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Mitochondria: More Than Just a Powerhouse

Review

Heidi M. McBride,[1] Margaret Neuspiel,[2] and Sylwia Wasiak[2]

Pioneering biochemical studies have long forged the concept that the mitochondria are the 'energy powerhouse of the cell'. These studies, combined with the unique evolutionary origin of the mitochondria, led the way to decades of research focusing on the organelle as an essential, yet independent, functional component of the cell. Recently, however, our conceptual view of this isolated organelle has been profoundly altered with the discovery that mitochondria function within an integrated reticulum that is continually remodeled by both fusion and fission events. The identification of a number of proteins that regulate these activities is beginning to provide mechanistic details of mitochondrial membrane remodeling. However, the broader question remains regarding the underlying purpose of mitochondrial dynamics and the translation of these morphological transitions into altered functional output. One hypothesis has been that mitochondrial respiration and metabolism may be spatially and temporally regulated by the architecture and positioning of the organelle. Recent evidence supports and expands this idea by demonstrating that mitochondria are an integral part of multiple cell signaling cascades. Interestingly, proteins such as GTPases, kinases and phosphatases are involved in bi-directional communication between the mitochondrial reticulum and the rest of the cell. These proteins link mitochondrial function and dynamics to the regulation of metabolism, cell-cycle control, development, antiviral responses and cell death. In this review we will highlight the emerging evidence that provides molecular definition to mitochondria as a central platform in the execution of diverse cellular events.

Introduction

The biochemistry of mitochondria has been the subject of intense investigation over the past 50 years. Within these organelles, sugars and long chain fatty acids are broken down, ADP is recycled back into ATP, steroids and lipids are synthesized, ancient DNA is replicated, transcribed and proteins are translated, along with numerous other reactions that are essential for human life. From a structural perspective, the mitochondrion is unusual since it contains two membranes that separate four distinct compartments, the outer membrane, intermembrane space, inner membrane and the matrix. The inner membrane is highly folded into cristae, which house the megadalton complexes of the electron transport chain and ATP synthase that control the basic rates of cellular metabolism. For the most part, the biochemistry of this organelle has been investigated in cell-free, isolated systems, leading us to imagine the mitochondrion as a lonely participant in the cell working tirelessly to provide the energy required for life. In the past 10 years, this view has changed as newer approaches have allowed the examination of dynamic mitochondrial function and behavior in response to cellular signals within intact cells. We now understand that mitochondria form a functional reticulum whose steady-state morphology is regulated by dynamic fission, fusion and motility events. Multiple proteins are involved in the remodeling of mitochondrial membranes [1]. Mitochondrial fusion is mediated through the action of at least three GTPases. Mitofusin 1 (Mfn1) and Mitofusin 2 (Mfn2) are integrated within the mitochondrial outer membrane, with their GTPase and coiled-coil domains exposed to the cytosol [2]. Mfn1 and Mfn2 exist as homotypic and heterotypic complexes that can form between adjacent organelles [3,4]. Mechanistically, it has been suggested that the carboxy-terminal coiled coils tether two organelles undergoing fusion, with the GTPase domains probably regulating the fusion reaction (Figure 1) [5–7]. Another dynamin-like GTPase, Opa1, resides in the intermembrane space, where it is associated with the inner membrane [8–10]. Opa1 exists as multiple splice and cleavage variants and its function in mitochondrial fusion has been linked genetically to that of Mfn1 (Figure 1) [11,12]. Mitochondrial fission relies on yet another GTPase from the dynamin family, DRP1 [13–15]. By analogy with dynamins, it has been suggested that DRP1 oligomerizes into ring-like structures around the fission sites, to constrict the organelle in a GTP-dependent manner. In fact, DRP1 forms puncta on the mitochondrial membrane, some of which mark future fission sites. The mechanism of recruitment of DRP1 into active fission complexes remains unclear, however. Also, other proteins have been demonstrated to function in tandem with the GTPases described above, and thus have been directly or indirectly linked to fission and fusion [1]. These proteins are listed in Table S1 (see Supplemental data published with this article online) and some of their roles in mitochondrial membrane remodeling as well as their wider impact on cellular events will be discussed below.

The identification and characterization of the protein machinery that controls mitochondrial membrane dynamics constitutes an important step towards a better understanding of mitochondrial behavior. Mitochondria are an integrated component of the cell and their actions are undoubtedly linked to cellular activities. The current focus of research within this field is to identify precise molecular links between mitochondrial

[1] University of Ottawa Heart Institute, 40 Ruskin St., Ottawa, Canada K1Y 4W7.
Email: HMcbride@ottawaheart.ca
[2] These authors contributed equally.

