiquitination can inhibit AMPK signaling by degrading the subunits forming the AMPK complex. Several ubiquitin ligases, such as CIDEA, MAGE-A3/6, TRIM28, and PIAS4, trigger the ubiquitination of specific subunits [110]. Finally, the interaction of AMPK with folliculin and folliculin-interacting proteins 1 and 2 (FNIP1 and FNIP2) results in inhibition of AMPK [33].

The intracellular localization of AMPK is dependent on *N*-myristoylation of the β subunit, which serves as a scaffold to recruit the complex to cellular membranes [111]. There is a nuclear localization signal in the α2 but not the α1 subunit, which suggests that α1-containing AMPK mostly phosphorylates cytoplasmic substrates associated with acute effects, whereas α2-containing AMPK mostly regulates targets that control gene expression and is associated with long-term effects [112]. In addition, there are location-specific activation mechanisms because LKB1-induced AMPK activation takes place at the lysosomal membrane [113].

The procatabolic and antianabolic roles of AMPK are partly mediated by mTORC1. AMPK activation results in mTORC1 inhibition. mTORC1 is a major coordinator of anabolism, promoting the synthesis of proteins, cholesterol, and nucleotides, among other essential building blocks for cell growth and proliferation. Inhibition of mTORC1 by AMPK is mediated by phosphorylation of RAPTOR, the defining subunit for mTORC1 [114]. However, the long reach of AMPK goes beyond mTORC1 because it often also regulates key enzymes of anabolic pathways. For example, it directly phosphorylates and activates the kinases ULK1 and ULK2, which promote autophagosome formation, as well as HMGCR, the limiting step of the cholesterol synthesis pathway, and represses the synthesis of fatty acids by phosphorylating acetyl-CoA carboxylase [21].

lumen to the cytoplasm, and release of Ca^{2+} through MCOLN1 is necessary for autophagy and lysosomal biogenesis [32]. In cells with chronic mitochondrial respiratory chain deficiency, low AMPK signaling results in loss of PIKfyve–MCOLN1 activity, accumulation of Ca^{2+} in the lysosomal lumen, and loss of lysosomal acidity and hydrolysis, which can all be recovered by pharmacological or genetic reactivation of AMPK activity [14]. Interestingly, the effect of AMPK on lysosomal function through PIKfyv–MCOLN1 is not rescued by inhibition of mTORC1 alone, suggesting that AMPK regulates lysosomal function in an mTORC1-independent manner. The detailed mechanism as to why AMPK signaling is decreased in chronic mitochondrial defects is not yet completely defined, but it is known that the tumor suppressor folliculin (FLCN) plays a role [14]. FLCN is one of very few proteins that are known to inhibit AMPK [33]. Interestingly, FLCN also inhibits TFEB. Notably, cells and tissues with chronic respiratory chain deficiency show increased levels of FLCN, whose silencing reactivates AMPK signaling and lysosomal function [14]. Interestingly, when cells are treated with a respiratory chain inhibitor, for the first few hours (ca 2–4 h) they show low FLCN and high AMPK signaling. As the respiratory chain inhibition persists, AMPK signaling subsides and FLCN protein accumulates [14]. It remains unclear what drives FLCN upregulation, although it seems likely to be a transcription-mediated effect because transcript levels for FLCN are also increased in cells with a chronic mitochondrial defect [14]. Notably, reactivation of AMPK in mouse models of mitochondrial disease was observed to have beneficial effects [34].

As often occurs when mitochondrial defects are involved, ROS have been suggested to play a role in modulating lysosomal biogenesis and function by signaling mitochondrial deficiency. Xu and

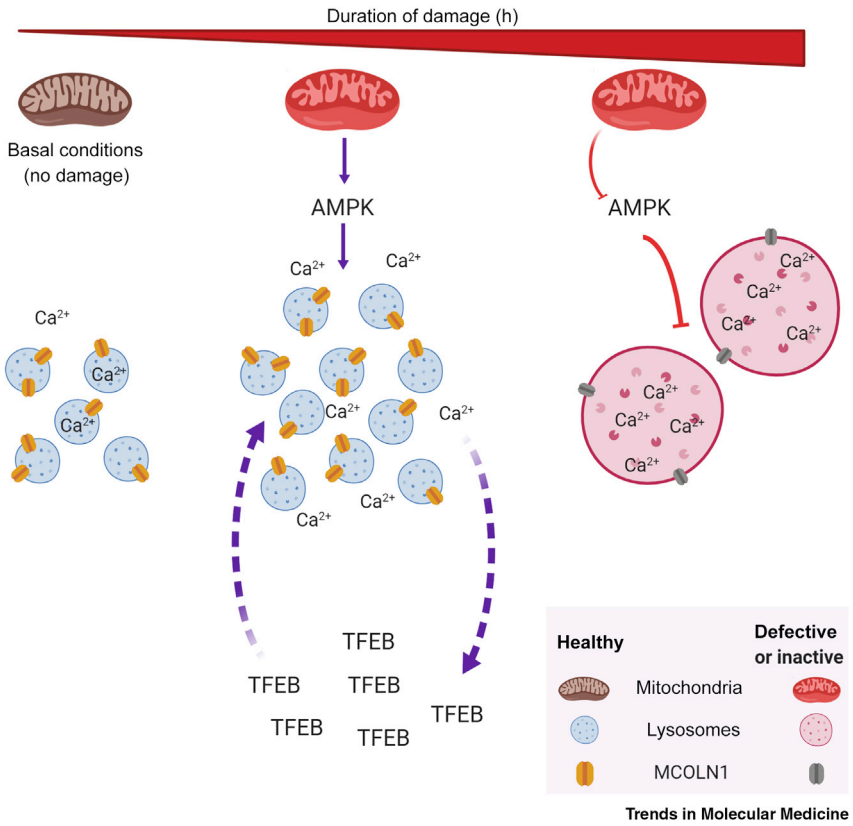

Figure 1. Mechanisms That Mediate the Effect of Acute and Chronic Mitochondrial Stress on Lysosomal Biogenesis and Function.

