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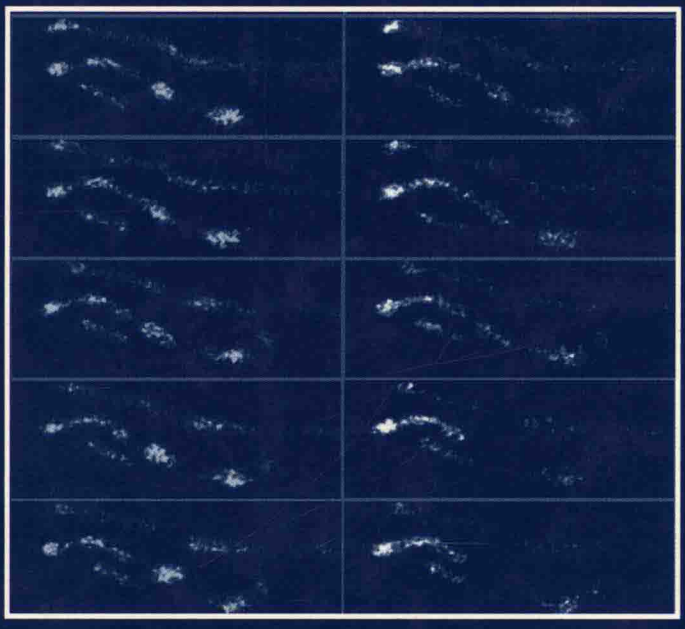
Kwang W. Jeon



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Contents

CHAPTER ONE

MITOCHONDRIAL DYNAMICS

Jürgen Bereiter-Hahn *and* Marina Jendrach

Contents

Abstract

Mitochondrial dynamics is a key feature for the interaction of mitochondria with other organelles within a cell and also for the maintenance of their own integrity. Four types of mitochondrial dynamics are discussed: Movement within a cell and interactions with the cytoskeleton, fusion and fission events which establish coherence within the chondriome, the dynamic behavior of cristae and their components, and finally, formation and disintegration of mitochondria (mitophagy). Due to these essential functions, disturbed mitochondrial dynamics are inevitably connected to a variety of diseases. Localized ATP gradients, local control of calcium-based messaging, production of reactive oxygen species, and involvement of other metabolic chains, that is, lipid and steroid synthesis, underline that physiology not only results from biochemical reactions but, in addition, resides on the appropriate morphology and topography. These events and their molecular basis have been established recently and are the topic of this review.

Key Words: Mitochondria, Mitochondrial movements, Mitochondrial morphology, Mitochondrial fusion and fission, Calcium, Cytoskeleton.

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Abbreviations

AD	Alzheimer's disease
Drp1	dynamin-related protein 1
ER	endoplasmic reticulum
Fis1	fission protein 1
FRAP	fluorescence recovery after photobleaching
HUVEC	human umbilical vein endothelial cells
IMM	inner mitochondrial membrane
KIF	kinesin superfamily
MEF	mouse embryonic fibroblast
Mfn	mitofusin
mtDNA	mitochondrial DNA
mtFP	mitochondrial fluorescent proteins
MTR	MitoTracker Red CMXRos
OMM	outer mitochondrial membrane
Opa1	optic-nerve-atrophy 1
PAGFP	photoactivatable GFP
PD	Parkinson's disease
PGC-1	peroxisome proliferator-activated receptor coactivator 1
ROS	reactive oxygen species
$\Delta\psi_{mit}$	mitochondrial membrane potential

1. Introduction

The production of ATP is the most important function of mitochondria. ATP production is made possible by the concerted action of redox systems forming the core of four electron transport complexes which generate a proton gradient across the inner mitochondrial membrane (IMM). Electron flow via the electron transport chain is a powerful source of reactive oxygen species (ROS), which stimulate cell proliferation and also cause mutations (Klaunig and Kamendulis, 2004) and are supposed to be the main cause for loss of proliferative activity and aging, as stated first by Harman (1956) and Harmann (1972). The proton gradient drives a molecular machine, the ATP-synthase, also termed complex V, phosphorylating ADP to ATP. Using the proton gradient, mitochondria can accumulate other cations such as K^+ and they act as powerful regulators of cytoplasmic Ca^{2+} (Gunter et al., 2000; Malli and Graier, 2010; Parekh, 2008; Werth and Thayer, 1994) and via calcium

