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Review

Staying cool in difficult times: Mitochondrial dynamics, quality control and the stress response ☆

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ABSTRACT

One of the critical problems with the combustion of sugar and fat is the generation of cellular oxidation. The ongoing consumption of oxygen results in damage to lipids, protein and mtDNA, which must be repaired through essential pathways in mitochondrial quality control. It has long been established that intrinsic protease pathways within the matrix and intermembrane space actively degrade unfolded and oxidized mitochondrial proteins. However, more recent work into the field of quality control has established distinct roles for both mitochondrial fragmentation and hyperfusion in different aspects of quality control and survival. In addition, mitochondrial derived vesicles have recently been shown to carry cargo directly to the lysosome, adding further insight into the integration of mitochondrial dynamics in cellular homeostasis. This review will focus on the mechanisms and emerging questions concerning the links between mitochondrial dynamics and quality control. This article is part of a Special Issue entitled: Protein Import and Quality Control in Mitochondria and Plastids.

1. Introduction: Mitochondrial proteases and the first line of defense

The question of mitochondrial quality control was first addressed with the discovery of a series of mitochondrial proteases that were evolutionarily conserved from bacteria. The matrix localized Lon protease has its origins in bacterial quality control pathways [1]. In mammalian cells, the AAA protease Lon has been shown to recognize oxidized cargo like aconitase [2], but it also can regulate mtDNA levels either directly [3], or through the degradation of the mitochondrial nucleoid-associated transcription factor A, TFAM, indirectly regulating mtDNA copy number [4]. As in bacteria, the regulation of protein turnover in the mitochondria is also subject by the "N-end rule", where the stability of the protein is dependent upon the N-terminal exposed residue. Protein stability of imported proteins can be tightly regulated through a second cleavage event mediated by matrix intermediate proteases. This cleavage has been shown to remove unstable N-terminal residues, effectively stabilizing the protein [5,6]. The N-terminal residues of mitochondrial proteins are therefore critical determinants of the half-life of a broad spectrum of proteins.

The importance of mitochondrial proteases in quality control has been demonstrated by the direct links to human diseases where AAA proteases are mutated. These include the inner membrane anchored AAA protease complex, AFG3L2 and Spg7, both of which have been linked to familial forms of human spastic paraplegia and ataxia [7–9]. The function of these proteases is broad, as they often have roles in import and processing of substrates, as well as in mitochondrial dynamics through the regulated cleavage of substrates like the inner membrane fusion GTPase Opa1. Opa1 is highly complex; with 8 splice variants, and at least two different cleavage sites. Some splice variants are cleaved constitutively by the intermembrane space protease Yme1 [10,11], but it was also shown that AFG3L2 participates in the regulation of Opa1 cleavage events, contributing to the balance of long- and short-forms of the GTPase in steady state [12]. Furthermore, upon the loss of mitochondrial electrochemical potential, another inner membrane protease Oma1, cleaves all of the Opa1 variants to the short form, effectively blocking mitochondrial fusion [12,13]. Opa1 has been a particularly complicated substrate, with many different mitochondrial proteases acting upon it, depending on the situation. For example, one of the Opa1 proteases, the rhomboid protein PARL [14], regulates mitochondrial dynamics through a conserved N-terminal matrix domain, which is subject to proteolytic cleavage in a manner dependent upon the phosphorylation state [15,16]. Importantly, PARL has also been identified as a Parkinson's disease gene, further implicating mitochondrial proteases in human disease [17]. The use of complex protease cascades in the regulation of protein turnover and function has become a recurrent theme, placing the mechanisms for protease regulation as a critical focus for future research.

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2. New discoveries explain mitochondrial clearance

A young graduate student named Derek Narendra in the lab of Richard Youle did an experiment that changed the field almost overnight. He simply added a mitochondrial poison, CCCP, which depolarizes the electrochemical potential across the mitochondrial inner membrane [18]. Over the course of an hour, Derek observed an almost complete recruitment of a ubiquitin E3 ligase called Parkin to the uncoupled and fragmented mitochondria. The continued treatment of the cells with CCCP eventually led to the clearance of all of the mitochondria through the autophagic pathway. If ever there was an Archimedes-inspired "Eureka!" moment in mitochondrial quality control, this was it. Neuroscientists had been intensely investigating Parkin, which is mutated in some forms of familial Parkinson's disease, for over a decade. They had established that the loss of Parkin led to mitochondrial dysfunction in *Drosophila Melanogaster* [19–21], yet the mechanistic links to mitochondria remained elusive. Since the publication of this result in 2008, there has been an exponential rise in publications focused on the role of Parkin and other PD related genes in mitochondrial quality control.