In acute mitochondrial stress, AMPK (AMP-dependent protein kinase) is activated, promoting autophagosome formation (not depicted), MCOLN1 (mucolipin-1) activity, and transcription factor EB (TFEB)/MITF-dependent lysosomal biogenesis, thus programming the cell to clean-up the dysfunctional mitochondria. When the defect persists, either because of genetic mutations that impair the function of the organelle or persistent treatment with respiratory chain inhibitors, FLCN (folliculin) is induced (not depicted), resulting in repression of AMPK activity, decreased MCOLN1 activity, lysosomal Ca^{2+} accumulation, and loss of lysosomal acidification, with consequent loss of lysosomal hydrolysis. This mechanism may represent a strategy to inhibit the degradation of mitochondria by autophagy during persistent mitochondrial malfunction.

colleagues showed that MCOLN1 is sensitive to ROS, which may explain its increased activity under acute mitochondrial stress [35]. However, it remains to be tested whether ROS-induced MCOLN1 activation is dependent on AMPK. Notably, ROS may also lead to AMPK activation [25], raising the possibility that ROS contribute to AMPK activation which, via its role in PIKfyve modulation, may lead to stimulation of MCOLN1 activity.

A summary of how mitochondrial perturbations affect lysosomal function is given in Figure 1. Other mechanisms employed by mitochondria to communicate with lysosomes are discussed in Box 3.

Impact of Lysosomal Perturbations on Mitochondria

The lysosome has garnered prominence in the past two decades owing to the discovery of its central role in metabolism beyond the cellular 'incinerator' function it was always known for. This is clearly emphasized by the physical and functional interaction of the lysosome with the master coordinators of anabolism and catabolism, mTORC1 and AMPK, respectively (Box 2).

Box 3. Other Mechanisms of Communication between Mitochondria and Lysosomes

In addition to the signaling pathways discussed in the main text, there are other mechanisms of crosstalk between mitochondria and lysosomes. Physical contact sites between mitochondria and lysosomes provide an evident platform for cross-organelle signaling. The formation of these contact sites is promoted by active Rab7, and their disassembly is triggered by deactivation of Rab7 [115]. However, the tether proteins that maintain the contact site in mammals remain unclear. The endosomes also form contact sites with mitochondria, which are dependent on Rab5 and are induced upon oxidative stress [116]. Some of the contacts between endosomes and mitochondria are transient 'kiss-and-run' contacts, and are particularly important in transfer of iron from endosomes to mitochondria [117,118]. In fungi, mitochondria–vacuole contact sites occur in proximity to the tethering structure of the mitochondria–ER contact site, and are responsive to metabolic activity [119,120]. The mitochondria–lysosome contact sites have been proposed to mark the sites of mitochondrial fission, but it remains unclear whether this is carried out with or without the involvement of the ER because the ER had previously been shown to wrap around the mitochondrial pre-fission site [121]. Interestingly, both mitochondria and lysosomes form contact sites with the ER, and these are fundamental for transfer of Ca^{2+} from ER to both organelles, as well as for transfer of phospholipids and cholesterol. These contacts are mediated by the lipid-transfer protein family Vps13 [122,123], and, whereas Vps13A tethers the ER to mitochondria, Vps13C tethers the ER to lysosomes/late endosomes [123]. It remains to be systematically determined whether and how the interplay between the different contact sites that involve mitochondria, lysosomes, ER, and other organelles (e.g., lipid droplets, plasma membrane) contributes to the regulation of mitochondria and lysosomal function. New advances in spectral imaging are likely to contribute to understanding of the interplay between contact sites [124]. Along these lines, there is evidence that one organelle can perturb the contact site between two other organelles: accumulation of undigested ganglioside GM1 (monosialotetrahexosylganglioside) as a result of lysosomal defects affects the membrane composition at mitochondria–ER contact sites, resulting in excessive mitochondrial Ca^{2+} uptake and consequent cell death and neurodegeneration [125].

Another important pathway relaying information from mitochondria to lysosomes is based on vesicular transfer via mitochondria-derived vesicles (MDVs) [126]. Several populations of MDVs with unique constitutions have been identified, some targeted to peroxisomes [127,128] and some to lysosomes [129]. MDVs targeted to the lysosomes are enriched in PINK1 and Parkin, and require syntaxin-17 to mediate their fusion with lysosomes [130].