signaling influence a wide variety of physiological processes (Ryu et al., 2010; Valero et al., 2008). Among those is the participation of mitochondria as a key component in the glucose-sensing machinery controlling insulin secretion by pancreatic β-cells (Lowell and Shulman, 2005; Maassen et al., 2004; Maechler and Wollheim, 2001). Their general association with the endoplasmic reticulum (ER) reflects the interaction of these two endomembrane systems in control of free cytoplasmic calcium. Among synthetic activities of mitochondria, those of steroids are the most important. Opening a mitochondrial transition pore causing release of cytochrome *c* and its interaction with proteins of the Bcl and Bac/Bax family comprise a central mechanism for apoptosis. Only a small fraction of mitochondrial proteins are encoded within mitochondrial DNA (mtDNA), and most of the genes for mitochondrial proteins are located in the nucleus. This renders mitochondria sensitive to genetic disturbances within the nucleus and within the mitochondria as well. Considering these many functions, mitochondrial integrity is a key requirement for cell performance. Being the main site of ROS production, mitochondria are the primary targets for oxidative damage, in particular because of very limited DNA-repair mechanisms as compared to the nucleus (Bohr and Dianov, 1999; Bohr et al., 1998). The dynamic nature of mitochondria provides quality control mechanisms stabilizing mitochondrial function.

Mitochondria appear spherical and ovoid or are elongated structures within cells. However, mitochondria continuously fuse and divide, and by this, the single mitochondrion represents only a transient manifestation of a reticulum termed chondriome. The nature of single mitochondria has been challenged (Park et al., 2001; Rizzuto et al., 2001) but has to be discussed in relation to the high mitochondrial dynamics (see Section 3.2.2). In a variety of vertebrate cells in culture, fusion and fission events are very frequent and take place within a few minutes per mitochondrion (Bereiter-Hahn and Vöth, 1994; Chen et al., 2003; Jendrach et al., 2005; Legros et al., 2002; Malka et al., 2005; Mattenberger et al., 2003; Twig et al., 2008, 2010) and exchange matrix (Liu et al., 2009; Twig et al., 2006), inner and outer membrane proteins (Busch et al., 2006; Muster et al., 2010), and mtDNA (Bereiter-Hahn and Vöth, 1996; Gilkerson, 2009). The same is true for plant cells (Arimura et al., 2004) and yeast (Hoppins and Nunnari, 2009). Therefore, it does not matter whether single mitochondria are devoid of nucleoids or not, because the next fusion process again can provide them with genetic material and allows for recombination of mtDNA (D'Aurelio et al., 2004; Kraytsberg et al., 2004; Legros et al., 2004; Nakada et al, 2001, 2009; Ono et al., 2001; Sato et al., 2009; Zinn et al., 1987). This overwhelming dynamics is accompanied by shape changes, branching and bending. In many cell types, mitochondria continuously change their position by saltatoric movements. They migrate along microtubules or other cytoskeletal elements. This locomotion as well as fusion and fission are essential for the physiological integrity

of neurons. Disturbances of mitochondrial dynamics are closely related to degenerative diseases like Alzheimer's, Huntington's, or Parkinson's Disease (Chen and Chan, 2009; Cox and Spradling, 2009; Gunawardena and Goldstein, 2001; Gunawardena et al., 2003; Hurd and Saxton, 1996; Reddy et al., 2009). But also in other cell types, mitochondrial dynamics is a prerequisite for functional integrity (Liesa et al., 2009; Zorzano et al., 2009), and if impaired, interrupts cell cycle progression (Karbowski et al., 2000) and leads to loss of mtDNA (Parone et al., 2008). Summing up, we can distinguish four types of mitochondrial dynamics:

- Movement within a cell which is strongly connected to associations with other organelles and at least in part depends on interactions with the cytoskeleton. These interactions may also be responsible for shape changes as are branching, extension, retraction, and bending.
- Fusion and fission events which provide a huge mixing machine establishing coherence within the chondriome.
- Dynamical behavior of cristae, nucleoids, and diffusion of proteins and lipids within the mitochondria.
- Formation and disintegration of mitochondria (mitophagy).