Importantly, parkin mediated mitophagy required that the mitochondria be fragmented, since the loss of mitochondrial fission blocked the clearance of the organelles [22]. The recognition of dysfunctional fragments by Parkin is essential in this pathway, as fragmentation alone does not trigger mitophagy [23]. In this way, we learned an important new function for mitochondrial fission — to allow for the removal of damaged organelles. It had been shown a few months before the Narendra study that mitochondrial depolarization occurred during a fission event, and this may function to continually "survey" the reticulum for the ability to recover from transient depolarization [24]. However, the delivery of the depolarized organelle to the autophagosome required a number of hours, so why don't they re-fuse with healthy mitochondria in the interim? As mentioned above, the loss of electrochemical potential leads to the activation of the inner membrane protease Oma1, which cleaves the inner membrane fusion GTPase Opa1 [10–13,25]. Once cleaved to the shorter form, Opa1 was released from the inner membrane, and fusion was blocked. In this way the lone, depolarized organelle becomes excommunicated from the reticulum awaiting the recruitment of parkin and final delivery to the cellular gallows.

We have since learned that additional PD related genes, including the kinase Pink1, are requisite for Parkin recruitment to depolarized mitochondria [22,26–29]. In addition, depolarized organelles recruit the chaperone p97/VCP, which facilitates the extraction and degradation of outer membrane proteins by the proteasome [30,31]. Together, these data provide links between mitophagy and the proteasome, suggesting that the removal of outer membrane proteins is requisite for the recognition of the fragment by the autophagosome. In addition, it was discovered that p97/VCP/Cdc48 are specifically recruited to mitochondria via the adaptor Vms1 in cells experiencing oxidative stress in multiple organisms [32]. The mechanism of retrotranslocation of mitochondrial proteins across the outer membrane to the proteasome is not yet established, but is known to regulate the turnover of many mitochondrial proteins [33–36]. With this, it is clear that ubiquitin-mediated protein degradation occurs in the absence of mitophagy as a mechanism for regulated mitochondrial protein turnover and quality control.

The established paradigm has been developed primarily using cultured cells. The best example of mitophagy within physiology is the example of the red cell. In this cell type, an outer membrane protein Nix (Nip3 like protein X) plays a critical role in the clearance of all mitochondria during development [37,38]. In this developmental system, the clearance of mitochondria is not precipitated by their dysfunction or loss of potential. Nix functions directly as an adaptor to couple the mitochondria to the autophagic proteins LC3 and GABARAP, leading to the engulfment of mitochondria by the autophagosome in a developmentally regulated manner [39,40]. Studies in this model system have also shown roles for Parkin [41], the outer membrane kinase Ulk1, and the cytosolic chaperones Hsp90 and Cdc37 [42]. In another tissue-specific model of autophagy within the heart, the Nix-related protein Bnip3 (Bcl2/E1B 19 kDa-interacting protein 3-like protein) was shown to be requisite for mitophagy in a mechanism that also requires mitochondrial fission and parkin recruitment [43]. From all these studies, it appears that two distinct steps must occur to facilitate mitophagy; the activation of the autophagic machineries, and the selection of the dysfunctional mitochondria, [41,44,45]. There is a growing complexity in the signals, adaptors and regulation of mitophagy that are currently under investigation [46].

In skeletal muscle, the regulated cellular program of muscle atrophy also requires active mitophagy [47]. In that study, mitochondrial fission was required in order to activate transcription of at least two genes encoding ubiquitin ligases required for atrophy-induced protein degradation, Atrogen-1 and MURF-1 [47]. This demonstrates that the process of mitophagy may also play a signaling role in determining cell fate in addition to the clearance of dysfunctional organelles.