As a result, lysosomal storage disorders (LSDs), which are primarily caused by mutations in lysosomal proteins, alter cellular metabolism beyond merely an inability to catabolize substrate. This is further illustrated by several reports of dysfunction in other organelles, especially mitochondria, gleaned from studies in LSDs, suggesting that there is functional interdependence between the organelles, thus making revisiting current views on lysosomal biology imperative. Notably, mitophagy is a cellular process that links lysosomes and mitochondria, although its mechanism and physiological relevance have been the subject of considerable debate. The gene products of *PINK1* and Parkin (*PARK2*/*PRKN*), which are mutated in familial forms of Parkinson's disease (PD), classically mediate mitophagy, which involves the recruitment of the E3 ubiquitin ligase, Parkin, to damaged mitochondria by the kinase, PINK1. Parkin-mediated ubiquitinated mitochondria are sequestered in autophagosomes and targeted for lysosomal degradation. Mutations in mitochondrial genes are often associated with impairment of the autophagy/lysosomal system which, among others, can involve reduced levels of the lysosomal protease cathepsin D [36]. As such, defective mitophagy and the consequent increase in the proportion of dysfunctional mitochondria is an expected outcome of LSDs. This is illustrated in Gaucher's disease, the most common LSD, in which the defective enzyme is glucocerebrosidase (GCase). Pharmacological inhibition of GCase in human dopaminergic cells recapitulates the accumulation of α-synuclein, a hallmark of the disease, leading to the accumulation of fragmented mitochondria with reduced membrane potential and progressive reduction of ATP synthesis [37]. These findings are corroborated by genetic ablation of GCase in mice, whose neurons accumulate dysfunctional mitochondria which are profoundly fragmented with reduced membrane potential and decreased activity of respiratory chain enzymes [37]. Indeed, decreased mitochondrial quality associated with reduced mitochondrial respiration and decreased levels of some tricarboxylic acid (TCA) cycle intermediates has been reported in bafilomycin- or chloroquine-treated primary cortical

rat neurons [38]. In addition, respiratory chain defects were already reported in Pompe's disease infants where the activity of several respiratory chain enzymes including complexes I, II, and III is reduced [39]. It is likely that the observed perturbation of Ca^{2+} homeostasis and mitochondria Ca^{2+} overload in LSDs [40] contribute to the mitochondrial fragmentation phenotype and its attendant decrease in mitochondrial function [41].

It is becoming clearer that cellular responses to lysosomal stress, for example, include not only the primary consequences of the lysosomal biochemical defects (e.g., storage of one particular metabolite) and the stress response elicited by that particular biochemical defect and consequent lysosomal dysfunction, but also perturbations in communication between lysosomes and other organelles, particularly mitochondria. It was recently shown that lysosomal malfunction is at the center of a transcriptional program that suppresses mitochondrial biogenesis and function [42]. In two lysosomal cholesterol/sphingomyelin storage diseases, patient cells and mouse tissues of acid sphingomyelinase (ASM) and Niemann–Pick type C (NPC) show a coordinated transcriptional program which mediates the repression of mitochondrial biogenesis and function (Figure 2). Interestingly, these findings were independent of the prevalent defective autophagy in ASM and NPC [42]. The underlying mechanism was triggered by defective sphingosine-1-phosphate (S1P) signaling. S1P is a signaling molecule that activates S1P receptors at the plasma membrane as well as in intracellular membranes. In ASM and NPC patient cells, the S1P receptor 1 (S1PR1) is mislocalized in the cell and is mostly absent from the plasma membrane [42]. This results in decreased S1PR1 downstream signaling, which in turn activates the transcription factor KLF2 [43]. KLF2 triggers the expression of another transcription factor, ETV1, and both KLF2 and ETV1 coordinately repress the expression of nucleus-encoded mitochondrial genes [42]. This culminates in robust repression of mitochondrial biogenesis and impaired mitochondrial respiration. Notably, S1PR1 can also mediate mitochondrial biogenesis and function via KLF2 and ETV1 in the absence of lysosomal defects. Intriguingly, modulation of the S1PR1 signaling node in ASM or NPC rescues mitochondrial defects in ASM and NPC cells at the expense of cell survival: reactivation of S1PR1 (and mitochondrial biogenesis) in the presence of lysosomal defects results in cell death [42]. Therefore, a picture emerges that, when lysosomes are defective, mitochondrial biogenesis is repressed in cells and tissues as a protective mechanism. It can be speculated that, because the ultimate destination of damaged mitochondria (the lysosome, last stop in the mitophagy pathway) is clogged, the cells prefer to shut down the production of an organelle (mitochondria) that can kill the cell when they are defective and not properly removed (Figure 3). Thus, it is possible that the crosstalk between lysosomes and mitochondria may also be responsible for maintaining a stoichiometric balance in the amount of functional mitochondria in healthy cells. It may be debated whether this paradigm is specific for mitochondria or if it also applies to other organelles whose homeostasis relies on selective autophagy, such as peroxisomes. Indeed, peroxisomal biogenesis is also repressed under lysosomal defects, albeit in a tissue-specific manner [42,44]. Additional mechanisms of lysosomal signaling towards mitochondria are discussed in Box 3.

Autophagy and Mitochondria

Because the status of lysosomes can impact on mitochondrial function, it is important to assess whether processes that culminate in the lysosome, such as autophagy and endocytosis, can affect and be affected by mitochondria.

