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Organellar vs Cellular Control of Mitochondrial Dynamics

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Abstract

Mitochondrial dynamics, the fusion and fission of individual mitochondrial units, is critical to the exchange of the metabolic, genetic and proteomic contents of individual mitochondria. In this regard, fusion and fission events have been shown to modulate mitochondrial bioenergetics, as well as several cellular processes including fuel sensing, ATP production, autophagy, apoptosis, and the cell cycle. Regulation of the dynamic events of fusion and fission occur at two redundant and interactive levels. Locally, the microenvironment of the individual mitochondrion can alter its ability to fuse, divide or move through the cell. Globally, nuclear-encoded processes and cellular ionic and second messenger systems can alter or activate mitochondrial proteins, regulate mitochondrial dynamics and concomitantly change the condition of the mitochondrial population. In this review we investigate the different global and local signals that control mitochondrial biology. This discussion is carried out to clarify the different signals that impact the status of the mitochondrial population.

Keywords

Mitochondrial Dynamics; Cell Cycle; Autophagy; Fusion; Fission; mtDNA; Mitochondrial Movement; Bioenergetics; Mitophagy

Introduction

Mitochondria exist as a dynamic network within living cells, undergoing fusion and fission events that facilitate inner and outer membrane fusion and the exchange of organelle contents such as solutes, metabolites, proteins, and mitochondrial DNA (mtDNA)[1-6]. Processes of fusion and fission are necessary for many cellular functions including mitosis, fuel sensing, ATP production, autophagy, and apoptosis [6-11]. Mitochondria are evolutionarily derived from the endosymbiotic relationship of prokaryotic bacteria within eukaryotic cells. Although the coexistence of these two organisms have become genetically integrated through natural selection, the individual mitochondrion is still in many ways its own organism [12-14]. In this review we explore the dynamic relationship between “global” cellular and “local” organelle specific control of individual mitochondria, and the implications of these controls on the mitochondrial population.

Mitochondrial proteins are encoded both by nuclear and mtDNA, however all mitochondrial proteins can be post-translationally modified, degraded, oxidized, or otherwise altered by

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conditions specific to the individual organelle. This process can be described as “local” control which refers to the effect of micro-environmental changes at the level of the individual mitochondrion that affect fission and fusion events. Local control can affect the capacity of the individual mitochondrion to function in energy production, sequester apoptotic stimuli, or propagate mtDNA. For example, loss of mitochondrial membrane potential ($\Delta\psi_m$) can lead to the isolation of the mitochondrial unit from the mitochondrial network, and increased probability of autophagic degradation [6;15].

In contrast to “Local” controls, “Global” controls are mechanisms that can either override or monopolize mechanisms of local control to alter the dynamic status of mitochondria in the entire cell. The cell cycle is a prime example of global control. A key phase of the cell cycle is G1-S mitochondrial biogenesis which is controlled by nuclear transcription factors PGC1 α , PPAR α , NRF1/2, and ERR α [16] [17]. Activation of these transcription factors leads to increased mitochondrial mass, respiratory capacity and energy production required during the etropic S phase of cell division [17]. Observational findings during the cell cycle have demonstrated that individual mitochondria act in a concerted manner at different stages of the cell cycle [17-20]. Specifically, network hyperfusion was found to occur at G1-S phase and be required for progression to S phase [16]. Additionally, hyper fragmentation of the mitochondrial network begins in S Phase [20]. Lastly, fragmented mitochondria localize to opposite telomeres of daughter mitochondria in M phase suggesting concerted movement and dispersion between daughter cells. A depiction of this process as well as the roles of global and local control can be found in Figure 1.