The loss of electrochemical potential is a primary trigger for mitophagy, yet it is not clear whether mitochondria significantly depolarize on a regular basis in vivo. In primary neurons, CCCP does not necessarily lead to global uncoupling of mitochondria. A recent study showed a differential effect depending on the carbon source provided to the cells [48]. When cells were grown on glucose, CCCP depolarized and parkin was recruited, however when grown on galactose which drives mitochondrial respiration, the drug did not lead to parkin recruitment. This is because mitochondria have evolved mechanisms to ensure they remain polarized in cells that require their services. For example, the addition of oligomycin to these galactose treated cells reverted the phenotype, and parkin was again recruited [48]. Oligomycin blocks the ATP synthase (complex V), which had been running in reverse to hydrolyze ATP, pumping protons back out of the matrix to regenerate the electrochemical potential. More recent work has confirmed the relative resistance of neuronal mitochondria to depolarization, but demonstrated a direct functional role for parkin recruitment in the neuronal survival [49]. Rather than the rapid parkin recruitment in transformed cells, it took 12–24 h before Parkin recruitment was observed in only 5–30% of neurons. This work demonstrates the universality of the paradigm. Future work will continue to focus on establishing the molecular details of parkin recruitment to depolarized mitochondria.

3. Mitochondrial fusion as cellular stress response

Equally exciting has been the realization that the mitochondria band together in times of stress (Fig. 1). In a term coined by Daniel Tondera in the lab of Jean-Claude Martinou, stress induced mitochondrial hyperfusion (SIHM) was observed in the hours following a number of cellular stresses [50]. Prolonged stress ultimately leads to mitochondrial fragmentation, due to accumulated damage and depolarization. Whether or not the fragmented mitochondria trigger apoptosis would depend on the extent and duration of the stress trigger. Once fragmented, dysfunctional organelles may be cleared through mitophagy, potentially restoring the reticulum to the normal state (see model, Fig. 1).

In the initial study, a prohibitin related protein Slp-2 was identified as requisite for the hyperfusion response, and its presence ensured the stability of Opa1 against stress-induced cleavage and degradation [50]. Although the precise mechanisms of Opa1 protection by Slp2 remain unclear, the data support the idea that the architecture of the inner membrane is likely very dynamic, responding to stress cues in various ways. The cristae themselves are tightly controlled through a conserved protein complex, recently named the MINOS (*m*itochondrial *in*ner membrane *o*rganizing *s*ystem), mitOS (*mit*ochondrial *o*rganizing *s*tructure) or MICOS (*mi*tochondrial *co*ntact *s*ite) complex present in yeast, *Caenorhabditis elegans* and mammalian cells [51–58]. This