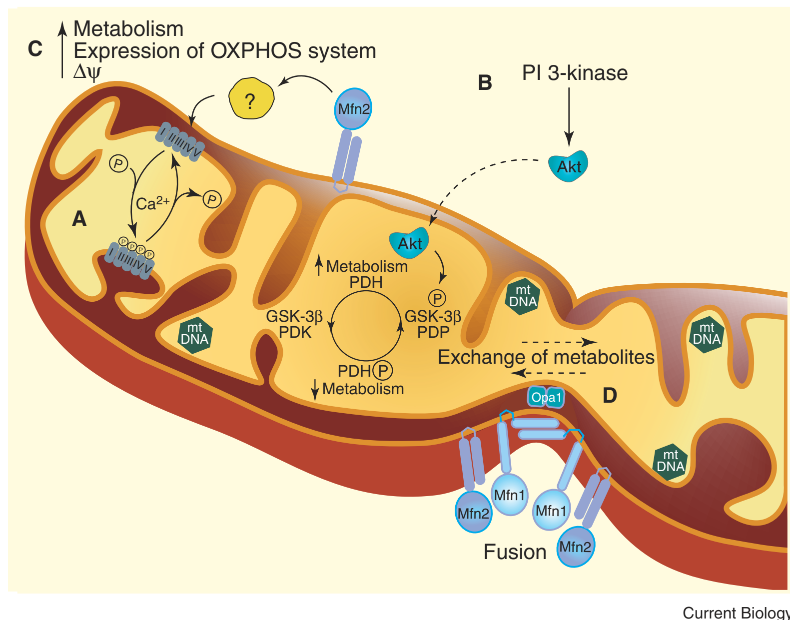

Figure 1. Regulation of mitochondrial metabolism through morphological changes and signaling. (A) Phosphorylation and dephosphorylation of metabolic enzymes, including subunits of the respiratory complexes I-V are modulated by calcium-mediated signaling. (B) In the mitochondrial matrix, phosphorylation and dephosphorylation of pyruvate dehydrogenase (PDH) are regulated through concerted actions of PDH kinases (PDK) and phosphatases (PDP). The activity of PDH is also modulated by a cytosolic signaling cascade that converges onto GSK-3β, leading to PDH dephosphorylation and activation, ultimately promoting metabolic activity. (C) The fusion GTPase Mfn2 regulates metabolism, electrochemical potential and expression of the respiratory complexes through unknown signaling partners. (D) Membrane fusion facilitates the propagation of metabolites, such as calcium, reactive oxygen species and mitochondrial DNA (mtDNA), within the mitochondrial reticulum. The GTPases Mfn1, Mfn2 and Opa1 coordinate the reaction of mitochondrial membrane fusion. Mfn proteins are required on both sides of the membrane, with Mfn1 mediating membrane tethering. Solid lines indicate protein interactions and reaction pathways, dotted lines represent protein and metabolite translocation events. See text for details and references.

function and the machinery that governs morphology. In addition, recent evidence suggests that bi-directional signaling exists between mitochondria and other cellular components. The implication of the GTPase switch, both regulatory and mechanical, in the governance of mitochondrial dynamics has recently been shown to couple mitochondrial function to cellular demand. This integration allows mitochondrial function, localization and biogenesis to be responsive to changes in cell metabolism, development, death and division. These new insights into mitochondrial biology reveal a more central function for this organelle than previously appreciated and constitute the focus of this review.

Regulation of Metabolism

Recent evidence demonstrates that the machinery that governs mitochondrial dynamics also participates in the temporal regulation of metabolism (Figure 1). These findings may not be surprising since the assembly of the mitochondrial cristae and apparent condensation of the matrix space observed by electron microscopy has been long considered to reflect the metabolic state of mitochondria [16,17]. In more recent years, tomographic reconstructions of isolated mitochondria revealed a detailed variety of cristae morphologies, from simple tubular structures to large, flat lamella and vacuolated intercristal spaces [18,19]. The dynamic shift in morphology coincides with a number of physiological events, such as transitions between different respiratory states and cristae remodeling during apoptosis [18,20,21]. Consistent with this, the loss or mutation of some inner membrane proteins affects cristae morphology and results in reduced respiration. For example, lack of non-essential subunits within the ATP synthase in yeast leads to the loss of cristae with the inner membrane forming ring structures within the mitochondria [22–24]. Similarly, deletion of the intermembrane space GTPase Opa1 causes vesiculation of the inner membrane, and loss of the inner-membrane-anchored Mitofilin leads to concentric sheets of inner membrane ring structures. In all these cases, aberrations in cristae morphology are accompanied by alterations in metabolism [9,25–27]. Furthermore, cells lacking Opa1 or Mitofilin are highly susceptible to apoptotic death, indicating the importance of maintaining inner membrane morphology [9,27]. The details of how these proteins contribute to the inner membrane architecture are unknown, but these findings illustrate the fact that cristae do not form spontaneously and are created by active, regulated processes. A number of other proteins required for the maintenance of inner membrane morphology have been identified from genetic screens in yeast [28–30], and are listed in Table S1.