Regulation and Monitoring of Dynamic Events

Recent findings have begun to elucidate the life cycle of a mitochondrion. It has been shown that mitochondria exist in networked and solitary phases within the cell, undergoing cycles of fusion and fission in response to a variety of stimuli [6;10;16;21-24]. Additionally, mitochondria can be subjugated from the dynamic life cycle and targeted for degradation by the autophagosome. [6;25;26]. Biophysical and morphologic parameters can be monitored for extended periods of time using mitochondrially targeted photo activatable GFP (mtPA-GFP) [27-29]. Real time tracking of fluorescently-labeled individual mitochondria enables quantification of size, motility, shape, $\Delta\psi_m$ as well as temporal properties of fusing organelles. Gerenser and Nicholls have developed the Optical Flow Method (OF) by which mitochondrial movement, redox status, and $\Delta\psi_m$ can be correlated using imaging techniques [30]. These experiments confirm that mitochondria exist in two states, networked and solitary, and that fusion and fission events are correlated to the bioenergetic state change of individual mitochondria.

In several cell types prolonged tracking (up to 2 hrs) of individual mitochondria demonstrated the organelle's ability to maintain a stable $\Delta\psi_m$ during the solitary period [6;15;27]. During most of that time (95% of the recording period) the $\Delta\psi_m$ of the individual mitochondrion was within ± 2.7 mV of its average baseline. This observation indicates that continuous deterioration in $\Delta\psi_m$ is an unlikely (or infrequent) route for the generation of depolarized mitochondria under normal conditions. The majority of fission events yield heterogeneous daughter mitochondria with opposite $\Delta\psi_m$ deflections, usually greater than 5 mV. Probability analysis has found that the depolarized daughter mitochondria are 6 times less likely to rejoin the mitochondrial network through fusion [6]. Non-fusing mitochondria can be identified using a procedure in which a small group of mitochondria are tagged by laser photoconversion of matrix-targeted mitochondrial photoactivatable green fluorescent protein (mtPA-GFP) and observed over time. These mitochondria fail to dilute their activated mtPA-GFP through fusion events. Co-staining with the membrane potential dependent dye TMRE revealed that non-fusing mitochondria are depolarized by ~ 7 mV

compared to average $\Delta\psi_m$, while co-staining with an OPA1 antibody, that distinguishes the various isoforms, shows that such mitochondria contain approximately 50% less total OPA1 levels compared to the typical mitochondria [6]. This identifies inhibition of fusion as a selective process or initial checkpoint that generates depolarized mitochondria. Characteristics of these depolarized mitochondria make them significantly less likely to fuse with the mitochondrial network and subsequently more likely to be degraded by autophagy [6]. The importance of these events to organelle functionality make the control by local and global signals of particular interest. A summary of the regulation steps discussed below and the appropriate references can be found in Table 1.

Factors Affecting Mitochondrial Fragmentation

In eukaryotic cells, mitochondrial fission requires local organization of FIS1 and recruitment of GTPase DRP1 for assembly of the fission machinery that subsequently leads to membrane scission [10;31-35]. Global cellular signals related to energetic status, mitosis, and cell death have the capability to affect fission. However, the recruitment, organization and assembly of these proteins ultimately can be regulated by the individual mitochondrion. Complete understanding of the regulation of this process has yet to be determined; however several levels of global and local control have been elucidated.

Secondary modifications of proteins and global ion signals have been shown to be strong global regulators of the fission machinery. DRP1 recruitment to the mitochondrial membrane has been found to be a calcium dependent process [34;36]. Dephosphorylation of cytosolic DRP1 by calcineurin has been shown to cause mitochondrial localization of DRP1 [21]. Both DRP1 and FIS1 have been found to interact with BCL-2 family members and thus globally regulated by apoptotic and anti-apoptotic machinery [18;36-39]. Additionally, DRP1 levels have been found to be regulated by sumoylation and other posttranslational modifications affecting mitochondrial fission [40;41]. The cell cycle has been found to cause uniform changes in the fission state of mitochondria, and in particular hyperfragmentation has been noted at the stage of M phase [17-19].