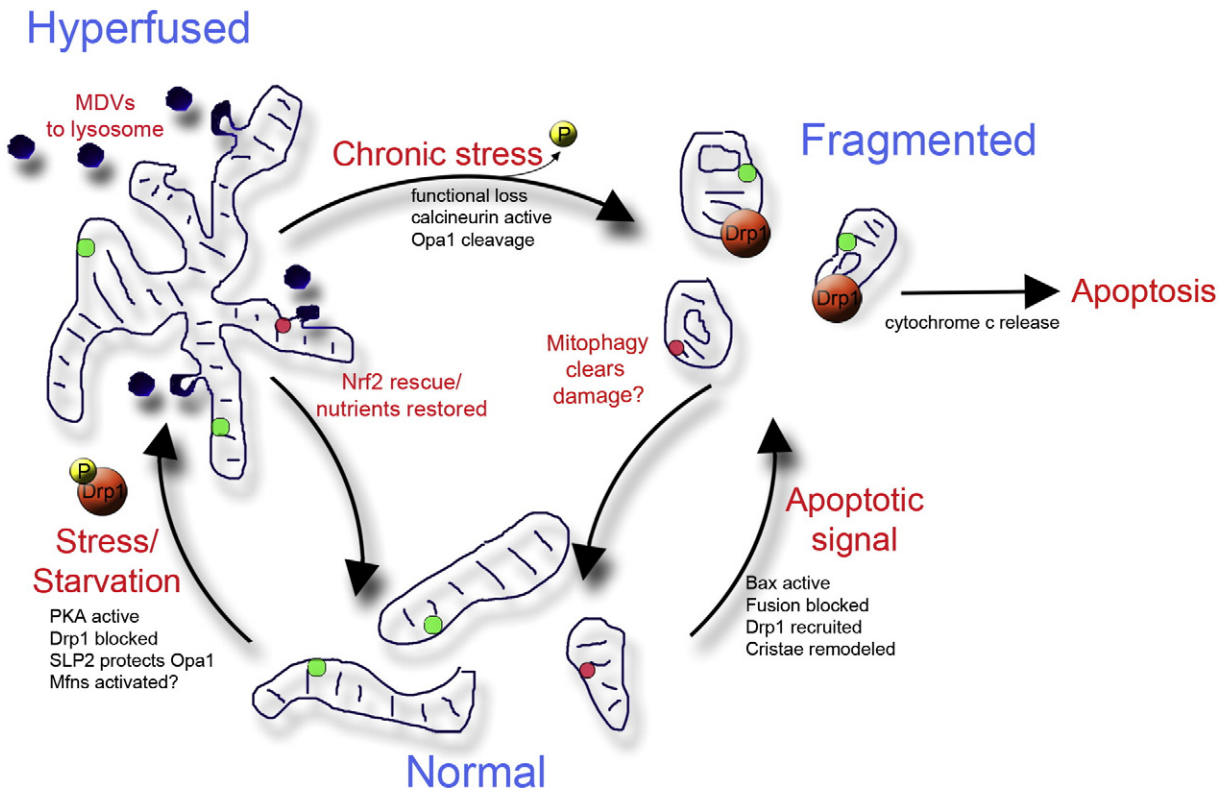

Fig. 1. Working hypothesis: mitochondrial hyperfusion as a stress response. Mitochondria containing green and red nucleoids are shown in the normal state. Green nucleoids contain wild type mtDNA, and the red indicates a heteroplasmic or mutant mtDNA. Upon dysfunction, the organelles containing the mutant genomes may lose potential and become degraded through mitophagy. Upon cellular stress or starvation, the mitochondria undergo hyperfusion. It has been shown that Drp1 becomes hyperphosphorylated and inactivated by PKA during starvation, although this has not yet been demonstrated specifically for all stressors. Slp2 protects Opa1 from cleavage and inactivation during stress, and it is likely that the outer membrane fusion machinery becomes activated in some specific manner. Once in the hyperfused state, damage can accumulate, and we have observed that mitochondrial derived vesicles (MDVs) are stimulated during oxidative stress, carrying mitochondrial proteins to the lysosome. The hyperfused reticulum protects the organelles from mitophagy and the cell from cell death. Rescue from this state could occur through the activation of the master transcription factor Nrf2, which upregulates the expression of antioxidant proteins, or through the restoration of nutrients. High levels and/or duration of cellular stress would ultimately lead to mitochondrial dysfunction. This activates the phosphatase calcineurin, which dephosphorylates Drp1 and activates mitochondrial fission. Should the electrochemical potential become compromised, Opa1 would be cleaved and fusion arrested. Once in the fragmented state, death may follow, or the potential clearance of damaged organelles through parkin-mediated mitophagy could again restore the reticulum. Mitochondrial fragmentation can also be triggered directly upon apoptotic signals. Upon Bax activation, mitochondrial fusion is arrested, Drp1 is recruited and the cristae become remodeled. Together, this leads to cytochrome c release and the execution of the death program. See text for details.

complex comprises a series of proteins that facilitate both cristae assembly and protein import. It has not been established how the MINOS complex may contribute to dynamic functional changes in mitochondrial shape and activity during cellular stress. Both Opa1 and the yeast orthologue Mgm1 are known to regulate cristae junctions [59,60], so it is likely that the oligomerization of these GTPases may play a critical role in the regulation of the MINOS complex. For example, loss of Opa1 oligomers occurs during apoptosis, releasing the cristae junctions to facilitate the exit of intermembrane space proteins [59,61]. The involvement of the MINOS complex in these activities, and potential links to the regulation of inner membrane fusion will certainly be the subject of future work.

Hyperfusion must require the specific activation of the fusion machinery, however it is not yet known how this might occur. The outer membrane fusion GTPases Mfn1 and Mfn2, have not been shown to become post-translationally modified upon stress. They are ubiquitinated by both MarchV/MITOL [62–65] and by Parkin [29–31,66,67], however these conjugates are responsible for their downregulation and degradation by the proteasome, which is not consistent with a pro-fusion function. Another mitochondrial E3 ligase MAPL/MULAN demonstrates inefficient ubiquitination activity in vitro [68,69], however biochemical and functional experiments suggest a primary role in the SUMOylation of mitochondrial substrates, including Drp1 [69]. Ectopic MAPL expression promotes mitochondrial fragmentation and inhibits fusion [69,70], making it an unlikely candidate in the activation of the mitofusins.

