Review

Positioning mitochondrial plasticity within cellular signaling cascades

Vincent Soubannier, Heidi M. McBride *

University of Ottawa Heart Institute, 40 Ruskin Street, Ottawa, ON, Canada K1Y 4W7

ARTICLE INFO

ABSTRACT

Mitochondria evolved from alpha-proteobacteria captured within a host between two and three billion years ago. This origin resulted in the formation of a double-layered organelle resulting in four distinct sub-compartments: the outer membrane, the intermembrane space, the inner membrane and the matrix. The inner membrane is organized in cristae, harboring the respiratory chain and ATP synthase complexes responsible of the oxidative phosphorylation, the main energy-generating system of the cell. It is generally considered that the ultrastructure of the inner membrane provides a large variety of morphologies that facilitate metabolic output. This classical view of mitochondria as bean-shaped organelles was static until in the last decade when new imaging studies and genetic screens provided a more accurate description of a dynamic mitochondrial reticulum that fuse and divide continuously. Since then significant findings have been made in the study of machineries responsible for fusion, fission and motility, however the mechanisms and signals that regulate mitochondrial dynamics are only beginning to emerge. A growing body of evidence indicates that metabolic and cellular signals influence mitochondrial dynamics, leading to a new understanding of how changes in mitochondrial shape can have a profound impact on the functional output of the organelle. The mechanisms that regulate mitochondrial morphology are incompletely understood, but evidence to date suggests that the morphology machinery is modulated through the use of post-translational modifications, including nucleotide-binding proteins, phosphorylation, ubiquitination, SUMOylation, and changes in the lipid environment. This review focuses on the molecular switches that control mitochondrial dynamics and the integration of mitochondrial morphology within cellular signaling cascades.

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1. Introduction

Mitochondrial morphology is tightly regulated by balanced fusion and fission events. These processes require several factors, many of them conserved during evolution. The fundamental importance of maintaining a highly dynamic mitochondrial reticulum is demonstrated by the essential nature of most of the proteins described in this section. Mice lacking the core fusion machinery are embryonic lethal, and there are a number of human diseases caused by mutations in both the fusion and fission proteins (reviewed in [1]). In the first section, we will introduce the machinery that has been characterized through the use of genetic and biochemical screens in multiple experimental model systems (Fig. 1). The second section of this review will expand upon the physiological importance of mitochondrial dynamics, and how these processes are integrated within cellular signaling pathways (Fig. 2).

2. Machineries that govern mitochondrial dynamics

2.1. Mitochondrial fusion machinery

2.1.1. Outer membrane fusion machinery

The central components of the mitochondrial fusion machinery include the mitofusin GTPases. Mammals have two *Mitofusin* homologues, namely Mfn1 and Mfn2, that are anchored in the mitochondrial outer membrane, while Fzo1 (*Fuzzy Onion 1*) is the single yeast orthologue [2]. These large, dynamin-like proteins contain an N-terminal GTPase domain and two coiled-coil domains both exposed to the cytosol [3,4]. Examination of the structure of the heptad repeats within Mfn1 suggests that Mfn1 tethers mitochondria by forming coiled-coil interactions with another Mfn1 protein in *trans* [5]. Data from isolated yeast mitochondria have shown that fusion requires GTP hydrolysis, suggesting that nucleotide hydrolysis by *trans*-paired Fzo1 may drive mitochondrial fusion forward [6]. Although Mfn1 and Mfn2 can form homo and hetero-oligomeric complexes and have partially overlapping functions [7], recent evidence suggests that Mfn2 may have evolved additional, more specialized roles relative to Mfn1 or Fzo1 [8]. For example, the GTPase activities of the two mitofusins are different, with Mfn1 possessing very fast rates of hydrolysis and low affinity for nucleotide, consistent with a dynamin-like function,