Recent evidence suggests that mitochondrial fission and fusion also regulate mitochondrial metabolism. The downregulation of Opa1 or both Mitofusin GTPases by RNA interference leads to fragmented mitochondria with greatly reduced oxygen consumption and electrochemical potential [26]. Although all three GTPases are known components of the mitochondrial fusion machinery [31], they are not directly linked to the metabolic machineries. Therefore it is unclear why the loss of these fusion proteins interferes with respiration. This effect is specific to the loss of the fusion proteins rather than the morphological transition alone since mitochondrial fragmentation induced upon

the stimulation of fission did not interfere with metabolism [26]. The metabolic dysfunction that characterizes fusion-incompetent mitochondria may be secondary to a requirement for exchange of specific cargo, for example mitochondrial DNA (mtDNA), calcium, and other metabolites (Figure 1). Complementation of mtDNA has been demonstrated to be one important aspect of mitochondrial fusion, since the nucleoids carrying the mtDNA are shared within the dynamic reticulum [32–35]. If a block in fusion results in small mitochondria that either completely lack mtDNA or become enriched with mutant mtDNA, these mitochondria would ultimately lose their functional electron transport chain, leading to compromised oxygen consumption. Interestingly, cells lacking Mfn1 and Mfn2 were maintained only in supplemented media containing high pyruvate and uridine, conditions that are also required to maintain *rho*^{0} cells lacking mtDNA [26]. In addition to the sharing of cargo, the fusion machinery may be required either directly or indirectly for the maintenance of inner membrane cristae dynamics, which, as mentioned above, regulate mitochondrial metabolism.

Consistent with the evidence that the loss of the fusion machinery leads to reduced metabolism, it has been recently demonstrated that overexpression of Mfn2 in cultured cells leads to an upregulation of the respiratory complexes, mitochondrial oxidation and glucose utilization by cells [36,37]. Interestingly, the expression of a fusion-deficient form of Mfn2 also increases the respiratory capacity with accompanying condensation of the cristae, suggesting that Mfn2 may have a role in metabolic regulation that is distinct from its role in fusion [37]. In addition, Mfn2 mRNA and protein expression within skeletal muscle is reduced in the Zucker rat model of obesity [36,37] and in human subjects with obesity and diabetes [38], highlighting the pathological implications of aberrant mitochondrial dynamics. Changes in Mfn2 expression are accompanied by altered mitochondrial network *in vivo* and reduced metabolism *in vitro* [37].

Experiments investigating the mitochondrial fission machinery are also consistent with the emerging functional link between the morphology and metabolic output of mitochondria. Cells exposed to high levels of glucose contain highly fragmented mitochondria [39,40] that are hyperpolarized and generate a transient increase in reactive oxygen species (ROS) production as a result of the increased respiration. Interestingly, the fragmentation event requires glucose entry into the cell, but precedes pyruvate uptake by mitochondria, placing it as an early event [40]. Importantly, the observed transition in mitochondrial morphology and increased ROS production return to baseline within 60 minutes, indicating that the process is reversible. In cells overexpressing Mfn2 or a dominant-interfering mutant of the mitochondrial DRP1, the mitochondria remain tubular and do not show any increased respiration, hyperpolarization or ROS production [40]. These data indicate that mitochondrial fragmentation is required for the hyperglycemia-induced increase in respiration. Interestingly, chronic hyperglycemia, as in the diabetic condition, results in downregulation of Mfn2 in muscle cells, which is consistent with a more stable transition of the mitochondria to the fragmented state [36,38]. Taken together, this accumulating evidence supports the idea that the machineries that govern mitochondrial fusion and fission are intimately coupled to the metabolic processes within the organelle.

Mitochondrial Kinases and Phosphatases in Metabolic Regulation

Given that there are no direct links between the fusion/fission machineries and the metabolic complexes, the obvious question that arises is how are these morphological transitions translated into altered metabolic output? One possibility is that the morphology machinery might be integrated into pathways that regulate post-translational modifications of the metabolic complexes. Experiments published almost 40 years ago demonstrated that phosphorylation of the matrix-localized pyruvate dehydrogenase (PDH) complex inhibits the conversion of pyruvate to acetyl Co-A, the first step in the citric acid cycle [41]. PDH kinases 1–4 were later identified and their expression is now known to be tightly controlled through transcriptional regulation [42]. For example, they are upregulated during starvation when they efficiently phosphorylate PDH, resulting in the inactivation of the enzyme when the substrate levels are low [43]. Interestingly, a mechanism for a rapid regulation of PDH has been uncovered that may complement the activity of the PDH kinases. Insulin stimulation activates protein kinase B/Akt in a phosphatidylinositol (PI) 3-kinase-dependent manner [44]. Akt controls a number of metabolic and survival pathways and was recently found to translocate into the mitochondrial matrix in response to insulin stimulation [45]. A known target of activated Akt is glycogen synthase kinase 3β (GSK-3β) [46], which, although mainly cytosolic, has also been found within mitochondria [47]. The non-phosphorylated form of GSK-3β is an active kinase that, among other substrates, targets PDH, leading to reduced metabolic activity [48]. Phosphorylation of GSK-3β by Akt inhibits its kinase activity, which allows PDH to be dephosphorylated and therefore activated, leading to an increase in metabolism. Although these data require further substantiation, it represents a mechanism for the dynamic regulation of metabolic components by extracellular stimuli.