Nevertheless, it is likely that the mitofusins will be activated through stress induced post-translational modifications that are yet to be defined. Work in yeast models has demonstrated that Fzo1 may be ubiquitinated during mitochondrial fusion [71,72]. These two laboratories have shown that the Skp/cullin ubiquitin E3 ligase Mdm30 ubiquitinates Fzo1 after GTP hydrolysis occurs. Using a series of tethering assays, biochemical and genetic studies, it appears that Fzo1 undergoes GTP hydrolysis upon mitochondrial docking, and this leads to its ubiquitination and cleavage by the proteasome. The Mdm30 orthologue has not been identified in mammalian systems, and ongoing work continues to explore these possibilities. Finally, mitochondria cannot fuse unless they are moved into close proximity. Links between Mfn2 and the Milton/Miro complex that regulates mitochondrial movement on microtubules also suggest that the activation of motility and fusion may be molecularly coupled as well [73]. At the mechanistic level, there is still a great deal to learn about how the fusion process is regulated.

Mitochondrial hyperfusion can also occur through the global inactivation of the mitochondrial fission machinery. The recruitment of the dynamin related protein Drp1 to the mitochondria is regulated by a series of post-translational modifications including phosphorylation, sumoylation and ubiquitination [74]. The best characterized among these is the role of phosphorylation, particularly in the regulation of Drp1 recruitment during cellular stress. Recent work in two independent labs has elegantly shown that the activation of Protein

kinase A (PKA) during cellular starvation led to the phosphorylation and inactivation of Drp1 [75,76]. This led to an increase in mitochondrial interconnectivity in both cultured cells, and within the muscles of mouse models. Although the process of hyperfusion obviously required the fusion machinery, it was not clear whether this machinery was actively stimulated, or whether fusion resulted due to the ongoing basal fusion activity in starved cells. It has been shown using a cell-free fusion assay that addition of cytosols isolated from forskolin-treated cells led to a two-fold increase in mitochondrial fusion over control [77]. This suggests that in addition to the arrest in mitochondrial fission, the activation of PKA by forskolin also stimulates the fusion process. However, it is not yet known what the molecular basis of this stimulation was.

A fused mitochondrial reticulum has also been associated with two other aspects of cellular physiology, the cell cycle and senescence. Mitochondrial hyperfusion has been observed at G_1S and manipulation of mitochondrial morphology has been shown to affect cell cycle progression [78]. The induction of a fused morphology triggers senescence-associated changes, suggesting that mitochondrial morphology as a whole is coupled to cell fate [79–81]. It is currently unknown whether there are distinct or common mechanisms regulating the hyperfused networks found in response to stress, starvation, cell cycle progression or senescence. Although the initial signals likely differ, at some point the mechanisms must converge to drive the fusion machinery.

4. Why mitochondria fuse?

What is the advantage of mitochondrial hyperfusion during stress or starvation? There are several intuitive answers to this question. First, it was established as early as 2001 that DRP1-deficient cells containing hyperfused mitochondria were resistant to apoptosis [82]. During the ensuing decade, it was revealed that the apoptotic cascade includes both the activation of Drp1 and mitochondrial fission, as well as the inhibition of mitochondrial fusion [83]. The intimate relationship between the mitochondrial shape-shifting machinery and the pro- and anti-apoptotic machinery has therefore been the subject of intense investigation in many different model systems. In one of the most robust examples, it was recently shown that Bax promotes fusion within non-apoptotic cells, however upon Bax activation during a death trigger, Bax then integrates into the membrane and fusion becomes arrested [84]. Therefore maintaining a hyperfused mitochondrial reticulum may protect cells, at least transiently, from death during stress. Indeed, during starvation, blocking the ability of mitochondria to fuse led to cell death, strongly supporting this idea [75]. The timing of the hyperfusion response may also be coupled to the transcriptional response that accompanies cell stress signals. For example, during oxidative stress, the transcription factor Nrf2 becomes stabilized, translocates to the nucleus and acts as a master regulator of anti-oxidant gene expression [85,86]. The expression of genes like superoxide dismutase and glutathione-related protective enzymes then relieve the damage signals, which may reverse the hyperfused phenotype. If the transcriptional response were insufficient, then the damage would accumulate, ultimately leading to mitochondrial dysfunction and fragmentation (Fig. 1). For example, the phosphatase calcineurin has been shown to become active once mitochondria become depolarized, which would dephosphorylate and activate Drp1 and fission [87–90]. Therefore, the hyperfused response provides a strategy to band together until help is called. If left too long, time runs out and the program would move towards fragmentation and cell death.