* Corresponding author. Tel.: +1 613 761 4701; fax: +1 613 761 5281.
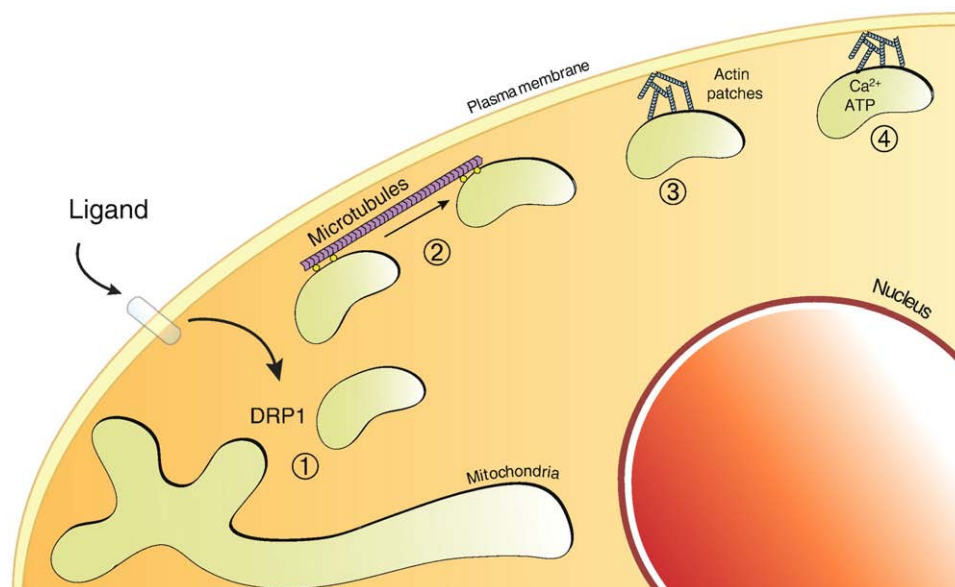E-mail address: hmcbride@ottawaheart.ca (H.M. McBride).

Fig. 1. Integration of mitochondrial dynamics within cellular signaling cascades. Evidence described within the text indicates that the activation of mitochondrial fission, fusion and motility can be regulated by cellular cues. The example shown in (1) refers to conditions of hyperglycemia where the uptake of glucose leads to the activation of Drp1 and mitochondrial fission. In (2), the long-range motility requires the action of kinesins, dyneins, Milton and Miro, and has been shown to be regulated by calcium. The regulation and activation of these proteins on the mitochondrial surface is not yet clear. In (3), the transition of mitochondria from long-range movement along microtubules to short range movement, or the anchoring of mitochondria to the actin cytoskeleton upon activation of TrkA receptors occurs in a PI(3)kinase-dependent manner. In another example, the NMDA receptor dependent movement of mitochondria into dendritic spines has been shown to involve the activity of WAVE1, which recruits the Arp2/3 complex to the mitochondria, thereby driving the production of actin filaments on the surface. Finally, in (4), mitochondria at their anchored destination fulfill important, cell type specific functions, including the production of ATP for the myosin driven movement of T-lymphocytes, the buffering of calcium within neuronal and immunological synapses, etc. See text for more examples of the regulation of these mitochondrial transitions in morphology and position.

whereas Mfn2 has a very low intrinsic rate of hydrolysis and high affinity for nucleotide [9,10], which is consistent with the more regulatory GTPases. An elegant *in vitro* tethering assay demonstrated a significantly enhanced role for Mfn1 in driving mitochondrial tethering relative to Mfn2, further highlighting their potentially unique contributions to mitochondrial docking and fusion [9]. In addition, the creation of a hydrolysis deficient mutant of Mfn2 led to the observation that GTP-bound Mfn2 stimulated mitochondrial fusion, which indicates that hydrolysis of GTP by Mfn2 is not required for mitochondrial fusion [10]. The stimulation of fusion while in the GTP-bound form is instead consistent with a role as a regulatory GTPase. Whether the nucleotide states of Mfn1 and Mfn2 are regulated by exchange factors or GTPase activating proteins is unknown, but future experiments will help to shed light on the functional role of their GTP switches. In addition to their roles in mitochondrial fusion, several lines of evidence suggest that the two mitofusins are also essential during development, in the progression of apoptosis, and in disease [8]. For example, the loss of both Mfn1 and Mfn2 results in embryonic lethality, however the loss of Mfn2 leads to a defect in placental implantation, whereas the loss of Mfn1 results in embryonic lethality a day or two later [7]. In conditional knock-out mice, Mfn2, but not Mfn1, was required for dendrite growth and spine formation, and the loss of Mfn2 led to the death of Purkinje cells [11]. These animal models show that Mfn1 and Mfn2 have distinct physiological roles and may begin to explain why only mutations in Mfn2 have been found in human disease like Charcott-Marie Tooth type 2A or autosomal dominant optical atrophy (reviewed in [12]).