Very recently the mitochondrial phosphoproteome was shown to be much more extensive than previously anticipated and, remarkably, there was a dynamic change in the phosphorylation state of numerous proteins in response to calcium signaling [49]. The mitochondrial response to calcium flux has long been considered a central aspect of its morphology and function, both as a calcium buffer and in the propagation of intracellular calcium waves during muscle contraction and synaptic vesicle release [50–53]. The proteins phosphorylated in response to calcium include many components of the electron transport chain and the citric acid cycle [49], indicating that multiple mitochondrial kinases and phosphatases regulate mitochondrial function in a dynamic manner (Table S1). The recent identification of a novel phosphatase, termed PTPMT1, as a permanent resident of the mitochondrial matrix compartment provides insights into

this exciting new area of research [54]. The loss of PTPMT1 by RNA interference leads to an almost two-fold increase in ATP production and increases the kinetics of insulin release both at steady state and upon glucose stimulation [54]. Although substrates of this phosphatase within the matrix have not yet been identified, components of the electron transport chain and ATP synthase are obvious candidates. Many cytosolic kinases have been found to associate specifically with the mitochondrial surface under different conditions [55]. These kinases include members of the Src family, whose mitochondrial localization has also been shown to affect oxidative metabolism and cell signaling [56–58]. Two new mitochondrial kinases have also been recently identified, the intermembrane space PTEN-induced kinase 1 (PINK1) [59,60], and the peripherally associated leucine rich repeat kinase 2 (LRRK2) [61,62]. The substrates and function of PINK1 and LRRK2 are currently unknown; however, both proteins are affected in Parkinson's disease, highlighting the importance of mitochondrial phosphorylation in human pathophysiology. In addition, PINK1 was recently found to interact genetically with a third Parkinson's disease gene, Parkin [63,64], which encodes a cytosolic ubiquitin E3 ligase that has been found in the mitochondrial matrix of dividing cells [65]. Future experiments will confirm whether the machinery that regulates mitochondrial morphology is functionally linked to metabolic regulation through post-translational modifications such as phosphorylation.

Mitochondria and the Cell Cycle

It is understood that functional mitochondria are required for all cell processes due to common energetic requirements. Recent data expand on this requirement, however, and place the emphasis on this organelle as a relevant signaling platform for cell-cycle progression. For example, two important studies have recently defined the molecular basis for the previously observed cell-cycle arrest under conditions of low energy. AMPK is a heterotrimeric kinase that is activated in high AMP conditions, making it a sensor of the AMP:ATP ratio [66]. Upon activation of AMPK, a phosphorylation cascade is initiated that alters both consumption and production of ATP. Recently, activated AMPK was shown to phosphorylate Ser15 of p53 [67], a modification that is known to protect the protein from degradation and promote cell-cycle arrest during DNA damage and aberrant growth factor signaling [68]. Upon deletion of p53, nutrient deprivation results in continued cellular proliferation, with an eventual loss in cell viability, confirming the essential requirement for p53 in nutrient-dependent cell-cycle arrest [67]. This discovery has led to a more precise mechanistic understanding of how low glucose and ATP leads to cell-cycle arrest and promotes cell survival [67]. In a series of experiments expanding on this theme in *Drosophila*, it was demonstrated that defects in the cytochrome oxidase subunit Va (CoVa) induced a cell-cycle arrest in the developing eye [69]. Surprisingly, the reduction in ATP production observed in the CoVa mutant was not sufficient to arrest cell growth or interfere with the differentiation program, since cells within the eye showed many hallmark features of differentiated neurons, rod and cone cells. Consistent with the work of Jones *et al.* [67], the block in cell division was due to the activation of AMPK and subsequent phosphorylation of p53, leading to the loss of cyclin E and cell-cycle arrest at the G1 to S phase transition. Interestingly, the *Drosophila* study proved that the secondary loss of either AMPK or p53 along with CoVa rescued progression from G1 to S phase, despite lower ATP production [69]. Together, these data define a novel low-energy cell-cycle checkpoint that monitors the metabolic activity of the mitochondria before committing to another round of cell division.

