Mitochondrial Dynamics: The Intersection of Form and Function

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Abstract

Mitochondria within a cell exist as a population in a dynamic morphological continuum. The balance of mitochondrial fusion and fission dictates a spectrum of shapes from interconnected networks to fragmented individual units. This plasticity bestows the adaptive flexibility needed to adjust to changing cellular stresses and metabolic demands. The mechanisms that regulate mitochondrial dynamics, their importance in normal cell biology, and the roles they play in disease conditions are only beginning to be understood. Dysfunction of mitochondrial dynamics has been identified as a possible disease mechanism in Parkinson's disease. This chapter will introduce the budding field of mitochondrial dynamics and explore unique characteristics of affected neurons in Parkinson's disease that increase susceptibility to disruptions in mitochondrial dynamics.

2.1 Introduction

Mitochondrial Dynamics refers to the observation that mitochondria within the individual cell go through fusion and fission events. Visually, this results in a morphological spectrum with contrasting degrees of elongation and fragmentation. Plasticity bestows the adaptive flexibility needed to adjust to changing cellular stresses and metabolic demands. Constant network remodeling also establishes a mechanism for quality control of the mitochondrial population with important ramifications for long-term function and health.

While our understanding of mitochondrial dynamics is just beginning, descriptions of morphological transitions by mitochondria can be traced to reports dating back nearly a century. In 1914, Lewis and Lewis elegantly describe witnessing fusion and fission events along with an incredible range of structures exhibited by mitochondria in cultured cells (Lewis and Lewis 1914). The body of knowledge surrounding mitochondrial dynamics has expanded greatly from these early studies and this chapter provides a brief introduction into this exciting field.

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2.2 Mitochondrial Dynamics Proteins

The state of balance between four dynamin-related proteins essentially controls mitochondrial fusion and fission. From yeast to humans, these highly conserved enzymes share homologous GTPase and transmembrane regions that form complexes and alter the curvature of the mitochondrial membranes. It is the relative activities of oppositional forces that together determine mitochondrial morphology. Complete network fragmentation can result from increased expression or activation of fission proteins. However the mitochondrial network will also fragment if fusion activity is inhibited. Similarly, elongated tubular networks occur with enhanced fusion activity as well as through blockage of fission. These extremes are reminders that antagonism between counteracting enzymatic forces sets the shape of mitochondria and therefore must always be considered simultaneously when deciphering network morphology.

2.3 Fusion

Fusion is categorized into two forms and three mitochondrial localized GTPases control the difference between transient and complete fusion events. Transient fusion involves only outer membranes while complete fusion requires merging of both inner and outer membranes. A complete fusion event occurs with a rapid diffusion of soluble mitochondrial components followed by a more gradual mixing of membrane elements (Twig et al. 2006; Partikian et al. 1998; Karbowski et al. 2004a; Jakobs et al. 2003; Jakobs 2006; Arimura et al. 2004; Busch et al. 2006). This process is believed to bestow complementation between units and increased homogeneity over the network. Complementation is a key mechanism by which mitochondria can rescue a damaged unit within the network. The effect of loss of fusion has been assessed in several model systems. Network fragmentation and susceptibility to apoptosis, decreased mitochondrial membrane potential and oxygen consumption, and increased ROS production are seen with blocking fusion and underscore its importance in maintaining mitochondrial integrity.

Two homologous proteins known as mitofusin 1 and mitofusin 2 (Mfn1 and Mfn2) function together to merge the outer membranes of mitochondria. Both proteins share relevant functional domains and connect adjacent membranes through coiled-coil antiparallel homotypic (Mfn1–Mfn1) and heterotypic (Mfn1–Mfn2) dimers. The GTPase activity of Mfn1 is higher compared to Mfn2, thus the relative proportion of dimer combinations has important functional consequences for fusion rates within the cell (Chen et al. 2003; Koshiba et al. 2004). Turnover occurs in part by polyubiq-uitination-mediated recruitment of chaperone proteins, such as p97, which mediate retrotranslocation of mitofusins and promote their proteasomal degradation (Tanaka et al. 2010a). Curiously, Mfn2 appears to have other crucial functions in the cell beyond mitochondrial fusion. One such function is tethering mitochondria and endoplasmic reticulum during calcium exchange between the organelles (de Brito and Scorrano 2008). In neurons, Mfn2 has been shown to play a role in motility by connecting mitochondria to the Miro/Milton transport complex (Misko et al. 2010).

Inner mitochondrial membranes are joined via the protein encoded by Optic Atrophy type 1 gene (OPA1) (Song et al. 2009). Expression of OPA1 is highly regulated at the transcriptional level with eight possible isoforms available through alternative splicing (Song et al. 2007). Imported OPA1 protein localizes to the intermembrane space and is further processed by several proteases to produce five additional isoform variations (Ehses et al. 2009). Functional differences between isoforms are not entirely understood but it is known that both long and short forms of OPA1 are needed to maintain fusion capacity (Song et al. 2007; Duvezin-Caubet et al. 2006). In both soluble and membrane associated forms, OPA1 exists in a complex with mitofusins (Cipolat et al. 2004). This interaction is crucial for complete fusion as cleavage of OPA1 disrupts the complex and limits mitochondria to only transient fusion events. In this way, proteolytic removal of long isoforms provides a mechanism for creating network fragmentation in response to stress (Griparic et al. 2007). The specific molecular signals that trigger processing of OPA1 remain largely a mystery but clearly both induction of apoptosis and dissipation of mitochondrial membrane potential induce OPA1 cleavage (Gottlieb 2006; Guillery et al. 2008; Lee et al. 2004; Olichon et al. 2007). This effect may represent a stopgap attempt to limit spread of damaged material within the mitochondrial network by isolating units that pose a risk or have been selected for mitophagy.