It is clear from the vast research in mitophagy that autophagy is a key component of mitochondrial homeostasis [45]. However, whether mitochondria can also regulate autophagy is much less well studied. Given the roles of mitochondria as a signaling platform connecting the metabolism of sugars and fatty acids to cellular bioenergetics and cell fate, and given that mitochondria can regulate lysosomal function, as discussed earlier, it is conceivable that mitochondria may also affect the regulation of other aspects of autophagy. A landmark study from the Nunnari laboratory showed, using budding yeast as the model organism, that deficiency in mitochondrial respiration can impair the formation of autophagosomes, autophagic flux, and the transcriptional activation of autophagy-related genes [46]. These effects triggered by respiratory chain deficiency are mediated by the activation of PKA. In mammals, chronic mitochondrial respiratory chain deficiency also represses both the expression of

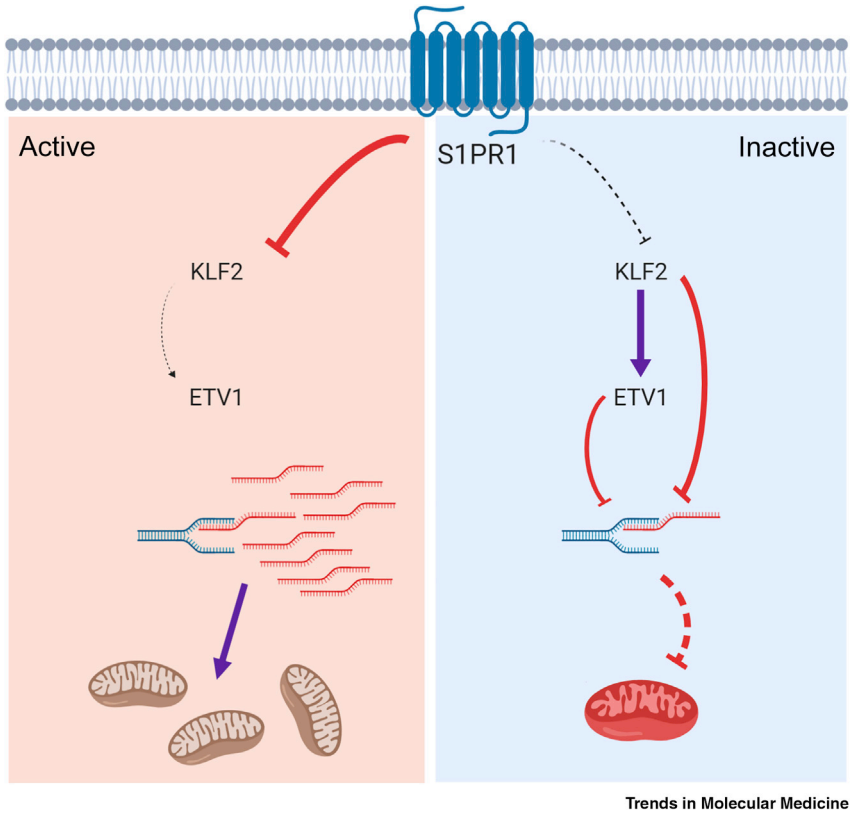

Figure 2. Regulation of Mitochondrial Biogenesis in Sphingomyelin- and Cholesterol-Storage Diseases. Mitochondrial biogenesis is responsive to the activity of the signaling lipid sphingosine-1-phosphate (S1P) through its receptor S1PR1. Activation of this receptor promotes mitochondrial biogenesis and function, which are reciprocally repressed by S1PR1 inhibition or loss of function. In two lysosomal storage diseases in which cholesterol and sphingomyelin accumulate in the lysosomes, signaling through S1PR1 is impaired (right panel), resulting in increased expression of the transcription factor KLF2, which induces the expression of ETV1. KLF2 and ETV1 cooperate to inhibit the transcriptional program of mitochondrial biogenesis, resulting in impaired mitochondria. In basal conditions (left panel), S1PR1 activity represses the transcription factor KLF2, ETV1 is not induced, and mitochondrial biogenesis occurs unperturbed, yielding functional mitochondria.

autophagy/lysosomal genes and autophagic flux by downregulating AMPK signaling [14]. It remains to be determined whether there is crosstalk between PKA and the yeast ortholog of AMPK, Snf1p, or if PKA is involved in the mammalian mitochondria-to-autophagy response.

Notably, the activity of the mitochondrial respiratory chain, and of complex I in particular, is necessary for autophagy induced by inhibition of mTORC1 [47]. The importance of the energy-generating role of mitochondria for autophagy is highlighted by two independent studies showing how mitochondria are protected from degradation during starvation-induced autophagy [28,29]. The cellular reliance on mitochondria during autophagy may have reasons that go beyond energy metabolism because contact sites between mitochondria and the endoplasmic reticulum (ER) can be used as source of membrane for new autophagosomes [48,49].

Another mechanism by which mitochondrial malfunction has been implicated in the stalling of autophagic flux concerns microtubule stability [50]. Cells with mitochondrial defects associated with PD show unstable microtubules as a result of decreased polymerization and increased depolymerization.

