Mitochondrial fusion, fission and autophagy as a quality control axis: The bioenergetic view

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The mitochondrial life cycle consists of frequent fusion and fission events. Ample experimental and clinical data demonstrate that inhibition of either fusion or fission results in deterioration of mitochondrial bioenergetics. While fusion may benefit mitochondrial function by allowing the spreading of metabolites, protein and DNA throughout the network, the functional benefit of fission is not as intuitive. Remarkably, studies that track individual mitochondria through fusion and fission found that the two events are paired and that fusion triggers fission. On average each mitochondrion would go through ~5 fusion/fission cycles every hour. Measurement of Δψm during single fusion and fission events demonstrates that fission may yield uneven daughter mitochondria where the depolarized daughter is less likely to become involved in a subsequent fusion and is more likely to be targeted by autophagy. Based on these observations we propose a mechanism by which the integration of mitochondrial fusion, fission and autophagy forms a quality maintenance mechanism. According to this hypothesis pairs of fusion and fission allow for the reorganization and sequestration of damaged mitochondrial components into daughter mitochondria that are segregated from the networking pool and then becoming eliminated by autophagy.

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1. Introduction

Mitochondrial dysfunction is suggested to play a central role in metabolic diseases such as diabetes and in a number of chronic conditions such as Alzheimer’s disease, Parkinson’s disease and aging. In these conditions accumulation of dysfunctional mitochondria leads to oxidative stress and impaired cellular functions [1–4].

Diverse mechanisms enable the turnover of damaged or misfolded mitochondrial proteins. A mechanism that functions inside mitochondria is the inner membrane AAA protease that digests inner membrane and matrix proteins [5]. Turnover of outer membrane proteins may be mediated by E3 ubiquitin ligase system, as in the case of mitofusin-2, implicating the ubiquitin proteasome in processing of mitochondrial membrane proteins and mitochondrial architecture [6]. Finally, turnover of a mitochondrion as a whole organelle is mediated by mitochondrial autophagy or mitophagy [7,8].

While autophagy is commonly considered involved in cell death pathways [9], it has a number of other functions required for cellular adaptation. For example, autophagy allows for adaptive reduction in the mass of organelles as observed in liver cells during the shrinkage of the ER in response to withdrawal of cytochrome P450-inducing drugs. Autophagy also functions as a mechanism to provide nutrients under starvation as seen for example in the hibernating myocardium [10]. Selective elimination of mitochondria by autophagy occurs in specific settings such as erythrocyte maturation [11] and specific targeting of sperm mitochondria during oocyte fertilization [12]. Large scale autophagy of mitochondria has been described during apoptosis where opening of the PTP was found to be essential for mitochondrial autophagy to proceed [7].

Although the molecular flags(s) that specifically targets mitochondria for autophagy has not yet been identified in mammals, autophagy has been associated with the dissipation of mitochondrial membrane potential (Δψm) [7,13–16]. This property makes autophagy a potential housekeeping process that targets dysfunctional mitochondria and maintains the bioenergetic efficiency of the cell. Inhibition of ATG genes in yeast resulted in higher levels of reactive oxygen species (ROS), reduced oxygen consumption, higher mitochondrial mutation rates and severe defects in mitocondrial degradation [17]. A similar phenotype was recently described in clonal (INS1) beta cells showing an increased sub-cellular heterogeneity in mitochondrial membrane potential, impaired oxygen consumption and accumulation of oxidized mitochondrial protein [16]. These observations suggest a rather individualistic and linear model of the mitochondrial life cycle: a mitochondrion gradually deteriorates to the point of Δψm dissipation, at which point it is targeted for recycling by autophagy.

2. Dynamics studies complicate the perceived mitochondrial life cycle

The discovery of mitochondrial dynamics has made this scenario rather too simplistic. Based on recent findings in mammalian models
and yeast, mitochondria exist in networks that are continuously remodeled through fusion and fission [18–21]. Fusion events allow rapid diffusion of matrix proteins [22–27], with slower migration of inner and outer membrane components [22,28].