After a short introduction on how to visualize mitochondrial properties, we will discuss these levels of mitochondrial dynamics in relation to their function. It is not intended to review the older literature (roughly before 1990) because of two reviews of the same author summarizing the structure and appearance of mitochondria as seen by light and electron microscopy (Bereiter-Hahn, 1990; Bereiter-Hahn and Vöth, 1994). The more recent work focuses on the molecular basis of mitochondrial dynamics on which we will emphasize in this review.

2. How to Visualize and Quantify Mitochondrial Dynamics

Visualization of mitochondria in living cells became an easy task (Fuller and Arriaga, 2003). Either small fluorochromes staining selectively mitochondria or fluorescent proteins are used. Uptake of small fluorescent molecules mostly depends on $\Delta\psi_{mit}$, for example, rhodamine 123, TMRME, DASPMI, JC-1 and JC-4, and mitotracker red (MTR). Others indicate mitochondrial pH or pCa^{2+} in the mitochondrial matrix, or the factors guiding dye accumulation are not really known (e.g., mitotracker green, NAO). MTR is very special among these dyes because its uptake is driven by $\Delta\psi_{mit}$, but then covalent binding to proteins stabilizes dye incorporation which now survives chemical fixation. Mitochondria themselves are fluorescent structures: Flavoprotein and NADH fluorescence

monitor redox states and can be quantified (Kajstura and Bereiter-Hahn, 1988; Mayevsky and Barbiro-Michaely, 2009).

Fluorescent proteins targeted to mitochondria opened a new area for research (Rizzuto et al., 1995) because these fluorochromes can be targeted not only to the matrix, rather all complexes of the respiratory chain and F_1F_0-ATP-synthase, and outer mitochondrial membrane (OMM) proteins have been labeled successfully. Multiple labeling either by transfecting with more than one fluorescent protein or combining fluorescent protein labeling with $\Delta\psi_{mit}$—sensitive dyes have been performed. All the mitochondrial fluorescent proteins (mtFP) vectors use the targeting sequence of cytochrome *c* oxidase subunit VIII to achieve mitochondrial matrix localization; IMM-PAGFP is the mitochondrial targeting sequence of ABCB10 fused with PAGFP (Twig et al., 2006).

Difficulties arise with plant mitochondria where a novel targeting model for protein import to mitochondria has been identified (Chatre et al., 2009). Therefore and because of interspecies differences, a different strategy for labeling with fluorescent proteins has to be followed (Logan and Leaver, 2000), replacing introduction of the GFP gene into the nuclear genome; this strategy is the use of a chimeric gene carrying appropriate regulatory sequences and a mitochondrial targeting signal (Köhler et al., 1997).

High-resolution light microscopy as 4pi microscopy provides excellent 3D images of the overall shape of mitochondria (Egner and Hell, 2005; Schmidt et al., 2008) but not of their inner membrane arrangements. Further advanced systems, that is, STED-microscopy or STORM (Huang et al., 2008) at the moment do not allow visualization of mitochondrial dynamics because of the relatively long duration needed to acquire single images. But this situation will change in future. In mitochondria with large distances between cristae, for example, in megamitochondria (e.g., Karbowski et al., 1999) or in some HeLa cell mitochondria, the fluorescence pattern of IMM-bound FPs comes close to the visibility of cristae and their dynamics. Electron microscopy has been used to show mitochondrial dynamics indirectly by correlation with light microscope observations (e.g., Bereiter-Hahn and Vöth, 1994).

Thus, visualization of mitochondria in living cells is performed almost exclusively by fluorescence techniques. The high contrast, that is, the grey level difference between the organelle of interest and its environment is a big advantage over previous contrasting procedures, because quantification of dynamics by a computer-assisted image analysis has become a realistic task today.