2.4 Fission

Fission is crucially involved in numerous important cell pathways including mitochondrial inheritance by daughter cells during cellular division, differentiation of post-mitotic cells such as neurons and cardiomyocytes, mitophagy, and forms of cell death (Lee et al. 2004, 2011a; Yu et al. 2005; Gomes and Scorrano 2008; Mendl et al. 2011; Grohm et al. 2010; Karbowski 2010; Jourdain et al. 2009; Wilkerson and Sankar 2011; Choudhary et al. 2011; Kane and Youle 2010; Shroff et al. 2009; Ishihara et al. 2009; Frank et al. 2001). Loss of fission results in increased mitochondrial connectivity, loss of mtDNA, bioenergetic deficiency, and alterations in apoptosis (Landes and Martinou 2011; Westermann 2010; Sheridan and Martin 2010; Parone et al. 2008). One cytosolic GTPase performs the division of fused mitochondria. Dynamin-related protein 1 (Drp1) translocates to mitochondrial scission sites and polymerizes into structures that surround the perimeter of the organelle (Fukushima et al. 2001). Polymerization activates the GTPase domain of Drp1, which literally causes constriction and pinching of a single unit into two individual daughters (Legesse-Miller et al. 2003). Sub-cellular localization and activity of Drp1 is regulated by several post-translational modifications, such as phosphorylation, ubiquitination, nitrosylation, and sumoylation (Figueroa-Romero et al. 2009; Cho et al. 2009; Wang et al. 2011a; Santel and Frank 2008; Braschi et al. 2009; Taguchi et al. 2007).

Network fragmentation occurs in response to various factors including intracellular calcium levels, mitochondrial membrane potential, and ATP availability (Yoon et al. 2003; Kong et al. 2005). For example, the calcium-sensitive phosphatase calcineurin promotes fission by dephosphorylating cytosolic Drp1, which causes translocation to mitochondria (Scorrano 2005). Two additional proteins that reside on the outer mitochondrial membrane act together as a receptor for organizing Drp1 to sites of fission. Mitochondrial fission 1 protein (hFis1) is a transmembrane protein that marks sites of division (Yu et al. 2005; Koch et al. 2005;

Serasinghe and Yoon 2008; Otera et al. 2010; James et al. 2003). Mitochondrial Fission Factor (Mff) interacts with hFis1 and serves as an adaptor that recruits Drp1 to promote polymerization (Otera et al. 2010). Recent studies have demonstrated that Mff is required for fission but hFis1 is dispensable (Otera et al. 2010; Huang et al. 2011a). This surprising finding suggests the existence of other proteins that can supersede hFis1 and act as alternative receptors for Mff and Drp1.

In summary, there are three key steps for mitochondrial fragmentation. First is the localization of fission adaptor proteins, such as hFis1 and Mff, to fission sites. Second Drp1 must be recruited from the cytosol and polymerize at fission sites. Finally, there must be an accompanying inhibition of fusion through cleavage of long Opa1 isoforms within mitochondria. These are the three minimal steps that are required for a continuous network of fused mitochondria to transition towards fragmentation.

2.5 Approaches for Measurement of Mitochondrial Dynamics

While early observational studies describe mitochondrial fusion and fission, direct experimental proof was first obtained using polyethylene glycol (PEG)-mediated cell fusion assays (Legros et al. 2002; Neuspiel et al. 2005). In this method, two separate cultures of cells have their mitochondria labeled with different molecular probes, such as green and red fluorescent proteins (GFP, RFP). Combining the two cell populations in the presence of PEG detergent promotes fusion of plasma membranes and subsequent mixing of mitochondrial populations. Fluorescence heterogeneity is detected in the resultant pool, with cells containing mitochondria purely expressing GFP or RFP while other units display a mixture of both colors. These studies provide direct proof of fusion between individual mitochondria but significant practical limitations of the methodology left ample room for improvement. Recently, more physiologically relevant studies have employed an expanded arsenal of probes in the form of dyes and proteins that allow precise, detailed data collection on a wide range of parameters.

The tracking of dyes that accumulate within mitochondria in a membrane potential dependant manner, such as Tetramethylrhodamine ethyl ester perchlorate (TMRE), was a significant advancement in the study of mitochondrial dynamics. Confocal imaging studies of cells labeled with TMRE reveal stable mitochondrial membrane potentials maintained for a period of 40–80 s followed by a sudden drop of more than 15 mV (Loew et al. 1993). These studies were pioneering in our understanding of mitochondrial biology but limited by an inability to assure that the detected mitochondrion did not fuse and/or divide during the recording time. For example, fission can occur without movement of the two daughter mitochondria or involve only the inner (but not the outer) mitochondrial membrane (Twig et al. 2006; Malka et al. 2005). Therefore fission 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 (Twig et al. 2006). The use of photoactivatable proteins was a breakthrough because it overcame these technical difficulties of imaging individual organelles that move and change morphology within a complex architecture (Betzig et al. 2006; Patterson and Lippincott-Schwartz 2002).

The creation of tools that allow laser-mediated photoactivation of mitochondrial matrix-targeted GFP (mtPA-GFP) facilitates improved biophysical and morphologic measurements as it is nontoxic for the cells and can therefore be used to make observations over an extended period of time (Karbowski et al. 2004a; Arimura et al. 2004; Busch et al. 2006). Overall fusion rates for a cell can be quantified by activating the mtPA-GFP in a subset of the mitochondrial population and then tracking diffusion of the fluorescence signal over time, as the mtPA-GFP spreads to non-activated fusion partners. While fusion rates vary across cell types and conditions, several studies have shown that when 10–20% of population is activated, the mtPA-GFP equilibrates across the entire network in approximately 45 min (Karbowski et al. 2004a, b; Twig et al. 2008a). This is predicted to result in homogeneity in protein content and function across the mitochondrial population. In addition to analyzing the properties of the entire mitochondrial network within a cell, mtPA-GFP can be used to assess attributes of an individual mitochondrion. These observable characteristics include the size, shape, membrane potential, motility, and temporal properties of fusion. For example, photoactivation of a selected mitochondrion enables real time tracking of that individual. Long-term monitoring of single mitochondrial units with activated mtPA-GFP in INS1 and COS7 cells has allowed for direct quantification of fusion rates. These studies revealed the frequency of fusion to be once every 5–20 min per mitochondrion (Twig et al. 2008a). The duration of fusion events is typically brief, lasting ~100 s and followed by fission (Arimura et al. 2004; Twig et al. 2008a). Thus mitochondria spend most of their time as individual solitary units. These studies provided the groundwork for a concept of the mitochondrial life cycle consisting of two stages, the pre-fusion period (solitary period) and the post-fusion period when mitochondria are connected together (networked period).