Local control of fission at the level of the individual mitochondrion appears to be largely regulated by the energetic status of the mitochondrion itself. Loss of membrane potential and ATP production can lead to OPA1 processing and mitochondrial fragmentation due to inhibition of its fusion activity [42-44]. Several studies have investigated OPA1 processing as a key regulator of mitochondrial fragmentation [18;31;44-46] [44]. OPA1 processing is dependent on membrane potential, metal ion levels, and ATP, and proteases PARL and Yme1 identified as key players, although the precise steps of this process still need to be elucidated [42;43;46-51].

Current understanding suggests three key steps to mitochondrial fragmentation: a) organization of FIS1 proteins around a fission site [52]; b) recruitment of DRP1 from the cytosol to the FIS1 sites [34]; c) inhibition of fusion in response to local processing of OPA1 in individual mitochondria. It should be noted however that inhibition of fusion has not been found to induce fission directly; however it does induce membrane fragmentation suggesting the importance of fusion/fission balance [32;33;53]. Of particular interest to the theory of local control of fission is that the energetic status of the individual mitochondria as dictated by Ca^{2+} , membrane potential, and ATP levels are key regulators of mitochondrial fragmentation [44;46;49;50;54]. These findings suggest that in most cases generation of a depolarized mitochondrion, a process regulated by fission, is therefore impacted by energetic status [55-57]. In this model fission events represent a checkpoint for mitochondrial depolarization and degradation by mitophagy. This suggests that through fission the cell has evolved a mechanism for “programmed organelle death” on a daughter

mitochondrion, a concept previously discussed in the literature[58;59]. Therefore, despite cellular control of the fission stimuli, the individual mitochondrial fission event is a locally controlled checkpoint for the generation of polarized and depolarized mitochondria. However, since fission events can also produce daughter mitochondria with equivalent membrane potential that interact normally with the mitochondrial network the fission event itself is not the key regulation [27;32;33]. Rather the process that allows fission to selectively generate a depolarized daughter mitochondrion and subjugate damaged or ineffective components will be the key regulation step. This area of research is of great interest and should increase our understanding of the regulation of mitochondrial dynamics

Local and Global Control of Mitochondrial Fusion

While fission can generate depolarized daughter mitochondria, it is fusion or rather lack of fusion of depolarized mitochondria back to the network that relegates it for degradation. The precise signal that initiates mitochondrial fusion has not been elucidated, although it is likely that it is initiated by both local and global signals. Fusion events allow rapid diffusion of matrix proteins and ions [8;32;33;60-62], with slower migration of inner and outer membrane components and mtDNA nucleoids [3;5;27;63]. Fusion events can be separated into two categories transient (outer membrane only) and complete (inner and outer membrane) fusion. Recent evidence suggests that these two types of fusion have alternate stimuli and purpose, however both play an important role in mitochondrial function [2;22].

In mammals, mitochondrial dynamic proteins MFN1, MFN2, and OPA1 have been shown to be critical for mitochondrial membrane fusion [18;64-68]. Further processing of these proteins, in particular OPA1, by proteolytic processing and interactions with BCL-2 family members has been found and speculated to affect fusion events and alter mitochondrial morphology [37;42;46;50]. Several examples of global control of this function exist. The proapoptotic protein BID was found to interact with OPA1 at the cristae junction and impair fusion [42;69]. Transcriptional stimulation of mitochondrial fusion by PGC1 is performed through selective induction of Mfn2 mRNA transcripts [10]. The cell cycle has been found to cause uniform changes in the fusion state of mitochondria, and in particular hyper-fusion has been noted at the stage of G1-S [18-20;37;70]. The local environment may regulate not only fusion itself but the type of fusion, transient or complete, as OPA1 processing could disrupt its interaction with MFN1 and block complete fusion [42;43;46]. Similarly GTP levels and processing of OPA1 by energy dependent proteases has been found to prevent transient fusion, complete fusion, or both depending on the conditions [42;44;45;49-51;71]. This suggests, as with fission, that fusion events have multiple regulation steps and it is a process regulated by energetic status. For a detailed review of the mitochondrial fusion proteins and their regulation see the following references [21;31-33;35;72].