The second intuitive answer that was alluded to above, is that hyperfused mitochondria are resistant to mitophagy. Particularly during starvation, banding together into an interconnected reticulum would render them too large to engulf [91]. In a number of systems, it has been shown that mitophagy was arrested in cells where mitochondrial fission was blocked [18,24,43]. This clearly indicated that fission is requisite for mitophagy. As a common physiological response to stress, mitochondrial hyperfusion may have evolved, at least in part, to evade the lysosome until nutrients are restored.

A third possibility to explain mitochondrial fusion is the concept of content mixing to rescue, or buffer, the damage that would increase during stress or starvation [92,93]. By sharing lipids, proteins, RNA, and mtDNA, fusion can ensure an even distribution of functional complexes throughout the reticulum. However, this may also have long-term detrimental effects since mixing damaged components could effectively "contaminate" the healthy organelles. In this case, a highly efficient process of fragmentation and mitophagy would be an essential aspect of recovery from the hyperfused state.

Finally, hyperfused mitochondria may be more metabolically efficient. It was observed that the density of cristae per micron of mitochondria was greatly increased upon starvation-induced hyperfusion [75]. Since the cristae house the respiratory machinery, it follows that an increase in their numbers may provide an increased metabolic efficiency when resources are scarce. For all of these reasons (and likely others we have not yet considered), hyperfusion appears to play a protective role in the early stages of cellular stress.

The fusion and fission machinery are highly conserved and ubiquitously expressed, and there is almost no doubt that there is active mitochondrial dynamics within differentiated tissue in vivo. However it must be noted that there is limited experimental evidence that has quantified the extent of content mixing in any given tissue. We know that starvation induces mitochondrial hyperfusion in mouse muscle in vivo, as measured using ultrastructural analysis [75]. It is also clear that the loss of fusion machinery within adult tissue compromises function in a number of organs including heart [94,95], brain [96], skeletal muscle [97] and the pancreatic islet cells [98]. However, a recent kidney specific knock-out of Mfn2 revealed totally normal renal function, unless the cells were exposed to metabolic stress [99]. This suggests that fusion may not be critical for the development and steady state functions of all differentiated cells. One caveat of the inducible and/or tissue specific knock out models is that the fusion machinery plays dual roles, where Opa1 (and the yeast Mgm1) also regulates cristae morphology [59,60] and Mfn2 is required for ER/mitochondrial contact sites [100]. Therefore the phenotypes that result from loss of these two genes cannot be blamed solely on an inability of the mitochondria to fuse. Indeed a new role is emerging for Mfn2 in regulating the ER stress response in a number of different model systems [101,102]. Nevertheless, from all of the emerging data, we would predict that mitochondrial fusion may not play a significant role in steady-state quality control, in fact fusion has been shown to exacerbate mitochondrial damage [24]. Instead, the primary role of fusion is likely in the survival of the cell under various forms of stress. One of the future challenges in the field will be to quantitatively monitor mitochondrial dynamics in differentiated tissue.

5. Avoiding heteroplasmy

It can be argued that the most important component of mitochondrial quality is the integrity of their genome. Since the mtDNA lacks robust repair mechanisms, they are highly prone to the accumulation of mutations with age and accumulated dysfunction [103,104]. There is no mechanism to selectively degrade mutant genomes, rather the idea is that mitochondria containing dysfunctional products of the mutant genomes would be recognized and removed through mitophagy. Consistent with this, Parkin-mediated mitophagy has been shown to rescue heteroplasmy in some cultured model systems [105,106]. Another protein Gimap3 (GTPase of immunity-associated protein 3) was also shown to play a role in heteroplasmic segregation in leukocytes [107], although the mechanisms are not yet clear.