In addition to the metabolic control of cell-cycle transition, efforts have been made to identify cell-cycle-dependent mechanisms that regulate mitochondrial inheritance during mitosis. In the yeast *Candida albicans*, it has been observed that the mitochondria fragment upon entrance into M phase [70]. Genetic screens were then developed to identify factors required for these morphological transitions in *Saccharomyces cerevisiae*. These screens examined mitochondrial morphology and positioning, and were the first to uncover many genes responsible for mitochondrial fission and fusion, as well as those implicated in the targeted migration of mitochondria into the bud [28,31,71–75]. However, even with this new list of essential proteins, it remains unclear how the machinery that mediates movement and division is modulated during the cell cycle. In addition to changes during mitosis, yeast mitochondria form a highly fused reticulum that is tightly associated with the nucleus during meiosis [76]. In the late tetrad state, just prior to sporulation, the mitochondria are again highly fragmented, a process that is required for the formation of viable spores [77]. In addition, one of the yeast proteins required for mitochondrial fusion, Fuzzy Onion (Fzo1p) is actively degraded by a proteasome-dependent process during mating, in response to G-protein-coupled receptor signaling initiated by the yeast α-mating factor [78]. Together, these data show that the machinery that governs mitochondrial morphology is intimately linked with the signaling cascades that initiate cell-cycle and mating transitions. In the mammalian system, overexpression of Mfn2, in addition to stimulating mitochondrial fusion, induces a cell-cycle arrest at the G1 to S transition [79]. An examination of the upstream signals affected by the expression of Mfn2 reveals that, upon addition of epidermal growth factor, the Ras GTPase is not activated and the ERK phosphorylation cascade is not engaged. The inhibition of proliferation and lack of ERK activation occurs even following expression of a form of Mfn2 that is not targeted to the mitochondria, arguing for a specific signaling role of Mfn2 in cell-cycle arrest [79]. These data suggest that Mfn2 is able to interfere with very early signaling events following receptor–ligand binding. It is unclear why this GTPase would have any effect on signaling proteins like Ras, but it is interesting to note that Ras and some of the downstream signaling effectors in this pathway, including ERK1/2, have been localized to the mitochondria (Table S1). Further studies are required to provide more conclusive insights into these mechanisms.

Mitochondria and Signaling

Unexpected Guests: K-Ras, p53 and NF-κB Signaling on the Mitochondria

Perhaps the best known example of the integration of mitochondria within an established signaling pathway is their central function within the apoptotic cascade. Morphologically, it has been determined that mitochondrial fragmentation and cristae remodeling are essential steps for cytochrome c release and cell death [80,81]. The field of apoptosis has been witness to a growing list of proteins that translocate to the mitochondria in a highly synchronized fashion, in addition to those that are systematically released from mitochondria in the dramatic cascade of events required to neatly package a dead cell. Interestingly, one of the key signal transduction GTPases, K-Ras, is a new addition to the list of proteins that translocate to the mitochondria and induce cell death [82]. K-Ras is primarily localized to the plasma membrane; however, upon phosphorylation by protein kinase C, a conformational change results in its extraction from the plasma membrane and rapid recruitment to the mitochondria [82]. Once positioned there, K-Ras interacts with Bcl-XL to promote activation-induced apoptosis of T cells [82]. The pro-death effects of mitochondrial-associated K-Ras are not completely understood, although its interactions with anti-apoptotic proteins like Bcl-XL and Bcl-2 may result in their sequestration and inactivation. Interestingly, this is not the first report of the recruitment of Ras family proteins to the mitochondria [83] and is consistent with the growing list of signaling adaptors and enzymes that can be found in mitochondrial fractions either biochemically or by fluorescence microscopy (Table S1). Given that K-Ras is known to partition selectively into lipid-based microdomains [84,85], it is possible that it could bring associated proteins, like Bcl-XL, into a novel type of mitochondrial platform that would change their functional activity. It is also conceivable that there are mitochondrial K-Ras exchange factors, GTPase activating proteins and effectors that may control the nucleotide state and function of Ras during its mitochondrial residence. In addition to K-Ras, the nuclear transcription factor p53 was found to translocate to the mitochondria during cell death and to interact directly with the anti-apoptotic proteins Bcl-2 and Bcl-XL [86]. Unlike K-Ras, mitochondrial p53 has also been shown to participate in the cytosolic activation of Bax, oligomerization of Bax and Bak and subsequent cytochrome c release [87]. The dynamic translocation of K-Ras and p53 to the mitochondria highlight the dual functionality of signaling proteins and their role in mitochondrial biology.

In the field of immunology, four independent groups recently identified a new mammalian protein called MAVS (mitochondrial anti-viral signaling protein). MAVS is an integral outer membrane protein that plays a central role in the signal transduction cascades that lead to the NF-κB stress response and type 1 interferon response [88–91]. MAVS functions as an adaptor between the viral-dsRNA-binding protein RIG-1 [92] and the effectors TRAF2 and TRAF6, which are required for the activation of NF-κB [88]. The NF-κB and interferon responses are critical for the cellular production of cytokines that alert the body to a viral infection. Interestingly, upon binding to TRAF2 and TRAF6, MAVS enters into a detergent resistant microdomain on the mitochondria, which regulates the efficacy of the downstream signaling cascades [88]. In an interesting twist, a protease encoded by the hepatitis C virus was shown to cleave MAVS, releasing it from the mitochondrial membrane and interfering with the host cell viral response [91]. Unexpectedly, these data place the mitochondria in the center of a signal transduction cascade that seemingly has no particular requirement for increased ATP consumption.