The combination of both TMRE and mtPA-GFP has two significant additional benefits in measuring biophysical properties of mitochondria (Twig et al. 2006; Molina and Shirihai 2009). First, it provides means for accurate determination of organelle boundaries that can be easily followed despite movement within a dense mitochondrial network. It is also beneficial because it allows comparison of the fluorescence intensities of the two probes to get a ratiometric value. This offers a tool for quantification of changes in membrane potential that are independent of exact focal plane. By avoiding the need to perform repeated imaging through the entire z-axis, monitoring can be done with greatly reduced phototoxicity. The combination approach with TMRE and mtPA-GFP extends the permissible recording periods for tracking mitochondria within a cell from minutes to hours. This advancement helped reveal that mitochondria maintain stable membrane potential during their solitary period for up to 2 h (Twig et al. 2008a; Wikstrom et al. 2007).

One major limitation of direct user-based microscopy studies is they tend to be labor intensive and therefore not amenable to high-throughput screening. Recent description of an innovative cell-free fusion assay addresses this shortcoming with a luciferase-based approach that will allow large-scale screens of modifiers of mitochondrial dynamics (Schauss et al. 2010). Specifically, the assay is based on a bimolecular complementation approach using both mitochondrial targeted yellow fluorescent protein (YFP) and luciferase constructs separated by a leucine zipper. The two split proteins are expressed separately in large cultures of cells from which mitochondrial populations are isolated and purified.

During the assay the two populations of mitochondria are mixed and through fusion the split proteins are able to combine to form functional molecules. This new system holds great promise as it provides multiple highly quantifiable readouts. Linking luciferase activity to fusion events provides a much-needed tool for rapid, large-scale screening of conditions that affect mitochondrial dynamics. The methods described here provided important insights into mitochondrial biology yet they likely represent a mere beginning, as creative new approaches expand boundaries in this emerging field.

2.6 Benefits from Mitochondrial Dynamics

Responsive mitochondrial dynamics is an essential part of an array of cellular processes including mitosis, fuel sensing, ATP production, mitophagy, and apoptosis (Arimura et al. 2004; Twig et al. 2008a; Nakada et al. 2001a, b; Skulachev 2001; Liesa et al. 2008; Molina et al. 2009). In some situations, entire network fragmentation is necessary to facilitate autophagic clearance of mitochondria (mitophagy), such as during erythrocyte maturation, sperm mitochondria in oocyte fertilization and apoptosis dependant on PTP opening (Takano-Ohmuro et al. 2000; Shitara et al. 2000; Elmore et al. 2001). On a local level, fusion allows mixing and complementation between two units. In a fused state, exchange of components such as solutes, metabolites, and soluble proteins occurs rapidly (Partikian et al. 1998; Arimura et al. 2004; Chen et al. 2003, 2005; Chen and Chan 2005; Shaw and Nunnari 2002; Griffin et al. 2006) while membrane embedded proteins and mitochondrial DNA spread more slowly (Twig et al. 2006; Legros et al. 2004; Gilkerson et al. 2008; Wikstrom et al. 2009). The ability of mitochondria to fuse together reduces content heterogeneity and thus is a first line of defence against dysfunction (Legros et al. 2002; Chen et al. 2005, 2011; Legros et al. 2004; Hori et al. 2011; Chan 2006; Ono et al. 2001; Mazzoni and Falcone 2011).

Maintaining quality control through mitochondrial dynamics simultaneously optimizes bioenergetic efficiency and reduces risks associated with oxidative phos-phorylation by removal of damaged material. For example, inhibition of mitochondrial fission leads to an increase in oxidized proteins along with decreased maximal oxygen consumption rates during uncoupled respiration. These findings suggest the accumulation of oxidized material is due to a loss of clearance rather than increased production of ROS. Failure to properly remove damaged components impairs mitochondrial function and limits reserve capacity. These outcomes are particularly important for long-lived cells with high metabolic demands.

The dependence of quality control on fission may stem from the ability to generate unequal daughter units. Most fission events produce heterogeneous daughters with opposite membrane potential "de flections," usually greater than 5 mV. Oxidized and damaged material is also inequitably distributed and this ability to regularly create uneven fission events suggests a selective mechanism of intra-mitochondrion segregation and separation. The net effect of numerous cycles of asymmetric divisions and selective isolation is the ability to concentrate undesirable material within a minimal number of units. Damage laden mitochondria ultimately get isolated and prevented from fusing with the rest of the network through reduction in fusion proteins (Twig et al. 2008a, b). In various cell types, loss of membrane potential leads to the polyubiquitination and proteasomal degradation of proteins

associated with the mitochondrial outer membrane, such as Miro and mitofusins. In addition to degradation of individual proteins, ubiquitination serves to recruit autophagy-related scaffold adaptors, such as p62 and HDAC6 (Huang et al. 2011b; Lee et al. 2011b). These scaffold proteins bind to polyubiquitin chains and serve essentially as receptors for autophagosomes to facilitate lysosomal degradation of the mitochondrial unit through mitophagy. Blocking autophagy is sufficient to cause a buildup of damaged material including mitochondria, particularly in energy intensive tissues such as brain, heart, liver, kidney, and pancreatic beta cells (Twig et al. 2008a ; Jung and Lee 2009 ; Taneike et al. 2010; Kimura et al. 2011). It is worth noting in these cases that dysfunctional mitochondria accumulate without requiring any additional toxins or mitochondrial stressors. Mitochondrial turnover is a major proportion of the basal autophagic processing within cells, especially those with elevated metabolic demands. Long-lived post-mitotic cells with chronic high levels of turnover are inherently vulnerable to disruptions in the quality control pathway (Terman et al. 2010).

2.7 Regulation of Mitochondrial Dynamics

Multiple levels of cell signaling are involved in regulating mitochondrial dynamics. Despite its complexity, the system can be broken down into two simple categories, global and local regulation (Hyde et al. 2010). Figure 2.1 illustrates regulatory elements during the mitochondrial lifecycle and Table 2.1 lists examples of global and local control during fusion, fission, and the solitary period. Control derived from the cellular macroenvironment that affects the entire mitochondrial network is categorized as global regulation of mitochondrial dynamics, such as during the cell cycle (Lee et al. 2004; Taguchi et al. 2007; Scarpulla 2002a; Arakaki et al. 2006). At different stages of mitotic cell division there is transcriptional control of dynamics proteins that lead to opposite extremes in network morphology. A concert of transcription factors mediates increases in mitochondrial mass, respiratory capacity, and energy production that are required during S phase (Scarpulla 2002a, b). Accordingly, there is hyperfusion of the network during G1-S phase while hyper-fragmentation occurs in late S and M phases.