It has been suggested that fusion provides a restorative function to depolarized mitochondria[32]. Fusion equilibrates matrix components as well as membrane potential of fused mitochondria in a range of several seconds [7;27;28;60]. Tracking of individual mitochondria demonstrated high probability for fission events briefly after fusion events, suggesting that fusion events are key regulator of the rate of dynamics [15;22;27]. Furthermore, tracking of fission and ψ_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 minutes as compared to their sisters generated during the same events ("fission-mate")[6]. This finding suggested that depolarized mitochondria may remain in the cell as non-fusing mitochondrion. Importantly, once the fission event has generated a depolarized mitochondrion, this depolarized mitochondrion meets a second checkpoint at the level of fusion. The ability of a mitochondrion to re-fuse to the network is a key determinant of its fate. At this checkpoint local responsiveness of the individual

mitochondrion to regulation of its fusion machinery can determine whether the mitochondrion rejoins the network or is relegated for autophagic processing. Thus, the fusion event establishes a secondary checkpoint for the fate of the individual mitochondrion.

Control of the Solitary Mitochondrion

Mitochondria spend the majority (Over 90%) of its life cycle as a solitary organelle [73]. In the solitary phase of the mitochondrial life cycle, the mitochondria does not directly participate in dynamic events. Of particular interest is what affects the length of this period, and how that impacts the rate of mitochondrial dynamics.

There is evidence to suggest that association of mitochondria with cytoskeleton and mitochondrial motility has a strong influence on the period of time spent between fusion events. Since fusion is initiated by tethering of MFN1/2 proteins on the outer membrane [31;47;48;66;67;74], movement may facilitate the chance for proper alignment between the engaging mitochondria. The work of *Yi et al* suggest that mitochondrial motility can be regulated by both calcium and ATP levels [75]. These results again point to energy status (ATP levels) as well cellular signals (Ca^{2+}) as regulators of individual mitochondrial motility and thus perhaps dynamic rate [22]. In H2c9 cardiomyoblasts motile mitochondria have higher chance to fuse compared to static organelles. Mitochondria were shown to travel along microtubules by association with dyneins and kinesins [76;77]. Specifically, mitochondria in neurons have been found to travel both anterograde and retrograde along axonal projections in a mitochondrial membrane and ATP production dependent manner [75;78;79]. Mitochondria with high membrane potential and elevated ATP production are directed to sites of vesicular release and ATP demand while depolarizing mitochondria are returned to the soma [76;80]. Additionally an increase in global ADP levels has been found to drive mitochondrial movement toward synapses [81]. Furthermore, the Ga12 receptor found on mitochondria regulates mitochondrial movement in a GTP/GDP dependent manner [82]. Recent work by *Gerencser and Nichols* using OF method of tracking mitochondrial movement suggests that mitochondrial movement can be directly impacted by energetic and redox status. In neurons, mitochondria with low $\Delta\psi_m$ travel more rapidly than those with high $\Delta\psi_m$, and are more likely to travel retrograde away from sites of high ATP demand [30]. Additionally high oxidative potential in individual mitochondria exhibit a loss of $\Delta\psi_m$. This suggests that oxidative damage may affect MMP and therefore movement of mitochondria. These results suggest that individual mitochondria have selective movement based on energy levels. This regulation of the solitary phase of the mitochondrial life cycle is likely to directly affect the rate of mitochondrial dynamics. Thus, stimuli that alter the energetic status of individual mitochondrion exert selective pressure not only during the networked state and during fission but also during the solitary phase.