Mitochondria as a Signaling Platform

One explanation for the utilization of the mitochondrial surface in the propagation of signaling cascades is the possibility that the mitochondrion provides a unique membrane environment for the assembly of dynamic complexes (Figure 2). The lateral movement of MAVS into a detergent-resistant microdomain is consistent with this notion. Given that the lipid composition of the mitochondria is distinct from those found on the plasma membrane, the nature of the microdomain must be unique. High levels of cardiolipin in the inner membrane, for example, clearly distinguish this bilayer from all others. The lipid ceramide has been shown to form microdomains that can lead to channel formation during apoptosis [93,94], and the ganglioside GD3, which is derived from ceramide, has also been shown to translocate to mitochondrial microdomains during cell death [95,96]. Interestingly, GD3 has been shown to interfere with the activation of NF-κB through unknown mechanisms [97]. It is possible that GD3 insertion in the mitochondrial outer membrane interferes with the translocation of MAVS into a functional raft, thereby inhibiting the NF-κB survival response.

Functional microdomains may also form on the mitochondria through the lateral recruitment and amplification of phosphatidylinositol phosphates (PIPs), activated Rab GTPases, and/or protein scaffolds. Although the identity of specific species of PIPs on the mitochondria has not been clearly demonstrated, mitochondria do contain enzymes that modulate the formation of PIPs. There are at least two mitochondrial PI (5) phosphatases — synaptojanin 2A [98] and Inpp5B [99] — and interference with synaptojanin 2A recruitment has been shown to affect mitochondrial morphology [98]. Rab GTPases are known to recruit a variety of effectors, including PI kinases, phosphatases, and regulators of motility, docking and fusion [100–102]. The recruitment of these effectors contributes to the formation of functional microdomains on almost all types of intracellular membrane. Two Rab GTPases have been linked to mitochondrial function. In mammals, Rab32 is recruited to the outer membrane, where it binds protein kinase A in order to modulate mitochondrial morphology through as yet unknown mechanisms [103]. The yeast Rab GTPase Ypt11p binds to the myosin V actin motor Myo2p, and both Ypt11p and Myo2p are required to tether mitochondria within the growing bud during cell division [104]. Although our understanding of the role of Rab GTPases in mitochondrial function remains limited, it is likely that they contribute to the formation of functional surface microdomains and mitochondrial activity (Figure 2).