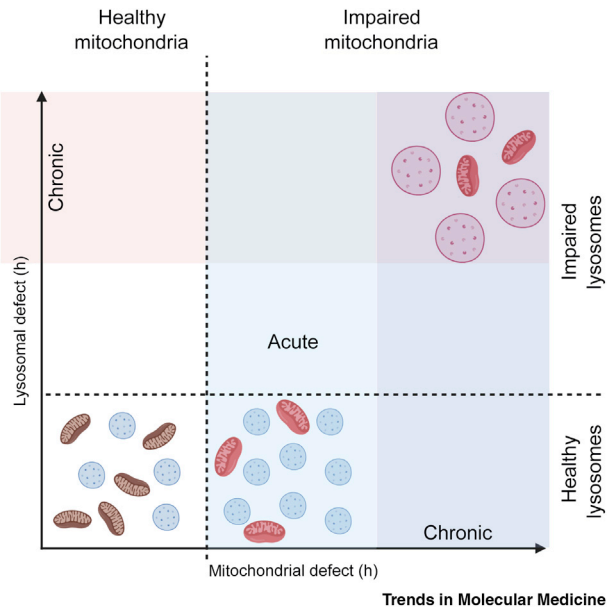

Figure 3. Interdependence of Mitochondria and Lysosomes.
Upon acute mitochondrial stress, lysosomal biogenesis is increased in an AMPK-dependent manner, and autophagy is stimulated. The cell attempts to respond to an acute mitochondrial crisis by removing the problematic organelles and gears up to do so (more autophagosome formation, more lysosomal hydrolytic capacity). When the mitochondrial defect persists, continuation of the previous paradigm would result in loss of all mitochondria, and the cell therefore shuts down AMPK signaling, which results in blockage of autophagosomal flux and downregulation of lysosomal function, resulting in damaged mitochondria and impaired lysosomes. Reciprocally, when the defect is lysosomal, the cell reacts by shutting down the production of new mitochondria, an apparent feed-forward mechanism with the goal of limiting future contributions to the autophagic flux via mitophagy. This scenario also results in damaged lysosomes and impaired mitochondria.

This results in an inability to transport autophagosomes to lysosomes for degradation, and in their accumulation in the cytoplasm.

The full impact of mitochondria on the autophagic pathway is still not completely explored. Although it is intimately related to the effects of mitochondria on lysosomes, it remains to be elucidated, for example, whether mitochondria-containing autophagosomes are preferentially degraded under conditions of acute mitochondrial stress, and if mitochondrial dysfunction affects the fusion of autophagosomes with lysosomes. Furthermore, because delivery of autophagosomes to lysosomes, under basal conditions, is necessary to maintain optimal lysosomal function [51], one can ask whether blockade of autophagic flux can result in perturbations of lysosomes, and in secondary perturbations of mitochondria (in addition to the accumulation of damaged mitochondria in the cytoplasm as a result of decreased autophagy).

Endocytosis and Mitochondria

The endocytic pathway brings extracellular signals and resources, and delivers them to endosomes, and eventually to lysosomes, for distribution to other cellular organelles. Given the role of endocytosis in growth factor signaling, and the importance of coordinating signaling and metabolism, it is expected that there is some crosstalk between mitochondria and the endocytic process. This is, however, a largely unexplored area.

There are different types of endocytosis, some clathrin-dependent and some clathrin-independent pathways. A recent review addresses these aspects in detail [52]. Mitochondrial uncoupling

results in strong inhibition of clathrin-mediated endocytosis [53]. The effect is not due to decreased ATP levels but instead to acidification of the cytoplasm. This is likely to impact on growth factor signaling, for example, through the epidermal growth factor receptor, which is dependent on endocytosis [54], as well as the uptake of key cellular components such as Fe (via the transferrin receptor) and cholesterol from low-density lipoparticles (LDL receptor). Furthermore, it has been reported that the inability of mitochondria to take up Ca^{2+} during action potentials results in increased endocytosis at the synapse without affecting the rate of exocytosis [55].

Endocytosis is often referred to as a compensatory process for exocytosis. Remarkably, mitochondria also participate in the regulation of exocytosis. It has been known for a long time that mitochondrial function affects the exocytosis of insulin-containing vesicles in pancreatic β cells, and decreased mitochondrial function in these cells can recapitulate symptoms of type II diabetes [56]. Notably, in neurons, the role of mitochondria in exocytosis seems to be unrelated to ATP production because synapses recruit glycolytic enzymes under energy stress [57]. The citrate cycle enzyme isocitrate dehydrogenase 3A is necessary for the production of α-ketoglutarate at the synapse, and a decrease in the synaptic levels of this metabolite causes defects in synaptic transmission [57]. These defects are not related to neurotransmitter (e.g., glutamate) or ATP availability. The size of mitochondria is also carefully regulated in the axons and presynaptic compartments of neurons [58]. The protein MFF (mitochondrial fission factor) recruits the GTPase DRP1 (dynamin-related protein 1) to the mitochondrial membrane, which then leads to mitochondrial fission [59–62]. MFF is pivotal in the regulation of presynaptic and axonal mitochondrial size, which in turn determines how much Ca^{2+} these organelles can uptake. Failure of MFF in the control of mitochondrial size results in bigger mitochondria in the presynaptic compartment, which then uptake more Ca^{2+} during synaptic transmission, thus decreasing presynaptic release [58]. MFF also works in peroxisomal fission [63], but the peroxisomal contribution to this effect remains to be determined.