Given the importance of mtDNA integrity, do mitochondrial dynamics play any active role in controlling mtDNA distribution or turnover? To date the links between mitochondrial nucleoids and mitochondrial shape are mostly indirect. Interference with

mitochondrial fusion or fission both lead to a loss in mtDNA [97,108–111], indicating that mtDNA replication is coupled to mitochondrial dynamics. It has also been noted through quantitative confocal microscopy that nucleoids are present in every mitochondrion, inferring a molecular mechanism to ensure their presence prior to a fission event [110]. The nucleoids are attached to the mitochondrial inner membrane which anchors them at specific sites [112,113]. Early studies in yeast suggested that Mmm1, an ER/mitochondrial encounter structure (ERMES) component, may somehow couple both mitochondrial membranes, anchoring the mtDNA in the matrix, to cytosolic actin [114–116]. The mammalian orthologues of these complexes are not yet known, yet it is likely that structures like these regulate the spatial distribution of nucleoids. Integration between these complexes and the activation of mitochondrial fusion or fission could provide a means to precisely control the fate of each nucleoid. Recent data suggests that the inner membrane fusion GTPase Opa1 binds to mtDNA within the matrix, which couples the fusion machinery directly to mtDNA [117].

The anchoring and placement of mtDNA along the inner membrane suggest that the transcription and translation of the gene products also occur very locally at sites of import. This allows the efficient assembly of the large protein complexes that contain proteins arriving from the cytosol and the matrix side. Should mutations accumulate within an individual nucleoid, then it is likely that a "patch" of dysfunctional complexes within the inner membrane may result. When this "patch" eventually becomes segregated through steady-state fission events [24], its dysfunction would be recognized by the mitophagy machinery. In this way, coupling the nucleoids to the membrane ensures the generation of distinct functional (or dysfunctional) domains along the bilayer that may allow their efficient recognition and removal when necessary. As a relatively stable structure, the positioning of the nucleoids would remain local even upon mitochondrial hyperfusion, limiting the spread of heteroplasmic, mutant genomes throughout the reticulum.

6. Mitochondrial derived vesicles and quality control

We recently discovered that the mitochondria have retained another feature common to their bacterial ancestors, the ability to release vesicles into the cytosol [118]. Bacterial shedding of vesicles plays critical roles in numerous aspects of their lives, from quorum sensing to the invasion of host cells [119]. The molecular mechanisms and regulation of this process in bacteria is now under intense investigation as the impact of bacterial vesicles in infection has become more apparent. Over the last few billion years, the mitochondria have co-evolved with an endomembrane system that employs a highly complex system of machinery to facilitate vesicle transport. However, unlike endocytosis and the biosynthetic pathways, there was no reason to believe that proteins would flow between the mitochondria and other organelles. Therefore it had never been considered that the mitochondria may participate in these types of behaviors. This view has now changed since we have shown that vesicle transport occurs between the mitochondria and at least two intracellular destinations. In the most intuitive example, we have shown that mitochondrial proteins are delivered to the multivesicular body/late endosome for degradation using a vesicular transport route [120]. We also demonstrated a direct pathway between the mitochondria and peroxisomes [70,121], although the functional significance of this route is still under investigation. Given that both the mitochondria and peroxisomes play roles in the beta-oxidation of fatty acids, this connection may relate to the common regulation of fat catabolism.

There are a number of implications that arise from this finding. First, the selection of mitochondrial proteins that are expelled from the organelle must be regulated in a manner similar to the mechanisms employed in other vesicular transport events. This means that cargo binding machinery, pinchases, GTPases, etc. must function at the mitochondria. It is clear that mitochondrial vesicles are generated in the absence of the fission GTPase Drp1 [70,120,121], so there must be an additional pinchase that functions in this pathway. Indeed, the diameter of the yeast Dnm1p spirals has been shown to be 100 nm [122], which is too large to constrict the small vesicles with a 20 nm neck. We have identified the retromer complex as critical in the delivery of the mitochondrial SUMO E3 ligase MAPL/MULAN to the peroxisome [121]. We must also identify the mechanisms used to target to specific acceptor compartments, for example tethering factors that can differentiate between the peroxisomes and late endosomes. It will take a great deal of future study to dissect and characterize these pathways.

The most urgent question is to understand the contribution of mitochondrial derived vesicles (MDVs) to mitochondrial quality control. Mitochondrial turnover utilizes at least 3 pathways, mitophagy, proteases and the proteasome, so why bother with a vesicular transport route as well? MDVs are released from intact, otherwise healthy mitochondria, and provide a means to remove protein complexes and lipids in a single structure [120]. MDVs also appear to function independently from Atg5-dependent autophagy, which may be important in conditions when the activation of autophagy machinery is detrimental to the cell. On the other hand, we cannot yet exclude that MDVs are engaged during autophagic programs, as there may be integration among the quality control pathways.