Time-lapse imaging of mitochondrial fusion and fission indicates that it is a rapid process. Laser mediated photoactivation of matrix-targeted photoactivatable GFP (mtPA-GFP) allows for selective labeling of mitochondria which is then followed by the spread of the photoactivated mtPA-GFP to conjoined, non-photoactivated, mitochondria. When 10–20% of mitochondria within a cell are photoactivated, the probe equilibrates across the entire mitochondrial population within ~45 min [16,24,29].

Given that the turnover of mitochondrial proteins is in the range of hours to days [30], it is predicted that the mitochondrial population within a cell will be homogenous in protein content and, consequently, in function.

In view of these findings, mitochondrial dynamics are expected to impact mitochondrial turnover and thereby the bioenergetic efficiency of the mitochondria population within a cell. The goal of this review is to propose a hypothesis in which the combined functions of fusion, fission and autophagy constitute a quality control mechanism that allows the sequestration, sorting and elimination of functionally impaired mitochondrial components. We address the paradoxical generation of depolarized mitochondria within a constantly mixing population of mitochondria and discuss a mechanism that targets dysfunctional mitochondria for autophagy and not for rescue by fusion with the network. Detailed reviews on the molecular machinery of mitochondrial fusion and fission, protein turnover and autophagy are available in these references [8,31–36].

3. Life cycle of the mitochondrion

Reduced mitochondrial membrane potential (hereafter referred as mitochondrial depolarization) may be the result of a gradual or spontaneous deterioration, or alternatively, may occur as a result of a regulated event. To investigate the events leading to appearance of depolarized mitochondria and the consequent mechanism(s) that target them to mitophagy or metabolic rescue, one must characterize the bioenergetic and biochemical properties of the life cycle of a mitochondrion.

A number of studies indicate that mitochondria go through continuous cycles of fusion and fission [19,20,40]. Although at a first glance this might appear to be in conflict with the tubular, web-like, structures described in HeLa cells and COS7 cells, a more detailed investigation showed that even in these connected webs mitochondria do not make a large continuum but rather continuously rewire the segments through fusion and fission [22,41–43]. Long term single mitochondrion tracking showed that the frequency of fusion events in COS7 and INS1 cells is once every ~5–20 min per mitochondrial [16].

In plants and mammals this behavior has been described as having a pattern of kiss and run, indicating that fusion is a brief event (~100 s in INS1 and COS7 cells) and is characteristically followed by fission [16,27]. As a result, mitochondria spend most of their time in their post-fission state as solitary units before entering a subsequent fusion. It is therefore suggested that the life cycle of mitochondria can be divided into two periods, the pre-fusion period (solitary period) and the post-fusion period when the mitochondrial network is connected to another (networked period).

4. The birth of a depolarized mitochondrion

Depolarized mitochondria may therefore be the result of a spontaneous depolarization during the solitary or networked periods or during the transition between them. A number of studies have reported on the monitoring of individual mitochondria over time and the observation of a specific depolarization event. Loew et al. provided one of the earliest quantitative measurements of individual mitochondria (as judged by imaging with the ΔΨm-dependent dye TMRE) that were tracked in the z-stack with high time resolution. They reported stable mitochondrial membrane potential for a period of 40–80 s that could be followed by a drop of more than 15 mV [44]. This pioneering study was however limited by the lack of technology to assure that the detected mitochondrion did not go through fusion and/or fission events during the recording time. Since fission can occur without movement of the two daughter mitochondria, or involved only the inner (but not the outer) mitochondrial membrane [22,45], it cannot be reliably identified by observation of separation of a mitochondrion into two segments. Similarly, the repositioning of a mitochondrion to become juxtaposed to another mitochondrion is not an indication that a fusion event occurred [22].