Because of the significance of mitochondrial dynamics for aging and a variety of diseases (see Brenner and Mak, 2009; Graham et al., 2010; Guglielmotto et al., 2010; Seo et al., 2010; Wallace, 2005), statistical methods have to be applied to monitor and to quantify these dynamics in response to different physiological and pathological conditions. Shape changes, intracellular trafficking, and membrane potential modulations are the main tasks for quantification. Classifications like "fragmented/spherical, swollen, elongated, and interconnected" are widely

used to characterize the overall mitochondrial shape (Jendrach et al., 2008; Mai et al., 2010; Michiorri et al., 2010). This approach is not very precise and contains subjective elements. These problems have been overcome by quantifying shape changes by determining the aspect ratio (ratio between the long axis and the extension vertical to it; deVos et al., 2005).

Short time dynamics can be estimated by adding up a time series of pictures yielding a single 2D image of the total area covered by mitochondria within the chosen time range. Normalization of area summation is reached by division of this area by the area covered by mitochondria as determined within a short time, excluding motion. The result provides a measure of shape changes plus locomotion. A more detailed automated analysis of 3D stacks of confocal images of human skin fibroblasts was presented by simultaneous measurements of mitochondrial morphology (number, length, and branching), mass, and $\Delta\psi_{mit}$ comparing fibroblasts from CI-deficient patients with those from healthy donors (Koopman et al., 2006, 2008). Mitochondria were stained with rhodamine 123, which allowed, at the same time, analysis of morphology and membrane potential. Furthermore, the analysis was extended to follow Ca^{2+} dynamics within the cells (Koopman et al., 2008; Willems et al., 2009). This elegant method, however, is restricted to very well spread out cells with almost no overlap of mitochondria.

The time course of $\Delta\psi_{mit}$ can be followed by fluorometry of mitochondria stained with the appropriate potential sensitive dyes which can easily exchange between mitochondria and the cytoplasm or extracellular space. In case the probes remain within the cytoplasm, measurements are limited to those compounds changing their quantum yield when becoming incorporated into the IMM (e.g., DASPMI; Ramadass and Bereiter-Hahn, 2008), otherwise $\Delta\psi_{mit}$ changes would be masked by the fluorescence increase in the cytoplasm. Using TMRE loading of mitochondria, the group of Aon and Cortassa succeeded in demonstrating $\Delta\psi_{mit}$ modulations in cardiomyocytes *in situ* as well after isolation, distinct from random behavior, with dynamics covering a range of at least three orders of magnitude (from milliseconds to minutes) under physiological conditions. The frequency distribution obeys a homogenous power law with a spectral exponent, $\beta = 1.74$ (Aon et al., 2006a,b). This type of computational analysis is remarkably powerful for the description of complex dynamics.

3. Levels of Mitochondrial Dynamics and their Functional Significance

3.1. Localization and movements of mitochondria within cells

In well spread out animal cells in culture and in neurons, some mitochondria permanently move toward the periphery and again back to the perinuclear region. There, most of the mitochondria remain almost stationary because they