Local regulation occurs at the level of the microenvironment of an individual mitochondrion. Fission events are controlled checkpoints for generation of polarized and depolarized mitochondria; therefore changes in membrane potential distribution are a mechanistic example of local regulation (Twig et al. 2008a; Wikstrom et al. 2007). Loss of membrane potential and ATP production causes cleavage and degradation of fusion proteins by mitochondrial proteases and the proteasome (Song et al. 2007; Chan and Chan 2011; Chan et al. 2011). Decreased fusion capacity results in increased time spent in the solitary phase, and if membrane potential is not recovered, the mitochondrion enters into the pre-autophagic pool (Cipolat et al. 2004, 2006; Baricault et al. 2007; Twig and Shirihai 2011; Rambold et al. 2011a).

Another crucial local regulator of mitochondrial dynamics is the degree of movement of an individual mitochondrion (Twig and Shirihai 2011). Movement greatly increases the chances of fusion perhaps in part because microtubule transport aligns mitochondria (Twig et al. 2010). Alignment facilitates pole interaction between mitochondria and thus increases the

likelihood of tethering between mitofusins. Motility depends on the calcium-sensitive mitochondrial Rho GTPase (Miro), which connects mitochondria to the ATP-dependant motor enzymes, dynein and kinesin (Boldogh and Pon 2007; Fehrenbacher et al. 2004; Saotome et al. 2008). Milton is a protein that complexes with Miro and is also required for transport of mitochondria (Glater et al. 2006; Rice and Gelfand 2006). Calcium binding to Miro inhibits motility by causing detachment of the motor protein complex from microtubules (Wang and Schwarz 2009a, b; Wang et al. 2011b). Calcium exchangers, serving a role in calcium buffering, are dependant on ATP, resulting in a dependency of the buffering capacity on mitochondrial ATP synthesis. This is leading to the detachment of mitochondria in calcium rich spots, thus creating a localized mechanism for selective delivery of mitochondria to cellular regions with unmet ATP needs (Yi et al. 2004). Elevated levels of cytosolic calcium inhibit motility by binding to Miro and decreased supply of ATP lowers the activity of ATPase-driven motor proteins. Kinesins themselves are unaffected by calcium levels, so by coupling both ATP availability and local calcium concentrations this local regulation specifically impacts mitochondrial movement and not general microtubule transport.

In neurons, proper distribution of mitochondria is of the upmost importance. The degree of dendritic arborization correlates with mitochondrial content and is dependant on Miro activity (Macaskill et al. 2009; Russo et al. 2009). Mitochondria with high membrane potential and elevated ATP production travel anterogradely to synaptic regions where there is a very high demand for energy (Miller and Sheetz 2004). Appropriately, global elevation of ADP levels in neurons increases delivery of mitochondria to synapses (Mironov 2009). On the contrary, there is fast retrograde transport of depolarized mitochondria with low membrane potential back to the soma to facilitate lysosomal degradation (Boldogh and Pon 2007; Gerencser and Nicholls 2008; Hollenbeck and Saxton 2005). In addition to supplying ATP, mitochondria fulfill a crucial role by buffering cytosolic calcium. The abundant neurotransmitter glutamate activates ionotropic NMDA receptors resulting in local increases in calcium influx. This relationship establishes an important regulatory mechanism for local inhibition of mitochondrial transport by Miro at active synaptic sites (Saotome et al. 2008; Wang and Schwarz 2009b; Macaskill et al. 2009).

The transcriptional and post-translational regulation of OPA1 serves as an excellent final example of both global and local control of mitochondrial dynamics. Through global signaling pathways, alterations in gene transcription can create eight isoforms of OPA1 (Landes et al. 2010). The distinct functions of the different forms of OPA1 are not well understood but clearly they can perform unique activities such as stabilizing cristae and protecting mtDNA (Semenzato et al. 2011; Merkwirth et al. 2008; Frezza et al. 2006; Elachouri et al. 2011; Yu-Wai-Man et al. 2010). A shift in isoform production affects all mitochondria undergoing protein import and therefore represents a form of global regulation. On the other hand, imported OPA1 is cleaved to produce variants of different lengths by several mitochondrial proteases, including MPP, OMA1, PARL, and Yme1L (Song et al. 2007, 2009; Ehses et al. 2009; Cipolat et al. 2004, 2006; Griparic et al. 2007; Guillery et al. 2008; Ishihara et al. 2004). This proteolytic processing is dependant on membrane potential, metal ion levels, and ATP availability. Both long and short isoforms are required for proper inner membrane fusion and so represents a means of local regulation.

For example, stress-induced cleavage of OPA1 long isoforms by OMA1 can disrupt interaction with Mfn1 and thereby block complete fusion (Ehses et al. 2009; Cipolat et al. 2004, 2006; Guillery et al. 2008). In this way, OPA1 is regulated both at the global and local levels to control fusion of mitochondria.

2.8 Mitochondrial Dynamics and Pathology

Alterations to mitochondrial fusion and fission have been demonstrated in several pathological conditions including neurodegeneration, obesity, and type II diabetes. Mutations in genes that encode for fusion proteins provide the clearest connection between mitochondrial dynamics and disease. Charcot-Marie-Tooth (CMT) disease Type 2A is caused by mutations in MFN2 and results in peripheral nervous system dysfunction (Ching et al. 2010; Casasnovas et al. 2010; Ouvrier and Grew 2010; Feely et al. 2011). The most common form of hereditary optic neuropathy is caused by mutations in OPA1 (Ferre et al. 2009; Nochez et al. 2009; Yu-Wai-Man et al. 2011a, b). These diseases confirm the importance of mitochondrial fusion in cell survival and also illustrate the existence of selective susceptibility amongst neuronal subtypes.