The use of photoactivatable proteins overcame a major technical difficulty of imaging individual organelles that move and change morphology within a complex architecture [46–48]. Simultaneous imaging of mitochondria stained with TMRE and expressing mtPA-GFP solved two major problems in monitoring biophysical activity of single mitochondria [22]. The first is the accurate determination of organelle boundaries that can easily be tracked despite movement within a dense mitochondrial architecture. The second is the use of mtPA-GFP with TMRE to derive a ratiometric value for ΔΨm that is independent of the exact focal plane. This approach spares laser radiation needed to image the entire z-axis and thereby reduces phototoxic damage. As a result, the recording period free of phototoxic damage can be extended from minutes to hours.

In COS7, INS1 and primary (β-cells prolonged tracking (up to 2 h) revealed that mitochondria retain a stable ΔΨm during the solitary period [16,49]. During most of the time (95% of the recording period) ΔΨm of the mitochondrion was within ±2.7 mV of its average baseline. This observation indicates that continuous deterioration in ΔΨm during the solitary phase is an unlikely (or infrequent) route for the generation of depolarized mitochondria under normal conditions.

5. Biophysical properties of individual fission events

In contrast to the remarkable stability of ΔΨm under control conditions during the solitary period, fission events are associated with major changes in ΔΨm. Most fission events yield daughter mitochondria with opposite ΔΨm, deflections, usually greater than 5 mV [16]. These observations indicate that while fusion mixes the content of the parent mitochondria, fission generates functionally divergent daughters. In support of functional asymmetry of fission events is data generated by EM tomography showing that NO-toxicity generates asymmetrical daughter mitochondria during fission [50]. The reported membrane structures of the two daughters suggest that the two would have disparate respiratory capacity [51]. Nucleoids that contain mtDNA were also shown to occasionally distribute asymmetrically during fission events [27].

It is unclear if the dissimilarity of the daughters is a result of an active or passive process that sorts active from inactive components and sequesters them to different segments within the mitochondrion. So far there are no data that oppose or support a “mitoskeleton” that would be involved in such function. An alternative possibility would be that the uneven distribution of functional components in the mitochondria is random and is mediated by diffusion.

In contrast to mitochondrial fusion, fission events do not require intact ΔΨm (see next section). This property is supported by numerous studies showing that depletion of ATP either by inhibiting ATP synthase [52,53], collapsing ΔΨm [45,54–56] or inhibiting Na/K ATPase [53] trigger general fragmentation of the mitochondrial web. Indeed, selective tracking of individual mitochondria with sustained depolarization revealed the occurrence of fission events. Remarkably, some
of these events could regenerate a daughter mitochondrion with an intact $\Delta \psi_m$, while the other daughter remains at a sustained depolarized level (unpublished data). Taking together, these data indicate that mitochondrial fission is a central metabolic event in the life cycle of mitochondria, being able to alter their energy state.

6. Fission as the autophagic checkpoint

Since mitochondrial fission occasionally generates depolarized mitochondria it is expected to feed autophagy [7,14,15]. Indeed, knockdown of FIS1 (by siRNA) or overexpression of a dominant negative isoform of DRP1 (DRP1K38A), reduces mitochondrial autophagy [16,50,57]. This reduction is selective to mitochondria and was not accompanied by a reduction in ER mass in autophagosomes (APs), rate of APs formation or in lysosomal mass.

Manipulations in FIS1 and DRP1 expression level were consistent with fission having a role in mitochondrial autophagy. Frieden et al. showed that overexpression of hFis1 reduced selectively the mitochondrial (but not ER) mass, consistent with fission increasing mitochondrial autophagy [60]. Arnoult et al. showed that overexpression of DRP1 facilitated mitochondrial elimination under various pro-apoptotic stimuli [57]. Qualitative evidence linking mitochondrial morphology and mitophagy was obtained in several studies in the CNS. In neurons, exposure to nitric oxide donor (50–200 µM of SNOC) resulted in fragmentation of the mitochondrial web, increased fraction of structurally damaged mitochondria and selective increase in mitochondrial mass in APs [50]. A similar relationship between mitochondria fragmentation and autophagy is found in Alzheimer’s disease where a chronic and progressive oxidative stress correlates with mitochondrial fragmentation that is accompanied by a selective increase in mitochondrial mass in APs [61–63]. Common to these observations is a stressor-induced mitochondrial fission and fragmentation, and a selective increase of mitochondrial localization in autophagosomes.