The identification of new binding partners for the mitofusins that demonstrate nucleotide specificity would also help to uncover the mechanisms that regulate mitochondrial fusion. Some binding partners have been identified, however their nucleotide specificity is not always clear. One regulator of the activity of the mitofusins has been identified as *Mi*tofusin-*b*inding protein (MIB), a cytoplasmic member of the medium chain dehydrogenase/reductase protein superfamily [13]. The function and localization of the proteins of

this superfamily are largely unknown. MIB was shown to interact with both Mfn1 and Mfn2 but the regulation of the targeting mechanism of MIB to the mitochondria remains to be elucidated. Overexpression of MIB led to mitochondrial fragmentation, by inhibiting Mfn1 activity [13]. In addition, MIB regulation of mitochondrial morphology requires the functional *c*oenzyme-*b*inding *d*omain (CBD). The modulation of MIB activity appears to be essential for cell growth but not for apoptosis. It was hypothesized that the CBD domain of MIB could recognize the GTP-bound form of mitofusins, thus controlling the GTPase activity in a manner linked to the dehydrogenase activity of the enzyme, but this has not been tested directly.

Crosslinking and immunoprecipitation experiments identified that *s*tomatin-*l*ike *p*rotein2 (Slp-2) interacts with Mfn2 [14]. Slp-2 is a mitochondrial protein that is processed upon import and remains associated with the inner membrane, facing the intermembrane space [14]. It was shown to also bind prohibitins and play a role in their stability, along with that of other proteins including complexes I and IV [15]. As a member of the stomatin and prohibitin/flotillin family of proteins, Slp-2 is also incorporated within cholesterol enriched microdomains [16–18]. Higher expression levels of Slp-2 have been associated with increased cellular proliferation, and it is emerging as a new marker for more aggressive cancer, however the links between these phenotypes and the mitochondrial function of Slp-2 are unknown [19–21]. Knockdown of Slp-2 showed a slight decrease in mitochondrial potential but did not induce a dramatic change in mitochondrial morphology, indicating that this protein is not essential for mitochondrial fusion [14]. It is not yet known if this interaction can functionally modulate Mfn2 activity, or how it may bind Mfn2 from its intermembrane space location. Further work is needed to determine the importance of this interaction in the regulation of mitochondrial dynamics.

Genetic screens in yeast identified additional proteins required to maintain mitochondrial morphology and distribution, and some of them appear to be conserved during evolution where others have no obvious mammalian counterpart. Ugo1p (Ugo means fusion in

Japanese) was identified from genetic screens in yeast as a required component for mitochondrial fusion [22]. No mammalian orthologues have been identified to date. It is a multipass transmembrane protein of the outer membrane containing a putative carrier domain and is found in a protein complex with both Fzo1 and the inner membrane GTPase Mgm1 (see below) [23–25]. This led to the speculation that Ugo1 may be an adaptor connecting these two GTPases in order to coordinate the fusion of the outer membrane with the initiation of Mgm1-mediated inner membrane fusion.

Regulation of mitochondrial fusion depends not only on protein complexes but also on changes in membrane properties. In mammals, an outer membrane, *mito*chondrial-anchored *pho*spho*lipase D* protein, MitoPLD, has been shown to be required for mitochondrial fusion [26]. The enzymatic activity of MitoPLD is localized to the cytosolic surface of the organelle where it hydrolyses cardiolipin to generate phosphatidic acid (PA), a fusogenic lipid. The role of PA in mitochondrial fusion is still undetermined; although it appears to function at a point after Mfn1/2-dependent tethering since its silencing leads to docked mitochondria that cannot fuse [26]. The local changes in lipid composition at a site of docking may alter the membrane curvature to facilitate fusion of opposing membranes, or recruit additional factors that may participate in this process.