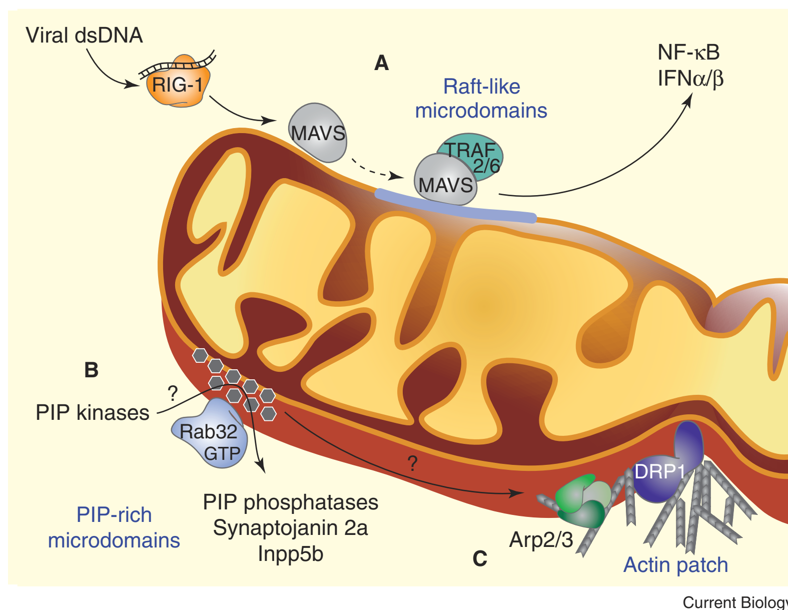

Figure 2. Assembly of lipid and protein microdomains on mitochondrial membranes. (A) Once activated by binding to viral dsRNA, the RIG-1 adaptor protein promotes incorporation of MAVS into detergent-resistent domains on mitochondrial membranes. From these raft-like domains, MAVS, together with TRAF2 and TRAF6, coordinates signals leading to NF-κB and IFNα/β activation. (B) PI phosphatases synaptojanin 2a and Innp5b and unknown PIP kinases potentially determine the composition of PIPs on mitochondrial membranes. The recruitment of these enzymes may be mediated through the action of regulatory enzymes like Rab32. The resulting enrichment in specific phosphorylated lipid species would act as a binding platform for dynamic protein complex assembly. (C) Oligomeric proteins like DRP1 localize to punctate foci on the mitochondrial surface. In yeast actin remodeling on mitochondrial membranes is performed by the Arp2/3 complex, and in the mammalian system actin promotes stable DRP1 association with mitochondrial membranes during stimulated fission. The hypothetical participation of underlying PIP domains in the assembly of DRP1 and actin polymerization is indicated by an arrow. Solid lines indicate protein interactions and reaction pathways, dotted lines represent protein and metabolite translocation events. See text for details and references.

Actin polymerization on the surface of intracellular membranes is also known to provide a stable platform for the assembly of protein complexes [105]. In yeast, the actin-nucleating complex Arp2/3 is recruited to punctate spots on the mitochondrial surface where it mediates anterograde mitochondrial motility along actin cables [106–108]. In mammals, the increased stabilization of DRP1 on mitochondria upon stimulation of fission is also regulated in part by actin polymerization [109]. Together, this indicates that actin patches, in combination with both lipid raft and PIP-rich domains, may modulate the assembly of diverse functional complexes on the mitochondrial surface.

Mitochondria and Development

The mitochondrial network displays remarkable plasticity during development of certain tissues. For example, electron microscopy studies have revealed a transition from elliptical and rod-like mitochondria in embryonic rat myocardiocytes and skeletal muscle cells to an interconnected reticulum in the cardiac muscle and diaphragm of adult animals [110–113]. This morphological transition possibly reflects timed induction of mitochondrial fusion. Supporting this idea, expression levels of Mfn2 increase during differentiation of myoblasts [36] and spermatocytes [114], correlating with the extensive mitochondrial remodeling observed in these cells.

The loss of either Mfn1 or Mfn2 is embryonic lethal, highlighting the importance of mitochondrial dynamics during development [3]. Interestingly, the Mfn2 null embryos appear relatively normal, but are resorbed by embryonic day 11.5 due to unsuccessful placental implantation. Conversely, Mfn1 null embryos, although developmentally delayed and resorbed by embryonic day 12.5, display normal implantation, suggesting that the placental malfunction observed in Mfn2 null mice is not directly related to a lack of mitochondrial fusion [3]. This is consistent with the emerging evidence that, in addition to its requirement for mitochondrial fusion, Mfn2 has additional roles both in protection from cell death [7,115,116] and in the regulation of metabolism [26,36,38]. In contrast, the data so far indicate that Mfn1 functions primarily as a core essential component of the fusion machinery. It has been shown that the heptad repeats of Mfn1 tether mitochondria together [4,5] and that Mfn1, but not Mfn2, participates with Opa1 in the fusion process [11]. In addition, the nucleotide-binding properties of Mfn1 and Mfn2 appear to be quite distinct, suggesting unique functional properties [5]. Mfn1 binds to nucleotide with low affinity and shows high rates of hydrolysis for GTP, consistent with its evolutionary relationship to the dynamin family of GTPases [5]. Although Mfn1 and Mfn2 have 60% sequence identity, Mfn2 binds nucleotides with high affinity and exhibits slow intrinsic hydrolysis rates reminiscent of the Rab family of regulatory GTPases [5,7]. Collectively, these findings strongly indicate that Mfn1 and Mfn2 are functionally distinct GTPases that are both essential for mitochondrial fusion and embryonic development.

Mitochondrial Responses in Neuronal Function

The genetic and cell biology systems used by neurobiologists have provided important new insights into the functional requirement and spatial positioning of mitochondria in polarized neurons both during development and within the adult. For example, recent experiments showed that local administration of nerve growth factor (NGF) to an axon of a primary neuron in culture increases mitochondrial movement into the stimulated region and triggers the arrest of anterograde-directed mitochondria. This arrest results in the accumulation of mitochondria within the vicinity

of the activated NGF receptor TrkA and is dependent upon activation of PI 3-kinase and actin polymerization [117]. These data imply that a signal transduction cascade downstream from TrkA results in the modulation of mitochondrial motility in active growth cones of developing neurons [117]. Interestingly, the actin-remodelling GTPase RhoA and its formin effector mDia1 play a role in the regulated arrest of mitochondrial motility in mammalian cells and in a *Drosophila* neuronal cell line [118], providing molecular cues responsible for actin-mediated modifications of mitochondrial movement.