Neurons in general are vulnerable to mitochondrial dysfunction due to extreme energy demands coupled with complex, polarized cell structures. Degeneration in Parkinson's disease (PD) occurs selectively in neurons that exemplify these combined susceptibilities and genetic studies strongly implicate defects in mitochondrial dynamics and quality control (Wang et al. 2011b; Braak and Del Tredici 2008; Braak et al. 2004; Narendra and Youle 2011; Dagda and Chu 2009; Whitworth and Pallanck 2009). Specific cellular morphological characteristics create inherent challenges to the networking of mitochondrial populations within PD-sensitive neurons. The A9 dopaminergic (A9-DA) neurons of the substantia nigra elegantly illustrate this principle (Braak and Del Tredici 2008; Braak et al. 2004; Ferrer et al. 2011).

The nigral A9-DA neurons are so polarized and branched that their somas account for less than 1% of total cell volume (Sulzer 2007). Massive neuritic arborization occurs in both axonal and dendritic compartments such that each A9-DA neuron may contain more than 300,000 synapses in its axonal field alone (Arbuthnott and Wickens 2007; Matsuda et al. 2009; Surmeier et al. 2010a, b). Extreme cellular morphology creates a major logistical hurdle and heightens susceptibility to disruptions in mitochondrial transport. Dispersion also limits protective mechanisms of complementation and quality control by decreasing the likelihood of fusion events. Synapses are the most energy-demanding region of the neuron as well as being sites of voltage-gated calcium influx. Proper mitochondrial distribution is therefore critical not only to provide ATP but also to buffer calcium levels (Oliveira 2010; MacAskill et al. 2010).

To reach the pre-synaptic compartment, mitochondria must travel along long, thin, and poorly myelinated axons in A9-DA neurons (Braak et al. 2004). Each of these characteristics increases both metabolic demand and the parallel risk of oxidative stress. Length correlates with surface area and longer axons have increased requirements for ATPase activity by the sodium potassium exchanger (Na^+/K^+ ATPase). Greater distances also increase energy

expenditures and travel time for motor proteins bringing cargo back and forth from soma to synapse. Longer retrograde transit times for damaged mitochondria autophagocytosed at synapses likely increases the risk and extent of oxidative damage en route back to lysosomes (Terman et al. 2010; Yue 2007; Yue et al. 2009). Like lanes on a road, width also impacts axonal transport. Thin caliber axons are spatially restrained and this reduces capacity for delivery of both mitochondria and autophagosomes. Thin A9-DA axons have higher surface area to volume ratios and therefore elevated energy demands due to higher basal Na^+/K^+ ATPase activity. Similarly, myelin limits the amount of surface area involved in ion exchange and this insulation dramatically impacts energy demands for maintaining ionic gradients required axonal conductance. Creating specialized sub-domains of the axon permits clustering of mitochondria in energy intensive micro-regions and that may promote mitochondrial fusion (Ohno et al. 2011). Poorly myelinated A9-DA neurons require high Na^+/K^+ ATPase activity over the entire length of the axon are thereby denied the potential benefits of mitochondrial clustering. Collectively, these characteristics of A9-DA neurons likely synergize to heighten sensitivity to disruptions in mitochondrial dynamics, motility, and mitophagy.

Studies of genetic mutations that cause recessive forms of familial PD support these predictions of heightened susceptibility of disruptions in mitochondrial dynamics and quality control in A9-DA neurons. Mutations in DJ-1, PINK1, and Parkin cause parkinsonism and originally these genes were thought to have disparate functions but recently their cellular roles were unified around mitochondrial dynamics and quality control (Narendra and Youle 2011; Dagda and Chu 2009; Whitworth and Pallanck 2009; Chu 2010a; Irrcher et al. 2010; Thomas et al. 2011). Together these genes provide protection against the extremes of mitochondrial membrane potential and ROS production. In this thermostat analogy DJ-1 targets mitochondria that produce excess ROS with normal to high membrane potential. The other extreme is handled by PINK1, which identifies mitochondria with little ROS production due to depolarized membrane potential. Parkin acts as a downstream effecter of both DJ-1 and PINK1 pathways to facilitate selective autophagic clearance of targeted mitochondria.

DJ-1 is a cytosolic chaperone protein that translocates to mitochondria in response to oxidative stress (Canet-Aviles et al. 2004; Moore et al. 2005; Xiong et al. 2009). Loss of DJ-1 function leads to aberrant mitochondrial morphology and function (Irrcher et al. 2010; Thomas et al. 2011; Goldberg et al. 2005; Krebiehl et al. 2010) as well as increased sensitivity to mitochondrial toxins and ROS (Canet-Aviles et al. 2004; Kim et al. 2004, 2005; Ved et al. 2005; Zhang et al. 2005; Paterna et al. 2007; Taira et al. 2004 ; Menzies et al. 2005 ; Meulener et al. 2005). The protective functions of DJ-1 are dependant on a specific cysteine residue located at site 106 and this single amino acid allows DJ-1 to function as a cytoplasmic sensor of mitochondrial oxidative stress (Irrcher et al. 2010; Canet-Aviles et al. 2004; Blackinton et al. 2005, 2009). In support of this role, mitochondria isolated from mice lacking DJ-1 display greater ROS generation and phenotypes associated with loss of DJ-1 are reversed with anti-oxidants (Irrcher et al. 2010; Thomas et al. 2011). Replacing wild-type protein rescues the phenotypes of DJ-1 knockout cells but not when the replacement protein is mutated at site 106. Exactly how DJ-1 regulates mitochondrial ROS production is not fully clear but two plausible mechanisms include modulation of complex I

activity and regulation of uncoupling protein expression (Hayashi et al. 2009; Guzman et al. 2010). Additionally, oxidation of DJ-1 results in binding with the cytosolic E3 ubiquitin ligase Parkin (Moore et al. 2005). DJ-1 functions upstream in this pathway as Parkin overexpression can rescue phenotypes associated with loss of DJ-1 (Irrcher et al. 2010; Thomas and Cookson 2009) but not the reverse (Dodson and Guo 2007).