We do not understand how proteins, complexes or lipids are selected for this pathway, rather than engaging the proteasome or proteases within the mitochondria. Vesicles can carry larger, membrane-associated complexes, which may be more easily degraded *en masse* in the lysosome, rather than ratcheting their subunits across a bilayer one by one for proteolysis. In addition, partially disassembled complexes may be dangerous since electron transport may become leaky and disrupted. It is also important to consider the role for MDVs in the degradation of oxidized mitochondrial lipids. Lipid peroxidation accompanies respiration and can become acute during times of stress. The remodeling of lipids like cardiolipin during stress has been studied extensively [123–125]. However, there is very little known about the turnover and degradation of mitochondrial lipids in steady state. We consider that the removal of oxidized lipids may be a central component of the MDV pathway, and we are working towards the reconstitution of MDV budding in vitro in order to better analyze the contents of the vesicles.

When thinking of the potential mechanisms that may trigger cargo incorporation, we look to the established mechanisms described for bacterial membrane shedding. In these cases, the trigger for vesicle formation is often a pH or other environmental change that induces specific periplasmic cargo proteins to oligomerize and form channels or aggregates within the outer membrane [119]. This generates curvature from the inside of the bacteria, and as it grows, the media will slough the vesicle off through shear forces. Clearly there are many differences at play within the mitochondria, however it is possible that protein and/or lipid oxidation could induce some level of aggregation that may be an initial trigger for vesicle generation. These potential aggregates would then need to signal the recruitment of cytosolic factors that would complete the task of budding the vesicle.

Finally, does the existence of MDVs help explain some pending questions in the field? One of the controversies is whether or not mitophagy in yeast requires mitochondrial fission or Dnm1 [126–129]. Depending on the triggers, it is possible that Dnm1-independent clearance of mitochondria could occur through MDVs. So far, it is not known whether MDVs are generated from yeast mitochondria. Although our studies show that MDV transport to the lysosome can occur in the absence of the autophagy machinery, this does not exclude that MDVs may play a role during autophagy. Therefore, future experiments may look at the generation of cargo-selected vesicles during autophagy in yeast in order to explain mitochondrial clearance in the absence of Dnm1.

The second controversy has been described above, which is the question of the role of Parkin in mitophagy in vivo. It is unclear

whether or not individual mitochondria lose potential within tissues. For example, dopaminergic neurons lacking mitochondrial respiration did not recruit Parkin to the mitochondria, even though these neurons died, resulting in a mouse model of Parkinson's disease [130]. One possibility may be that MDVs play a significant role in vivo to extract damaged mitochondrial cargo from organelles that globally retain their electrochemical potential. Again, the challenge is to find ways to quantitatively determine the contribution of each of these pathways in mitochondrial quality control.

7. Conclusions

The field of mitochondrial dynamics has accelerated exponentially with the realization that mitochondrial dysfunction may be more causal to disease than previously thought. We have here discussed the links between the Parkinson's disease genes and the mitochondria. This emerging area of research has provided a compelling model to explain how accumulated mitochondrial damage may ultimately kill dopaminergic neurons in Parkinson's patients. This review has also focused on the new role of mitochondrial hyperfusion as a cellular stress response. An inability to fuse during times of stress was shown to render cells to be susceptible to death, consistent with the identification of mutations in the fusion machinery that lead to degenerative diseases. We have yet to learn whether errors in MDV transport are linked to human disease, as we must first identify the critical components of the pathway. However, it should be noted that one of the retromer subunits Vps35 was recently identified as a new Parkinson's disease gene [131,132]. As a requisite player in MDV transport to the peroxisome, perhaps this link is not coincidental. It is very likely that as the field continues to progress mechanistically, we will gain a clearer understanding of how mitochondrial damage is controlled. When one considers the high levels of respiration in so many tissues, it is no wonder that the mechanisms that control oxidative damage are complex, and include multiple layers of redundancy. The challenges remaining are to focus on the development of tools to improve the efficiency of these quality control pathways. Clearing the mitochondria of damage and mtDNA heteroplasmy is an urgent goal as this will help us find ways to reverse the myriad of degenerative human conditions.

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