Notably, many proteins that manipulate membranes in endocytosis have closely related isoforms that work in mitochondria. For example, dynamins (dynamin-1, -2, and -3) carry out membrane constriction and fission in endocytic pits, whereas dynamin-like protein DRP1 catalyzes mitochondrial fission [59,61,64]. Endophilins A (endoA1, A2, and A3) are involved in different types of endocytosis, and endophilin B1 regulates mitochondrial morphology [65]. Synaptojanin, a phosphatase that is also involved in multiple types of endocytosis, also has isoforms dedicated to endocytosis (synaptojanin-1) and to mitochondrial dynamics (synaptojanin-2) [66]. It thus seems that the cell has coopted the endocytic pathway to dedicate a specific set of membrane-remodeling proteins to mitochondria as well as to other intracellular organelles. This is important to note because, for example, DRP1 also catalyzes peroxisomal fission [59,61], and a brain-enriched DRP1 isoform was found to associate with lysosomes, late endosomes, and the plasma membrane, and also to regulate endocytosis independently of mitochondrial fission [67,68]. Another membrane-remodeling protein associated with recycling endosomes, EDH1, was also found to regulate mitochondrial fission [69]. The biochemical and genetic interactions between the endocytosis-specific isoforms and the mitochondria-specific isoforms require further investigation. Similarly, the significance of the presence of some of these proteins in mitochondria and other organelles remains to be elucidated. This question is particularly enticing because mutations in many endocytic adaptors have been found to increase the risk of neurodegenerative diseases, particularly PD [69]. Most of the known genetic risk factors for PD impact on mitochondria, autophagy, and endocytosis, raising the possibility that crosstalk between mitochondria and the endolysosomal system is involved in the pathology.

AMPK and mTORC

AMPK signaling is at the center of mitochondrial and lysosomal stress responses. AMPK promotes mitochondrial biogenesis [70] and fission [27], as well as lysosomal biogenesis [14,20] and lysosomal function [14], in addition to its roles in promoting autophagy [21]. Therefore, AMPK coordinates the

entire process of autophagy, from autophagosome formation and lysosomal degradation to lysosomal biogenesis. The induction of folliculin expression in the presence of chronically impaired mitochondria results in decreased AMPK signaling and impaired lysosomal function as a result of low PIKfyve activity [14]. This finding also places AMPK at the core of the crosstalk between mitochondria and lysosomes. Notably, repression of AMPK signaling in chronic respiratory chain deficiency is at the root of the loss of lysosomal function [14]. This effect could be rescued by AMPK reactivation but not by mTORC1 inhibition, implying that chronic mitochondrial stress regulates lysosomes in a AMPK-dependent but mTORC1-independent manner.

In several mouse models and human patient tissues with mitochondrial disease, AMPK downregulation and mTORC1 upregulation were observed, thus placing AMPK and mTORC1 as promising therapeutic targets. Indeed, the treatment of MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) patient fibroblasts [71], as well as mice with deficiency in mitochondrial respiratory chain complex IV, with AMPK activators attenuated the pathological phenotype [34]. Mice lacking the subunit Ndufs4 of mitochondrial respiratory chain complex I (NDUFS4-KO mice) display hyperactive mTORC1, and their treatment with rapamycin alleviated the phenotype and increased lifespan [72], albeit by unclear mechanisms. It is noteworthy that treatment with rapamycin seems to be effective mostly in cases of mTORC1 hyperactivation [73]. mTORC1 regulates mitochondrial biogenesis at the translational level, and inhibition of mTORC1 results in decreased mitochondrial function [74], and mTORC1 inhibition in the context of mitochondrial disease may therefore seem to be counterintuitive. It is worth emphasizing, however, that mTORC1 inhibition in these settings may serve to maintain energy balance by shutting down processes that consume large amounts of cellular ATP (e.g., ribosome biogenesis, translation, and mitochondrial biogenesis).

Reactivation of TFEB and lysosomal biogenesis seems to be a common thread between AMPK activators and mTORC1 inhibitors [75]. For example, treatment of worms with metformin increases the lysosomal biogenesis pathway [76]. Metformin is a widely used drug for type II diabetes, and acts by inhibiting respiratory chain complex I; it also affects v-ATPase-mediated regulation of AMPK and mTORC1 by repressing mTORC1 and activating AMPK [77]. In this context, it is important to also consider the role of the zinc-finger DNA-binding protein ZKSCAN3, which is a transcriptional repressor of autophagy and TFEB-induced lysosomal biogenesis [78]. Under basal conditions ZKSCAN3 is localized in the nucleus, but starvation or mTORC1 inhibition trigger its relocation to the cytoplasm, further enabling autophagy and lysosomal biogenesis [78]. The combined effect of mTORC1 inhibition, release of mTORC1- and ZKSCAN3-mediated TFEB inhibition, and AMPK activation results in AMPK- and TFEB-dependent lysosomal biogenesis. Importantly, rapamycin has recently been implicated in the activation of the lysosomal Ca^{2+} export channel mucolipin-1 independently of mTORC1 [79]. However, this is debatable given that mTORC1 was previously shown to directly phosphorylate and inhibit the mucolipin-1 channel [80]. Notwithstanding, rapamycin-and/or mTORC1 inhibition-dependent mucolipin-1 calcium export mediates TFEB nuclear translocation, a key component of lysosomal biogenesis and autophagy activation [32,79]. Because AMPK and mTORC1 have an antagonist regulation, the combined effect of AMPK activation and mTORC1 repression is often entirely attributed to mTORC1 repression. Nevertheless, AMPK activation independently of mTORC1 is necessary for the maintenance of lysosomal function via the AMPK–PIKfyve–MCOLN1 branch [14], and the benefits of mTORC1 repression for lifespan in some instances require AMPK activity [81]. Furthermore, it has been reported that mTORC1 may be hyperactive in the presence of mitochondrial defects even if AMPK is also upregulated [82], highlighting that mTORC1 is more than an effector of AMPK activity. Indeed, mTORC1 integrates inputs from several other signaling nodes [22]. Therefore, the combined activation of AMPK and inhibition of mTORC1 seems to be a more promising therapeutic strategy for mitochondrial diseases than simply activating AMPK or repressing mTORC1 alone.