Mitochondrial Dynamics and Mitochondrial DNA

Fusion and fission allow for diffusion and redistribution of mtDNA and reduces the heterogeneity in mtDNA content between mitochondria within the cell [3;4;31;32;35;62;83]. Increased heterogeneity in mtDNA content has been correlated with several aging related diseases [35]. Our recent findings as well as the findings of others suggest that fission is an initial step in quality maintenance that ends up with the removal of bioenergetically compromised mitochondria by autophagy. [6]. This suggests that mtDNA mutations that lead to respiratory dysfunction and loss of $\Delta\psi_m$ would be selectively excluded from the mitochondrial network by fission and subjugated for autophagy. This process results in the removal of bioenergetically compromising mutations and offers a mechanism of natural selection. In support of this idea, it has been shown that mtDNA nucleoids tightly regulate their genetic material and do not freely exchange between nucleoid units [5]. This infers that

mtDNA nucleoids constitute the basic mitochondrial unit, and that mutations within nucleoids can be propagated or eliminated based on degradation or proliferation of the mitochondrial unit. As we discussed mitochondrial dynamics are linked to the degradation and production of mitochondria within the cell and thus the global and local control of this process has the potential to impact mtDNA mutation and overall cellular mtDNA heteroplasmy. However, the duration between fusion events (5-30 minutes) is significantly shorter than the half life of most of the respiratory chain subunits. Therefore, it is likely that inhibition of mitochondrial fusion for durations that exceed the lifespan of respiratory chain components (range of days) may be essential for producing a bioenergetic phenotype from mtDNA mutations and deletions; thereby allowing their sequestration and elimination. It is therefore possible that adaptation to a new environment could benefit from an extended period where fusion is inhibited and the mitochondrial network is fragmented.

Future Topics

Based on the quantifications of fusion and fission events, time spent in networked and solitary phases, specific rates of fusion/fission the total number of events in the cellular life cycle can be predicted. This could imply that the cell evolved a required amount of fusion/fission events for each life cycle. This number may be a function of the need for complementation and homogenization of mitochondrial components in preparation for cell division or the need for proper dispersion of mtDNA copies. The matrix PA-GFP diffusion assay is a method for quantification of this, as it measures the time it takes for matrix contents to be equilibrated across the cell through mitochondrial dynamics and the number of dynamic events required for this process. Additionally, the OF method developed by Gerschlager and Nicholls can be used to quantify organelle velocity offering an accurate method for quantification of the solitary period and mitochondrial movement [30]. Interestingly, primary cells, which are less proliferative than their differentiated counterparts, display a slower rate of matrix diffusion [38]. This finding may implicate the rate of mitochondrial dynamics and thus diffusion as intrinsic parts of the cellular life cycle. As the mechanisms that regulate mitochondrial dynamics, both at a local and global level, become elucidated, it will be important to determine the impact of modulation of these factors on the ability of the mitochondria to network and properly diffuse components. The cell cycle exerts global control which may up or down regulate fusion/fission, yet ultimately individual events are responsive to the local mitochondrial status. Therefore in future work it will be critical to examine diffusion and dynamic event rates in response to alterations in local and global control mechanisms.

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Abbreviations

	Mitochondrial membrane potential
Bcl-2	B-cell Lymphoma -2
Bid	BH3 interacting domain
Drp1	Dynamin related protein 1
ERR	Estrogen related receptor alpha
Ga12	GPCR a 12
mtDNA	Mitochondrial DNA
mtPAGFP	Mitochondrial photo activatable green fluorescent protein
MFN1/2	Mitofusin 1 and 2
NRF1/2	Nuclear respiratory factor 1/2
OF	Optical flow method
OPA1	Optic atrophy protein 1
PARL	presenilin-associated rhomboid-like
PGC1	PPAR gamma coactivator 1 alpha
PPAR	Peroxisome proliferation activated receptor alpha
TMRE	tetramethylrhodamine ethyl ester
Yme1L	yme1-like protein

Figure 1. Mitochondrial Life in the Cell Cycle

This diagram depicts the normal life cycle of an individual mitochondria during the G₀ phase of the cell cycle. The mitochondrion undergoes fusion, fission, depolarization, and degradation by autophagy. This process is depicted as one of local control where by mitochondrial events are largely dictated by the local energetic status and associated local signals. During the cell cycle Global signals cause concerted changes in the mitochondrial population, as noted by Hyperfusion in G₁-S and fragmentation during M phase. These global population effects are governed by the cells demand for energy required by cell division and the need for homogenization and sequestration of cellular components during met-phase. The cell cycle serves as an elegant example of the parities of local and global control.