are more integrated into reticular superstructures (Bereiter-Hahn et al., 2008; Yaffe et al., 2003). Wrapping of a mitochondrial reticulum around a nucleus is a widespread phenomenon from animal cells to plant cells (e.g., apical shoot cells of *Arabidopsis*), to green algae (Atkinson et al., 1974; Blank and Arnold, 1981; Calvayrac et al., 1972; Hermann and Shaw, 1998; Sequí-Simarro et al., 2008; Yaffe, 1999, 2003; Zadworny et al., 2007). Also, tethering of mitochondria to the nuclear envelope and membrane bridges between the organelles have been described (Prachař, 2003). This morphology favors the support of mitochondria by nucleus-encoded proteins (see Section 3.1.1) and mutual control of gene expression according to external signals and functional requirements (Ryan and Hoogenraad, 2007). Similar to the role of ER–mitochondria associations, also those close to the nuclei can be involved in regulation of nuclear Ca^{2+} changes (Alonso et al., 2006). Close apposition of nuclei and mitochondria was speculated to be a mechanism to expose the nucleus to hypoxia and thus to protect nuclear DNA against ROS (Skulachev, 2001). This hypothesis is not immediately convincing because $\Delta\psi_{mit}$ in perinuclear mitochondria is often higher than of peripheral mitochondria, showing that hypoxia is not in a range-reducing respiration. But using isolated cytoplasm, Niethammer et al. (2008) demonstrated that $\Delta\psi_{mit}$ can still be high (close to its maximum) also at low oxygen concentrations, corresponding to our previous findings that inhibition of respiration in living cells does not necessarily perturb $\Delta\psi_{mit}$ (Bereiter-Hahn et al., 1983). Despite high $\Delta\psi_{mit}$, ROS production can still be high (Guzy and Schumaker, 2006; Niethammer et al., 2008), $\Delta\psi_{mit}$ alone does not determine ROS production levels (e.g., Dikov et al., 2010). Finally, reduced oxygen levels paired with high $\Delta\psi_{mit}$ might well provide a mechanism for protection against ROS by mitochondria accumulated around nuclei, but experimental evidence is still missing. Two additional reasons for accumulation of mitochondria in the perinuclear area have to be mentioned, a potential high energy demand of the nucleus and dysfunctional motor molecules. Trapping of mitochondria by structures with high energy demand, for example, plasma membrane with high activities in ion transport or signaling, is a well-established phenomenon in many cell types, as revealed by electron microscopic studies (Bereiter-Hahn, 1990; Fawcett, 1981; Germer et al., 1998a, 1998b; Hollenbeck and Saxton, 2005; Munn, 1974; Riva et al., 1999), and may result from immobilization by increased ADP concentrations (Bereiter-Hahn and Vöth, 1983) or from interactions with cytoskeletal elements (e.g., Perkins et al., 2010; Sung et al., 2008; see Section 3.1.2). Loss of $\Delta\psi_{mit}$ renders mitochondria immobile and promotes their accumulation in the cell center. This can also be achieved by factors interfering with the cytoskeleton and motor molecules including members of the dynamin family (Pitts et al., 1999) or the CLU1 gene product in Dictyostelium discoideum and *Saccharomyces cerevisiae* (Fields et al., 1998). However, just an accumulation of immobile organelles in the central part of a cell, aside the nucleus, has to be distinguished from mitochondria wrapping around the nucleus with extensive branching and high $\Delta\psi_{mit}$.

Slow dislocations are often accompanied by the formation of branches or shape changes making mitochondria creep through the cytoplasm like worms (Bereiter-Hahn, 1990; Bereiter-Hahn and Vöth, 1994), but also these deformations may result from the interaction of mitochondria with cytoskeletal elements and be driven by the activity of motor molecules connecting the OMM to cytoskeletal fibrils. Small spherical mitochondria can reach a speed up to 0.5 μm/s without any visible shape changes. In neurons, speed might even be higher, as Misgeld et al. (2007) determined peak velocities of 1.02 μm/s for anterograde movements and 1.41 μm/s for retrograde movements in mouse neurons. Elongated mitochondria only rarely reach these high speeds, probably because of simultaneous activity of motor molecules driving into different directions. Velocities up to 2 μm/s have been determined for the saltatory movements along microtubule tracks in the filamentous fungus *Neurospora crassa* (Steinberg and Schliwa, 1993). In budding yeast, mitochondria extend from the mother cell to a growing bud at a speed of 49 $\pm$ 21 nm/s (Simon et al., 1995).