Mitochondria are highly responsive to synaptic stimulation. In hippocampal neurons, dendritic mitochondria rapidly fragment and cluster in the proximity of dendritic spines in response to neuronal activity [119]. Activity also decreases mitochondrial motility, arguing for the existence of specific signals regulating mitochondrial behavior. Interestingly, overexpression of DRP1 results in an increased number of mitochondria within the dendrite, as well as an increased density of dendritic spines and synapses [119]. Conversely, expression of a dominant-negative DRP1 mutant results in fused mitochondria that remain primarily within the soma, resulting in very few mitochondria migrating into the dendrite [119]. Depletion of dendritic mitochondria upon expression of the mutant DRP1 has an adverse effect on spine development, suggesting that mitochondrial function is required and limiting for the formation and maintenance of synapses.

In the adult neuron, mitochondria are enriched at the synapse, which is commonly explained by the important functional role of mitochondria in buffering calcium fluxes during synaptic transmission and providing energy for synaptic vesicle release and recycling. However, experiments designed to remove mitochondria selectively from the synapse suggest that many of these assumptions are overly simplistic. Mutations in DRP1 in *Drosophila* are semilethal, with the survivors suffering neurodegeneration and lack of coordination (termed the *Fratboy* mutant) [120]. The mitochondria within mutant neurons are clustered and fused within the soma, with few migrating into synapses. Interestingly, although the resting cytosolic calcium within these synapses is increased, neurotransmitter release remains normal upon calcium stimulation. In addition, synaptic vesicle endocytosis proceeds normally, indicating that, even in mitochondria-depleted terminals, there is sufficient energy to drive this process. Intriguingly, prolonged stimulation reveals defective migration of the reserve pool of synaptic vesicles to the membrane [120]. This phenotype is due to a specific requirement for mitochondrial ATP in the activation of myosin motors required to mobilize the reserve pool of vesicles to the plasma membrane. Since the role of mitochondria in calcium buffering and local energy production has been well established, it is quite surprising that these neurons are so functional in the absence of mitochondria.

Mutations in *Drosophila* Miro1, an integral outer membrane protein with two Rho-like GTPase domains and calcium-binding EF hand motifs result in a loss of anterograde mitochondrial delivery into the axon [121]. Flies lacking dMiro1 die at the larval stage due to numerous defects, and the mitochondrial distribution in muscles and neurons is limited to the cell body. Similar to the DRP1 mutants, both basal and fast calcium-stimulated neurotransmission are relatively normal, with functional defects observed mainly upon chronic stimulation. At the neuromuscular junction, the presynaptic boutons in the dMiro mutants are more abundant, smaller, structurally deformed and positioned close together. The increase in synaptic boutons in the absence of mitochondria is unexpected and also in contrast with the observed decrease in dendritic spines observed in mitochondria-depleted neurons expressing mutant DRP1 [119]. In general, the seemingly complex involvement of mitochondria in developmental processes suggests that the positioning of the mitochondria may be selectively required for the propagation of developmental signaling cascades. Future experiments will certainly shed more light on these exciting new aspects of mitochondrial function.

Conclusions and Perspectives

This review has focused on the idea that the mitochondria are intimately embedded in the signaling cascades and programs that operate within the cell. Evidence is emerging that these links include functions that extend beyond the primary role of mitochondria as ATP generators. We have supplied examples to illustrate mechanisms by which the mitochondria can receive cellular signals and propagate a targeted response. These examples include intuitive responses, such as altering the position of mitochondria within the cell in order to provide a local ATP supply, but also more surprising responses, such as the control of cell cycle and the dynamic modulation of respiratory capacity. In many cases, we have suggested that the interface between the cellular signal and mitochondrial response hinges upon the activity of a new cast of proteins that modulates mitochondrial morphology and movement. Some of these proteins appear to be unique mitochondrial proteins and enzymes, and others derive from the more familiar families of proteins, such as GTPases, lipid-modifying enzymes, kinases and phosphatases (Table S1).

By delving into the complex interconnected nature of mitochondria and cellular physiology, we will contribute to the development of intelligent treatment strategies for diseases characterized by mitochondrial dysfunction. Many of these diseases result from mutations in proteins that modulate mitochondrial behavior. Mutations in Mfn2 result in the disease Charcot-Marie Tooth Type 2A, characterized by a loss of peripheral motor neurons [122], and mutations in Opa1 (and recently also Mfn2) are responsible for autosomal dominant optic atrophy, causing progressive blindness [8,123]. Similarly, understanding the links to cell cycle and apoptosis will provide new concepts in the treatment of cancer, and the emerging understanding of the kinases and phosphatases involved in Parkinson's disease will shed new light on neurodegenerative conditions. In addition to the monogenic mitochondrial diseases, a better understanding of dynamic metabolic regulation will help to control diabetes, obesity, and complex mitochondrial myopathies. The fundamental insights to be gained through the investigation of dynamic mitochondrial function and the integration

of this organelle in cell signaling pathways will have far-reaching implications in many areas of biology.

Supplemental data

A supplemental table listing the proteins that modulate mitochondrial morphology and signaling is available at http://www.current-biology.com/cgi/content/full/16/14/R551/DC1/.

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