Mitochondrial fission is more likely to be permissive for autophagy since only a minor fraction of fission events yield daughter mitochondria that will be eliminated by autophagy. For example, in an unstressed individual beta cell, a population of 300 mitochondria will generate 500–1000 fission events per hour, but less than 100 mitochondria-containing autophagosomes per hour. That reduction in mitochondrial size is not sufficient to induce autophagy is also exemplified by conditions of OPA1 overexpression which result in reduced mitochondrial size as well as reduced autophagy [58,59].

This raises the hypothesis that mitochondrial fragmentation, which can be induced by various insults, is a common stress response that permits the segregation and elimination of dysfunctional mitochondria from the web. However, the function of fusion as a rescue mechanism may not allow efficient segregation of dysfunctional mitochondria from the networking population. The emerging question is how mitochondrial fusion relates to depolarization and whether it acts to rescue damaged mitochondria.

7. Is fusion a rescue mechanism?

Mitochondrial fusion was suggested as a complementation mechanism through which mitochondria compensate for certain metabolic depletions by transferring soluble as well as membranous components. While ample data supports that fusion allows for the diffusion of multiple components between the involved mitochondria [20,27,28], there is no evidence to indicate that this process functions to rescue mitochondria that are bioenergetically compromised. Several studies revealed the existence of potential mechanisms that would prevent fusion of mitochondria when mitochondrial membrane potential is dissipated by an uncoupler [45,54,55,64–66].

8. Fusion is a selective and primarily exclusive mechanism rather than a rescue one

While the above observations conclude that depolarized mitochondria do not fuse with each other, more recent studies determined whether depolarized mitochondria can fuse with those that have intact $\Delta \psi_m$. Simultaneous tracking of fission and $\Delta \psi_m$ shows that
depolarized mitochondria generated during fission events are 6 times less likely to become involved in a consecutive fusion event within the next 10 min as compared to their sisters generated during the same events (“fission-mate”) [16]. This finding suggested that depolarized mitochondria may remain in the cell as non-fusing mitochondria. By selectively tagging in vivo a group of mitochondria with mtPA-GFP and observing them over time, non-fusing mitochondria can be identified as those that fail to dilute their mtPA-GFP. Co-staining with TMRE or OPA1 antibody revealed that non-fusing mitochondria are depolarized by ~7 mV compared to average Δψm and have approximately 50% less OPA1 compared to the rest of mitochondrial web [16]. The biochemical mechanisms that link OPA1 processing to bioenergetic parameters have been studied by a number of groups. Herlan et al. were the first to show that mgm1 (OPA1 equivalent in yeast) undergo splicing (also referred as alternative topogenesis) to s- and l-isofoms in an ATP-dependent manner [67]. Indeed, consequent studies in mammalian models revealed that the l-isofoms of OPA1 undergo cleavage (or degradation) under mitochondrial depolarization or depol- leton in ATP [68–71]. Since both l- and s-isofoms of OPA1 are pre- requisite for mitochondrial fusion [70], a decrease in the driving force for ATP synthesis (i.e., Δψm, depolarization) triggers mitochondrial fragmentation by regulating OPA1 isoforms. A number of proteases have been implicated in OPA1 cleavage, including Rhd1/Pcp1 in yeast and presenilin-associated rhomboid-like protease (PARL), m-AAA Paraplegin, and AFC3L2 proteases in mammals [69,72,73]. These proteases have been found to be regulated both by ATP levels and by Δψm, implicating mitochondrial energetic status as a regulator of OPA1 processing. Recent studies by Baricault et al. have shown that OPA1 processing in mammalian cells may involve direct interaction of OPA1 with respiratory chain complexes [74,75]. Taken together, these findings suggest that mitochondrial fusion is primarily a selective and exclusive process rather than an unselective rescue mechanism. Remarkably however, certain mtDNA mutations have been shown to spread across the mitochondrial network through fusion events. In a heteroplasmic cell, exchange of mitochondrial DNA may allow for complementation and recovery of function [76]. Ono et al. isolated two types of respiration-deficient cell lines with pathogenic mutations in mitochondrial tRNAlle[Leu(UUR)] genes from patients with mitochondrial diseases [77]. Following PEG induced fusion of the two cell lines, the coexistence of their mitochondria within hybrids restored their normal morphology and respiratory enzyme activity. These observations do not necessarily contradict the selective fusion hypothesis since the effect of each of these mutations on mitochondrial membrane potential and OPA1 is unknown, and it is unclear if fusion of depolarized mitochondria occurs in these cells. Future studies need to unravel why and how under certain circumstances mtDNA inter-mitochondrial complementation compensates for the mutated mtDNA in a manner that skips the selective fission.