Maintenance of homeostasis within the mitochondrial population is not complete with only protection from excess ROS by DJ-1. Production of ROS is connected to membrane potential and when a mitochondrion becomes depolarized it decreases ROS output. In this situation the dysfunctional mitochondrion would be undetected by the DJ-1-mediated quality control mechanism. An additional surveillance mechanism is needed to guard against this other potential extreme situation. PTEN-induced kinase 1 (PINK1) is a protein kinase with a mitochondrial targeting signal and a putative transmembrane domain (Chu 2010a, b; Mills et al. 2008). PINK1 is continuously imported into the intermembrane space where it is immediately targeted for degradation by several mitochondrial proteases. This normal turnover is interrupted when mitochondria depolarize and PINK1 accumulates, allowing for kinase signaling to bring about selective mitochondrial removal (Jin et al. 2010; Narendra et al. 2010a). In this way PINK1 provides a mechanism for monitoring mitochondrial function that is not based on ROS production. Loss of PINK1 function leads to a buildup of damaged mitochondrial material along with decreased membrane potential and ATP synthesis (Dagda and Chu 2009 ; Exner et al. 2007 ; Liu et al. 2009, 2011; Grunewald et al. 2009; Marongiu et al. 2009; Dagda et al. 2009a, b; Wood-Kaczmar et al. 2008; Gandhi et al. 2009). Mitochondrial dysfunction occurs prior to the onset of any neu-rodegeneration in mice lacking the PINK1 gene (Gispert et al. 2009; Narendra et al. 2008, 2009, 2010). One downstream event of PINK1 stabilization is the binding and phosphorylation of Miro/Milton mitochondrial transport complexes (Wang et al. 2011b; Weihofen et al. 2009). This PINK1 effect may isolate depolarized mitochondria by limiting their transport and help in the clearance by mitophagy.

Another consequence of depolarization-induced PINK1 accumulation related to mitophagy is the mitochondrial recruitment of Parkin. Cytosolic Parkin is selectively recruited to depolarized mitochondria, which facilitates autophagic elimination of the dysfunctional units (Narendra et al. 2008, 2009, 2010a) and PINK1 is required for this process (Geisler et al. 2010a, b; Vives-Bauza et al. 2010a, b, c; Vives-Bauza and Przedborski 2010). By attaching lysine 48 (K48) linked polyubiquitin chains, Parkin promotes proteasomal degradation of mitochondrial outer membrane proteins such as Miro, mitofusins, and several transporters (Tanaka et al. 2010a, b; Chan and Chan 2011; Chan et al. 2011; Ziviani et al. 2010; Ziviani and Whitworth 2010; Poole et al. 2008, 2010 ; Tanaka 2010). In this way K48-mediated proteasomal turnover of outer membrane proteins immobilizes and isolates damaged mitochondria to increase the likelihood of autophagic clearance. In addition, Parkin also creates recruitment signals for mitophagy via lysine 63-linked (K63) ubiquitin chains. Scaffold proteins, such as HDAC6 and p62/SQSTM1, bind to K63-ubiquitin and facilitate localization to the aggresome and clearance by mitophagy (Huang et al. 2011b; Geisler et al. 2010a; Lee et al. 2010; Okatsu et al. 2010; Narendra et al. 2010b; Ding et al. 2010a).

Quality control of mitochondrial performance occurs through two pathways in which either DJ-1 or PINK1 utilize Parkin as a downstream effector, summarized in Fig. 2.2. Age of onset studies from PD patients support this conceptual hierarchy. Mutations in DJ-1 and PINK1 cause an early onset of Parkinson symptoms relative to the sporadic disease (~30–50 compared to ~60–80 years of age) (Abou-Sleiman et al. 2004; Mizuno et al. 2006). In accordance with being downstream in both pathways, Parkin mutations tend to have very early disease onset with a large number of juvenile cases occurring before the age of 30 (Kitada et al. 1998; Nisipeanu et al. 1999, 2001; Oliveri et al. 2001; Lucking et al. 2000).

2.9 Open Questions and Controversies

1. Studies have shown that Mff is required for fission and hFis1 is not (Otera et al. 2010). This raises the potential for the existence of other mitochondrial outer membrane proteins that can bind and anchor Mff for Drp1 recruitment. The identity of these additional proteins is not known nor is it known how they differ functionally from hFis1. Finally, hFis1 is definitely involved in mitochondrial fission but its function is not required. Could hFis1 be important for a specific form or aspect of fission, such as in the mechanism behind the generation of unequal division?

2. One crucial area of future study is understanding of the regulatory mechanisms for mitochondrial dynamics and how they connect to quality control. These are of particular interest as therapeutic targets since strategies aimed at maximizing upkeep of mitochondrial performance would have broad application across health. Can enhancement of mitochondrial dynamics, quality control, and turnover be a viable therapeutic strategy for treatment of chronic diseases such as diabetes and neurodegeneration?

3. A large portion of publications connecting the DJ-1-PINK1-Parkin pathways were performed with cell lines in nonphysiologic conditions. The degree to which these pathways exist in neurons, surprisingly, remains a matter of debate (Van Laar et al. 2011). It is also worth acknowledging that most patients with Parkin mutations lack Lewy Bodies, the intracellular neuropathological hallmarks of PD (Ahlskog 2009). Assuming the role of Parkin in neurons is to execute mitophagy downstream of DJ-1 and PINK1 signaling, the question of what connects Parkin-mediated mitophagy to Lewy Body formation is another tantalizing question facing the field of PD research.