Neurons represent the most extended cell types in animals. Therefore, mitochondrial locomotion is of utmost significance in these cells. Perturbation of mitochondrial locomotion impairs fusion and fission of mitochondria and inevitably induces neurodegenerative diseases, as mentioned before (Gunawardena and Goldstein, 2004). In developing neurons, mitochondria accumulate near growth cones (Morris and Hollenbeck, 1993) and locate to synaptic terminals in dendritic spines as well as axonal buttons (Chada and Hollenbeck, 2004; Li et al., 2004; Rowland et al., 2000; Shepherd and Harris, 1998; Spacek and Harris, 1998; Spirou et al., 1998) where they are required to maintain synaptic transmission (Chan, 2006; Stowers et al., 2002). Remodeling of the actin cytoskeleton on neuron depolarization by *N*-methyl D-aspartate (NMDA) induces the Wiskott–Aldrich syndrome protein verprolin homologous-1 (WAVE1) to bind to mitochondria where it promotes fission and trafficking from dendritic protrusions to dendritic spines and filopodia, and thus stimulates spine morphogenesis and enhances synaptic plasticity (Sung et al., 2008). The tension applied to mitochondria by motor molecules may promote recruitment of Drp1 to form the scission ring (Ingerman et al., 2005) which then can move back and forth along the mitochondrion (Bereiter-Hahn, 1990), similar to tension-induced dynamin assembly (Roux et al., 2006). WAVE1 targeted to mitochondria becomes part of a complex of other cAMP-regulated signaling molecules, including glucokinase and BH3 pro-apoptotic Bcl-2 homolog (BAD; Danial et al., 2003) impacting on organelle network and respiration (Carlucci et al., 2008).

In embryos, mitochondria accumulate around the nuclei or germinal vesicles, and a second group stays in the subcortical cytoplasm (Barnett et al., 1996; Bavister and Squirrel, 2000). Another type of multiple mitochondrial aggregates has been found during *Drosophila* oogenesis in functionally

impaired mutants of the genes *clu* and *park*, and thus establishing *Drosophila* mutants as models for Parkinson's Disease (PD; Cox and Spradling, 2009).

In plant cells, mitochondria appear to be carried along with other organelles within the streaming cytoplasm, but motion analysis showed that the motions of different mitochondria and of other vesicular organelles are not synchronized, thus more specific interactions have to be considered. In filamentous hyphae of fungi, vivid mitochondrial movements occur back and forth and make them accumulate at the growing tip (Fuchs et al., 2002; Scheckhuber et al., 2007; Suelmann and Fischer, 2000), while in the spherical cells of budding yeast, slow extensions of mitochondria participate in bud formation (Altmann et al., 2007, 2008; Drubin et al., 1993).

3.1.1. Interactions of mitochondria with endoplasmic reticulum

Among the organelles interacting with mitochondria and contributing to their dynamics, the ER is of utmost significance because of the delicate interplay in Ca^{2+}-messaging (e.g., Pizzo and Pozzan, 2007; Rizzuto and Pozzan, 2006; Willems et al., 2009). Thus, an ubiquitous occurrence of ER–OMM interactions can be assumed. This interaction was shown in a large variety of developing oocytes (Dumollard et al., 2006) as well as in many metazoan cells in culture. In well spread out heart endothelial cells and other vertebrate cells in culture, mitochondria are never found in areas devoid of ER, and mitochondria exclusively move along ER traits (Bereiter-Hahn and Vöth, 1983). Joint trafficking of ER and mitochondria has also been found in HEK cells stimulated by carbachol (Brough et al., 2005). This points to rather stable or at least continuing connections.

The existence of special ER areas tethered to mitochondria was suggested by cosedimentation of membranes from both organelles in cell lysates (Ardail et al., 1993; Camici and Corazzi, 1995; Gaigg et al., 1995; Shiao et al., 1995; Vance, 1990; Zinser et al., 1991). These membranes were termed "mitochondria associated membranes" (MAM). Electron microscopy revealed a distance between 10 and 60 nm between ER and OMM (Achleitner et al., 1999). The same group estimated 80–110 contacts of the ER with mitochondria in a yeast cell, considerably more than to any other organelle. About 20% of mitochondrial surface may be in direct contact with the ER (Rizzuto et al., 1998) which was confirmed by electron tomography (Mannella et al., 1998; Renken et al., 2009). Although both organelle systems are highly dynamic, MAM–mitochondria interactions seem to be stable for at least some minutes, otherwise copurification should not be possible (Lebiedzinska et al., 2009). Improper spacing between the MAM and OMM alters their function, that is, small spaces increase the susceptibility to mitochondrial Ca^{2+} overload and may elicit opening of the mitochondrial permeability transition pore, while widening of the may reduce Ca^{2+}-sequestration by mitochondria (Csordás et al., 2006). On the OMM, interactions can take place with the voltage-dependent anion