9. Fusion, fission and autophagy as a bioenergetic quality control mechanism

The observation that mitochondrial fusion is a Δψm-dependent process ensures that potentially dysfunctional organelles will avoid fusion, while intact mitochondria benefit from sharing of metabolites. Segregation of dysfunctional mitochondria from the fusing population shifts their balance from fusion to fission resulting in the generation of small and depolarized mitochondria. The finding that autophagy targets depolarized mitochondria places autophagy at the end of the axis of quality control as a receiver of the segregation output. Fig. 1 summarizes schematically how the combination of fusion, fission and autophagy acts as a quality control mechanism. This view suggests that the absolute rate of fission events per se (and not merely the balance between fusion and fission) determines the efficacy of the proposed quality control axis. The higher the fission frequency, the higher is the probability that dysfunctional units will be segregated and eliminated. In this respect it should be emphasized that while in the absence of stress inducers most fission events occur as part of a fusion–fission cluster [16,27], under stress, fission events will occur independently of fusion events. This may facilitate the segregation of damaged mitochondria in conditions that are characterized by increased damage to mitochondria or those that require adaptation of mitochondria through positive selection.

10. Enrichment arm of the quality control mechanism — a theory

Thus far we have been describing the selectivity characteristics of fusion as a key component of the quality control axis. However a number of observations suggest the possibility that mitochondrial fusion might play an active role in the quality control. Although fusion allows for the equilibration of mtPA-GFP between the fusing pair of mitochondria [22,24], membranous components and DNA do not necessarily become equilibrated [27,50]. Therefore, fusion may lead to functionally uneven distribution of components involved in respiration and to the generation of dissimilar daughter mitochondria. The daughter that ends up with higher content of impaired components may become depolarized and therefore segregated from the networking population, then removed by autophagy. Parallel to removing a daughter enriched in impaired components, such process would also result in the enrichment of the remaining daughter with undamaged material. The hypothesis of enrichment by redistribution, fission and autophagy offers a mechanism for the maintenance of the bioenergetics efficiency of a cell and may function to prevent aging related deteri- roration. However, a number of key points are yet to be studied and assessed. These include the mechanism by which mitochondria redistribute material in an uneven manner, prior to fission. This process might be stochastic or may involve active sorting of material between the two ends of a mitochondrion tubular structure. Moreover, it is yet unclear why this mechanism would not work in a number of mitochondrial diseases where heteroplasmasy remains stable with age even when the mutation is compromising the respiratory function and can lead to cellular death. Future investigation on the mechanisms underlying fission-induced asymmetry is a key for testing additive pathways of how mitochondrial fusion benefit mitochondrial metabolic quality.

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