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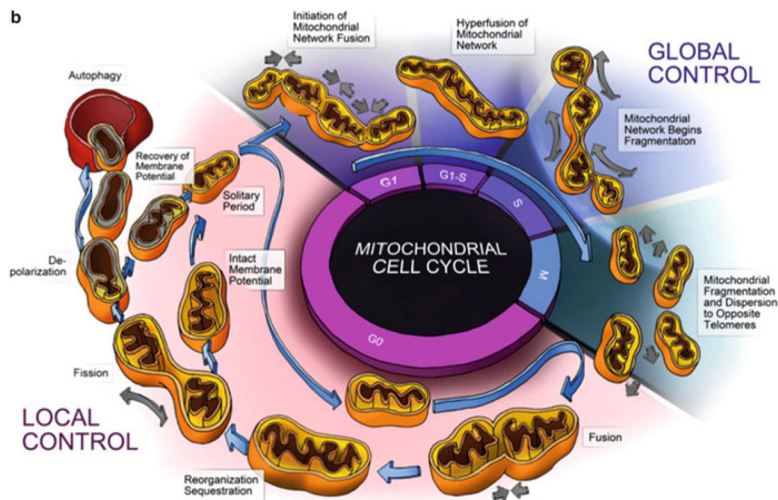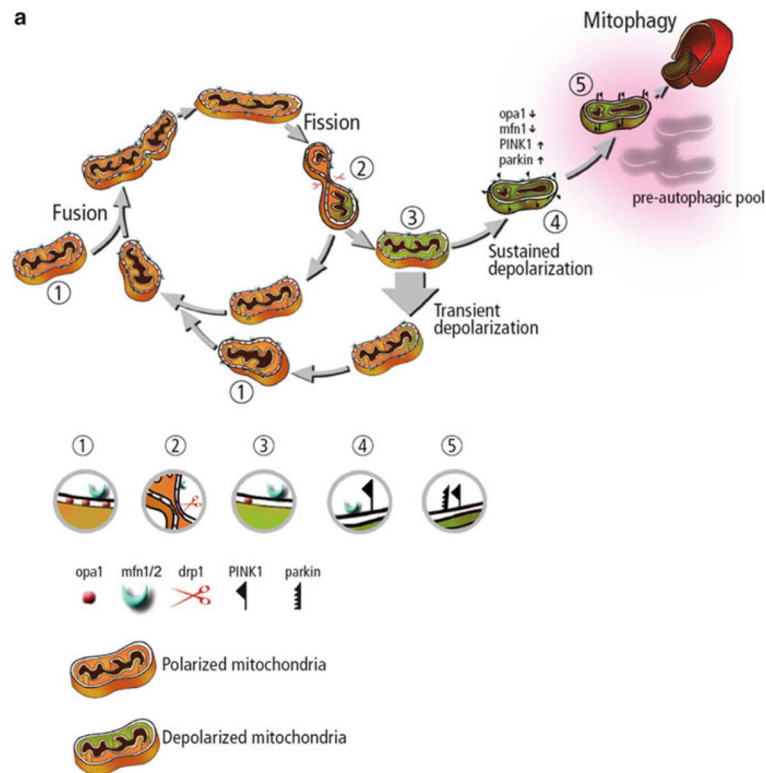
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Fig. 2.1.

Organellar and cellular controls of the mitochondrial life cycle. The mitochondria life cycle. (**a**) The mitochondria life cycle. Mitochondria go through continuous cycles of fusion and fission. Each cycle last 5–20 min. Fusion is brief (1) and triggers fission events (2). A daughter mitochondrion may maintain intact membrane potential (*orange*) or depolarize (3, *green*). When depolarized a subsequent fusion event is unlikely to occur, unless the mitochondrial re-polarizes. As a result, depolarized daughter mitochondria remain solitary. Depolarized and solitary mitochondria (4) remain for 1–4 h in a pre-autophagic pool before

being consumed by the autophagic machinery. (**b**) The interaction of the mitochondria life cycle with the cell cycle—this diagram depicts the normal life cycle of an individual mitochondrion 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 whereby 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 the G1-S and fragmentation during the M phase. These global population effects are governed by the cellular 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
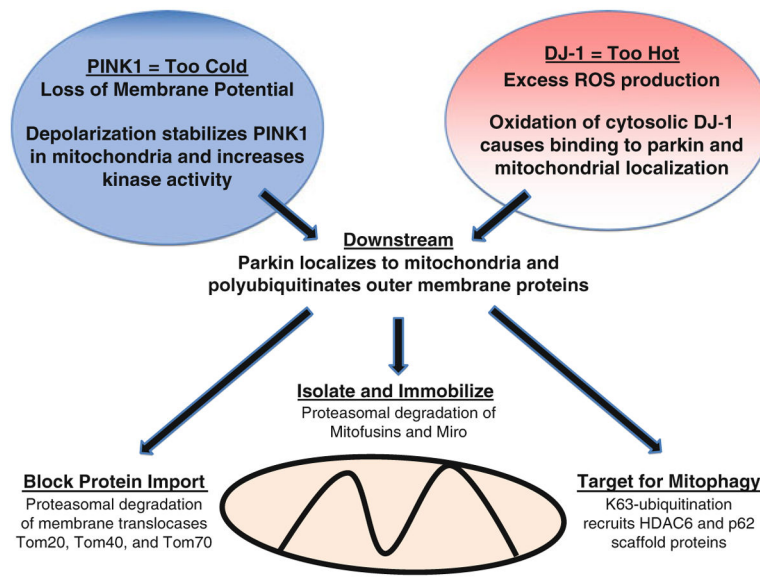
Fig. 2.2.

The thermostat model of mitochondrial quality control. Separate pathways maintain mitochondrial homeostasis by safeguarding against functional extremes. In the cold extreme, PINK1 is stabilized within mitochondria upon membrane depolarization leading to increased kinase signaling. Alternatively, oxidative activation of cytosolic DJ-1 occurs in response to the hot extreme of excess ROS production. Downstream of both PINK1 and DJ-1 pathways is the recruitment of the E3 ligase Parkin and attachment of K63 and K48 polyubiquitin chains to mitochondrial outer membrane proteins. Damaged mitochondria are isolated and immobilized by proteasomal degradation of K48-tagged proteins, such as mitofusins and Miro. Proteasomal clearance of mitochondrial translocases prevents repopulation of the outer membrane with newly synthesized replacement proteins. Finally, K63 polyubiquitin chains selectively identifies mitochondria for autophagic clearance by recruitment of scaffold proteins, such as HDAC6 and p62