Table 1
Regulation of Mitochondrial Dynamics

	Control Mechanism	Outcome	Reference
Fission	<i>Global Cellular Control</i>		
	Ubiquitination of DRP1 (MARCH V)	Inhibition of Fission	[84]
	cAMP Dependent Phosphorylation of DRP1	Inhibition of Fission	[85]
	Calcium dependent recruitment of DRP1 from Cytosol	Fission induced by global calcium	[34] [84]; [36]
	Dephosphorylation of DRP1 by Calcineurin regulates translocation to mitochondria	Fission induced elevated cytosolic calcium	[85] [86]
	Increase of Bax/Bak induces Fission. Bax and Bak in healthy cell controls fusion through Mfn2	Interacts at mitochondrial fission sites to promote fission	[37;38]
	Sumoylation of DRP1 at fission sites	Sumoylation protects DRP1 from degradation and allows fission to proceed	[40] [41]
	<i>Local Organelle Control</i>		
	Loss of Membrane potential leads to OPA1 processing by metalloproteases	Increased OPA1 processing inhibits fusion of individual mitochondrion	[46] [50] [51] [45]
	Increased OPA1 processing in response to local membrane potential and ATP levels	OPA1 processing inhibits fusion and induces mitophagy	[6;44;50]
Fusion	<i>Global Cellular Control</i>		
	Activation of PGC1a/PGC-1b/ERRa induces MFN2 mRNA. PGC-1b induces mitochondrial fusion through Mfn2.	Increased Fusion/Mitochondrial Biogenesis	[87] [10]
	BID disrupts the OPA1 cristae junction complex	The size of cristae junction is regulated independently of OPA1 mitochondrial fusion activity	[88]
	Mito tubularization and network fusion at G1-S of cell cycle.	G1-S stimulates global mitochondrial fusion, mitosis stimulates fission	[16;20] [19]
	<i>Local Organelle Control</i>		
	Functional interaction of OPA1 with MFN1.	Functional interaction of Mfn1 and Opa1 but not Mfn2.	[43;89]
	OPA1 processing by Metalloproteases blocks fusion	Fusion inhibited at the local level by protease activity	[50], [51], [45] [89] [44]
	Low Local GTP level induces outer membrane tethering	Initial fusion promoted in energy deficient environments	[71]
	High Intra-mitochondrial GTP levels required for inner membrane fusion	Complete fusion regulated by energetic status	[71]
	Mitochondrial movement on microtubules is essential for fusion	Inhibition of movement arrests fusion	[24]
Solitary Period	<i>Global Cellular Control</i>		
	Increase of cellular calcium increases mitochondrial motility and fusion (Miro)	Release of cellular calcium stores increases mitochondrial movement	[75] [90]
	Global ADP levels increase Mitochondrial movement to synapses	ADP signals mitochondrial motility	[81]
	GPCR Ga12 is expressed in mitochondria and regulates motility	GPCR sensitive to GDP/GTP levels regulate mitochondrial motility	[82]
	<i>Local Organelle Control</i>		

Control Mechanism	Outcome	Reference
Local ATP level/membrane potential regulates motility	Increased energetic capacity increases movement	[75]
Mito movement along Microtubules occurs in an energy dependent manner	Individual mitochondria move at different rates along microtubules based on ATP levels	[78] [79]; [75]
Local redox status of Mitochondria impacts MMP and Velocity of movement	Elevated Oxidation leads to Loss of MMP. Loss of MMP leads to increased motility	Gerenscer and Nicholls 22