However, it remains to be determined which pools of AMPK are activated in response to acute and chronic mitochondrial stress because different AMPK intracellular pools (cytoplasmic, mitochondria, lysosomal, and others) respond to specific activation mechanisms and may target different pathways

[83]. It is noteworthy that stress-induced AMPK activation is not necessarily pathological because exercise can also trigger the pathway [84].

The behavior of AMPK and mTORC1 in response to mitochondrial and lysosomal defects has predictable downstream implications given the importance of these regulatory nodes in cell functions. For example, protein synthesis is under tight regulation by mTORC1, and is strongly inhibited in acute mitochondrial stress, but is activated in chronic mitochondrial stress [85]. It remains unclear whether these effects are related to changes in AMPK; however, the fact that mTORC1 and translation are inhibited when AMPK is hyperactive (acute mitochondrial stress), and active when AMPK is repressed [14,17], suggests that AMPK might be involved. Furthermore, AMPK can promote cellular survival during energy stress by activating mTORC2, which in turn leads to increased Akt signaling, the key 'survival hub' of the cell [86]. This is likely to be a time-dependent effect because prolonged activation of AMPK in mice with defective mitochondria results in synapse loss [87] and neuronal cell death [25].

Pathological Mechanisms

The importance of mitochondria–lysosome crosstalk is evidenced by the wide range of pathologies in which the communication between these organelles is perturbed. As discussed above, many perturbations of lysosomal function in mitochondrial diseases have been reported (Figure 3). Reciprocally, most lysosomal storage diseases have reports of mitochondrial dysfunction, of which Gaucher disease is a pivotal example [37], as well as other diseases caused by defects in lysosomal catabolism of sphingolipids [42], glycogen [88], or glucosaminoglycans [89]. Furthermore, many neurodegenerative diseases show signs of defects in both mitochondria and lysosomes. This is particularly clear in PD [89] (discussed in more detail in Box 4). In Alzheimer's disease, a novel neuronal pathway of mitochondrial activation by mTORC1-dependent lysosomal amino acid sensing has been shown to be inhibited by the β-amyloid oligomers that characterize the disease [90]. Therefore, the mitochondria–lysosome axis is a promising target that has been receiving increasing attention.

Therapeutic strategies aimed at increasing lysosomal biogenesis have been focused mostly on the overexpression or activation of TFEB, and have been attempted in several models of neurodegenerative disease including PD [91], Alzheimer's [92], Huntington's [15], and amyotrophic lateral sclerosis [93].

A novel approach was also attempted in Niemann–Pick type C, a lysosomal sphingomyelin and cholesterol storage disease. Fernandez-Checa and workers found that the mitochondrial pools of glutathione (GSH) were decreased in a mouse model of the disease (*Npc1*$^{-/-}$) as well as in patient fibroblasts [94]. GSH is the major cellular redox buffer and has a pivotal role in countering oxidative stress as well as in cellular signaling [95]. They tested whether replenishing the mice and patient fibroblasts with a GSH derivative, GSH ethyl ester (GSH-ee), would have a beneficial effect. Treatment with GSH-ee *in vivo* resulted in normalization of mitochondrial GSH pools in brain and liver of the *Npc1*$^{-/-}$ mice, and a similar result was obtained in patient fibroblasts [94]. This approach illustrates that multiple layers of biochemical interactions between mitochondria and lysosomes can be explored for therapeutic strategies.

Concluding Remarks

The coordination between mitochondria, lysosomes, metabolism, and cell signaling is pivotal for cellular function. The function of these organelles extends beyond their metabolic roles, and the importance of their in-phase functioning is demonstrated by disruption of their function or communication in multiple pathologies, particularly neurodegenerative disorders. The importance of the lysosomal–mitochondrial communication axis is further underscored by its early evolution – already in trypanosomes and fungi an inability to acidify the vacuole results in perturbations of mitochondrial function [96,97].

Clinician's Corner

Mitochondria and lysosomes are pivotal organelles for cellular metabolism and also have important roles as signaling platforms. Many diseases are characterized by impaired mitochondrial or lysosomal function. These are often referred to as 'mitochondrial diseases', which have a primary cause in mitochondria, or as lysosomal storage diseases. Although each specific syndrome is usually a rare disease, many different syndromes share common patterns. It is therefore important to understand the generic consequences of mitochondrial and lysosomal impairment. Furthermore, the function of these organelles is coordinated, and compromises to such coordination are commonly observed in disease. Notably, diseases caused by primary defects in one of these organelles often display secondary perturbations in the other (Figure 3).

Therefore, understanding the mechanisms that underlie the coordination between mitochondria and lysosomes is fundamental to harnessing those mechanisms for possible therapeutic strategies. This is particularly important given that, for many of these syndromes, the therapeutic intervention is mostly focused on symptom management. Holistic comprehension of the pathways affected by mitochondrial and lysosomal dysfunction, as well as those involved in communication between the organelles, might provide therapeutic targets that are closer to the root of the problem – ablation or manipulation of the pathological signaling pathways may be sufficient to minimize or avoid pathology [9].