Table 2.1

Local (organellar) versus global (cellular) controls of mitochondrial dynamics

Control mechanism	Outcome	References
Fission		
Global cellular control		
Recruitment of Drp1 to mitochondria is calcium dependent and regulated by calcineurin	Elevated cytosolic calcium levels activate calcineurin to dephosphorylate Drp1	Yoon et al. (2003), Kong et al. (2005), Kaddour-Djebbar et al. (2010), Hom et al. (2010), Cribbs and Strack (2007), Cereghetti et al. (2008, 2010), Tan et al. (2011), Wang et al. (2011c)
Prolonged exercise increases transcription and expression of Fis1 while decreasing mitofusins	Acute increases in metabolic demands of skeletal muscle stimulate fission	Ding et al. (2010b)
BH3 only proteins and Bax/Bak induce fission, Bax/Bak in healthy cells control fission through MFN2	Interact at mitochondrial scission sites to promote fission	Karbowski (2010), Shroff et al. (2009), Karbowski et al. (2002), Wu et al. (2011), Sheridan et al. (2008)
Sumoylation of Drp1 occurs by multiple enzymes and is present at fission sites	Protects Drp1 from degradation and increases fission activity	Figueroa-Romero et al. (2009), Braschi et al. (2009), Harder et al. (2004), Dimmer and Scorrano (2006)
High levels of oxidative stress causes fragmentation of the mitochondrial network	Drp1 phosphorylation and Bid translocation increase fission activity	Grohm et al. (2010), Qi et al. (2011)
Amino acid and other nutrient deprivation causes hyperfusion by downregulation of Drp1	Fused mitochondrial network evades autophagic degradation during starvation	Rambold et al. (2011a, b)
Drp1 expression is transcriptionally activated by p53 protein in response to apoptotic stimuli	The miR-30 family of micro-RNA limit fission by suppressing p53 expression and Drp1 activation	Li et al. (2010)
Inhibition of histone deacetylases induces mitochondrial elongation	Fused networks occur due to decreased Fis1 expression and reduced Drp1 translocation	Lee et al. (2012)
Phosphorylation of Drp1 has opposing effects on fission depending on the kinase	Example: phosphorylation of Drp1 by cAMP kinase increases yet PKC delta decreases fission	Cribbs and Strack (2007), Qi et al. (2011), Kim et al. (2011)
Local organelle control		
Knockdown of Mff promotes elongation of the network and overexpression of Mff promotes fission	Mff recruits Drp-1 to fission sites on the outer membrane independently of hFis1	Otera et al. (2010), Gandre-Babbe and van der Bliek (2008)
Blocking the mitochondrial Na$^+$/Ca^{2+} exchanger increases interaction between Drp1 and Fis1	Elevated levels of mitochondrial calcium increases fission activity	Kaddour-Djebbar et al. (2010)
Treatment with cysteine-alkylators inhibits fission and fast mitochondrial movement	Loss of movement coincided with microtubule-dependent thin mitochondrial extensions	Bowes and Gupta (2005, 2008)
Conformation specificities and self-interaction dictate the ability of Fis1 to recruit fission machinery	Fis1 activity is regulated by two interaction interfaces and its ability to oligomerize	Serasinghe and Yoon (2008), Zhang and Chan (2007)
Fusion		
Global cellular control		
Activation of PGC1a/PGC-1b/ERRa induces MFN2 mRNA. PGC-1b induces mitochondrial fusion by Mfn2	Increased fusion activity accompanies mitochondrial biogenesis	Liesa et al. (2008), Soriano et al. (2006)

Control mechanism	Outcome	References
Mitochondrial tubularization and network fusion at G1-S of cell cycle	G1-S stimulates global mitochondrial fusion, mitosis stimulates fission	Lee et al. (2004), Taguchi et al. (2007), Arakaki et al. (2006), Kashatus et al. (2011)
Variation in isoform expression of OPA1 occurs via alternative gene splicing	Expression of the eight splice variants differs across tissues and alter fusion and apoptotic activity	Song et al. (2007), Olichon et al. (2007), Frezza et al. (2006)
The promoter region of MFN 2 is a target of the tumor suppressor protein, p53	Mfn2 mRNA and protein levels are up-regulated in a p53-dependant manner	Wang et al. (2010)
Local organelle control		
Functional interaction of OPA1 with MFN1 and physical interaction between mitofusins and OPA1	Protein complex spans the two mitochondrial membranes and permits fusion activity	Cipolat et al. (2004), Guillery et al. (2008)
OPA1 processing by metalloproteases can block fusion activity and alter cristae structure	Provides a mechanism for fusion inhibition at the local level by protease activity	Song et al. (2007, 2009), Ehses et al. (2009), Duvezin-Caubet et al. (2006), Griparic et al. (2007), Guillery et al. (2008), Baricault et al. (2007), Kieper et al. (2010)
Low levels of local GTP induce outer membrane tethering while complete fusion events require high intra-mitochondrial GTP levels	Initial fusion is promoted in energy deficient environments yet complete fusion is regulated by energetic status of the organelles	Meeusen et al. (2006)
G-protein beta2 is enriched in the mitochondrial membrane and interacts with Mfn1 to regulate fusion	Gbeta2 regulates the mobility of Mfn1 within the outer membrane and promotes fusion	Zhang et al. (2010)
Bcl-x(L) increases rates of fusion and fission with an observed overall network elongation	Bcl-x(L) increases mitochondrial mass concurrent with elevated dynamics cycling	Berman et al. (2009)
Solitary period		
Global cellular control		
Global ADP levels increase mitochondrial movement to synapses	ADP signals mitochondrial motility	Mironov (2009)
G-protein coupled receptor, Gα2, is expressed in mitochondria and regulates motility	GPCRs are sensitive to GDP/GTP levels and can regulate mitochondrial motility	Andreeva et al. (2008)
Bnip3 expression induces Drp1 mediated fission and parkin translocation in adult myocytes	Increased fission activity and parkin translocation enhanced mitophagy	Lee et al. (2011a)
Local organelle control		
Mitochondrial movement along microtubules occurs in an energy-dependent manner	Individual mitochondria move at different rates along microtubules based on ATP levels	Yi et al. (2004), Miller and Sheetz (2004), Guo et al. (2005)
Local redox status of mitochondria impacts membrane potential and velocity of movement	Elevated oxidation leads to depolarization and to increased motility	Gerencser and Nicholls (2008)
PINK1 and Parkin target Miro, mitofusins and other outer membrane proteins for proteasomal degradation and promote mitophagy	Proteasomal degradation of Miro and mitofusins isolate and immobilize mitochondria which increases the pre-autophagic pool	Tanaka et al. (2010a), Wang et al. (2011b), Weihofen et al. (2009), Ziviani et al. (2010), Ziviani and Whitworth (2010), Poole et al. (2008, 2010), Yang et al. (2008), Glauser et al. (2011), Rakovic et al. (2011), Gegg et al. (2010)