Because of the major roles of mitochondria and lysosomes in metabolism, these organelles are strongly integrated with major hubs of cellular signaling, such as AMPK and mTORC1, which coordinate the balance between

Box 4. Parkinson's Disease

PD is the most common movement disorder and the second most common neurodegenerative disorder, having a prevalence of ~2% in people older than 65 years old [131]. PD is characterized by loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) leading to a dopamine deficit in the dorsal striatum [132]. A pathological hallmark of PD is the aggregation of α-synuclein, ubiquitin, neurofilaments, and molecular chaperones that present as intraneuronal inclusions called Lewy bodies (LBs) [132]. However, the exact mechanism by which their aggregation occurs is poorly understood, although some lines of evidence implicate mitochondria and autophagy dysfunction as possible primary causes [133–136]. At least six mutated genes that are linked to familial PD impact on mitochondria and autophagy, including α-synuclein (*SNCA* or *PARK1*), Parkin (*PARK2* or *PRKN*), ubiquitin carboxyhydroxylase L1 (*UCH-L1* or *PARK5*), PTEN-induced putative kinase 1 (*PINK1* or *PARK6*), DJ-1 (*PARK7*) and leucine-rich repeat kinase 2 (*LRRK2* or *PARK8*) [137]. Sporadic and genetic PD appear to have mitochondrial dysfunction as a common hallmark [138]. Reports have described disruption of the mitochondrial electron transport chain (ETC) with a prime involvement of complex I and impaired electron transfer [139], mutations of genes involved in mitochondrial function in genetic forms of PD [140,141], deletions in mtDNA [142], as well as defects in the regulation of mitochondrial dynamics, all in the context of PD pathogenesis [143]. Alterations in mitochondrial structure and function can lead to ROS-dependent cell damage culminating in neuronal loss [9,12,25]. Disruption of mitochondrial quality control mechanisms, particularly mitophagy, has also been observed in PD.

The *PINK1* gene encodes a putative mitochondrial serine/threonine protein kinase with a mitochondrial target sequence, which is normally imported to the inner mitochondrial membrane in a mitochondrial potential-dependent manner. Decreased mitochondrial potential or mutations in *PINK1* may result in the accumulation of PINK1 in the outer mitochondrial membrane, which allows Parkin, an E3 ubiquitin ligase protein, to bind to depolarized mitochondria inducing mitophagy [144]. PINK1 mutations are involved in PD by altering mitochondrial physiology, namely by inducing mitochondrial cristae fragmentation and also leading to increased oxidative stress [145]. Parkin deficiency in PD patient fibroblasts leads to dysfunction of the retromer, a trimeric cargo-recognition protein complex responsible for protein trafficking in the endosomal compartment [146]. Notably, despite the proposed roles of Parkin and PINK1 in mitophagy, this process occurs relatively unperturbed in flies and mice, including in dopaminergic neurons [147,148]. These two proteins have, however, been found to inhibit the formation of mitochondria-derived vesicles (MDVs) and mitochondrial antigen presentation, which are both induced when PINK1 and Parkin are absent [149]. In addition, PINK1 and Parkin have been reported to inhibit the release of inflammation-inducing molecules by mitochondria, an effect underscored by strong inflammatory signaling in *Pink1*$^{-/-}$ or *Prkn*$^{-/-}$ mice [150]. Furthermore, PINK1 seems to play a role in the immune system, demonstrated by the susceptibility of *Pink1*$^{-/-}$ mice to develop PD symptoms after intestinal infection with Gram-negative bacteria [151]. Therefore, PINK1 and Parkin link mitochondrial signaling and endolysosomal function to the systemic pathology of PD.

catabolism and anabolism. These kinases are well studied and pharmacologically targetable. Although repression of mTORC1 has been successful for some mitochondrial diseases, it has several drawbacks. The potential of pharmacological AMPK activation was confirmed for some mitochondrial defects, but remains to be tested systematically, both in mitochondrial and lysosomal diseases.

Finally, the mechanisms that underlie the dysfunction of both mitochondria and lysosomes and their role in the pathology of neurodegenerative diseases, of which PD is a case in point, may provide extraordinary therapeutic potential, particularly in the modulation of inflammation induced by mitochondrial or lysosomal dysfunction.

The accumulation of evidence demonstrating that the functional roles of mitochondria and lysosomes are interdependent warrants further understanding of these organelles in a holistic perspective in which not only their primary functions but also their roles as components of a multi-organelle network are considered. Furthermore, the role of other organelles, particularly the ER and peroxisomes, in crosstalk between mitochondria and lysosomes remains to be elucidated, as do the particularities of tissue-specificity, developmental stage, and age progression (see Outstanding Questions).

In addition, understanding the higher-level connections between different organelles may reveal so far unexplored targets that can be of therapeutic value. For example, the activation of AMPK, a hub that coordinates both mitochondrial and lysosomal function, remains to be systematically explored in mitochondrial and lysosomal diseases, as well as in neurodegenerative syndromes that encompass defects in these organelles. Another outstanding question likely to be addressed in the coming years concerns the role of the major metabolic hubs (AMPK, mTORC1) in the regulation of contact sites and vesicular trafficking between organelles – and in particular whether and how these hubs can modulate the immune phenotypes recently described in PD.

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Outstanding Questions

What is the role of mitochondria–lysosome contact sites in the crosstalk between these organelles?

How do other organelles contribute to mitochondria–lysosome crosstalk?

Are there tissue-specific effects on mitochondria–lysosome crosstalk?

What are the pitfalls of activating AMPK as a therapeutic strategy for mitochondrial and lysosomal diseases?

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