2.1.2. Inner membrane fusion machinery

Mitochondrial fusion is a complex reaction due to the presence of two membranes that require the existence of two distinct machineries. *In vitro* fusion assays using mitochondria isolated from yeast cells revealed that the two fusion events are distinct and have different energy requirements [6,27]. The large GTPase OPA1 (*O*ptical *A*trophy 1), or Mgm1 (*m*itochondrial *g*enome *m*aintenance) in yeast, plays an essential role in mitochondrial inner membrane fusion [2]. Mgm1 and OPA1 are found in the intermembrane space associated with the inner membrane and their function is linked to that of Fzo1 and Mfn1 respectively [23,24,28]. OPA1, but not Mgm1, is present in eight mRNA splice forms that can undergo proteolytic processing in mitochondria, producing a complex mixture of long and short OPA1 forms [29]. Recently, it has been clarified that the presence of both long and short OPA1 or Mgm1 products is important for mitochondrial fusion activity [30–35]. Early studies demonstrated that Mgm1 is cleaved by Pcp1/Rbd1 (*p*rotease of *c*ytochrome *c* *p*eroxidase 1/*Rhom*boid protease 1), a rhomboid protease localized in the inner membrane [30–32]. The balance between the two cleaved forms of Mgm1 is governed by a mechanism called alternative topogenesis that depends on the mitochondrial import motor and on the matrix ATP level [36]. It has been suggested that this mechanism may prevent mitochondria presenting unfavorable bioenergetics from fusing into the rest of the mitochondrial network [33,36].

Although the function of OPA1 and Mgm1 in regulating mitochondrial fusion is conserved, OPA1 processing appears to have evolved quite differently. Understanding the identity and regulation of the proteases that cleave OPA1 has been made technically challenging due to the fact that there are two proteolytic sites S1 and S2 within the 8 splice variants which generate a number of short isoforms. Perhaps it is not surprising then that the identity of the protease activated in this condition is currently the subject of intense investigation and some controversy. Several studies have demonstrated that the rhomboid protease PARL, the mammalian orthologue of Pcp1, is not the primary protease that cleaves OPA1 at S2 [37]. Instead, it appears that mitochondrial AAA proteases are involved in this process. The i-AAA protease Yme1L appears to be a central protease responsible for OPA1 cleavage at the S2 site [34,35,38], although it is also possible that matrix-AAA proteases and prohibitins contribute to this process [39–41]. The reason for this evolutionary change in the mechanism of OPA1 cleavage is not yet understood. However while the metabolic status of the mitochondria determines the topology of Mgm1 during import, OPA1 cleavage at the S1 site and the degradation of the long forms is stimulated upon the loss of electrochemical potential, and during apoptosis [33,38]. This may reflect an improvement in the responsiveness of mammalian mitochondria to damaging cellular triggers relative to yeast.

2.1.3. Regulation of the mitochondrial fusion machinery

The list of proteins that function as core machinery in the fusion reaction remains relatively short. However, there are a number of other proteins implicated in the fusion reaction that likely play a more regulatory role. These proteins modify the function or protein levels of the core machinery and are described below.

Ups1 (*Un*processed 1) is a yeast mitochondrial intermembrane space protein peripherally associated with the inner membrane [42]. Ups1 regulates the alternative topogenesis of Mgm1p in the inner membrane but is not part of the protein import machinery. Ups1 expression is required for the formation of the short, cleaved form of Mgm1p when cells are grown in the fermentable carbon source glucose. This regulation by Ups1 was not observed when cells are grown on non-fermentable carbon source. The specific requirement for Ups1 suggests that this protein is essential to assume control of mitochondrial dynamics under certain metabolic conditions. PRELI (*p*rotein of *r*elevant *e*volutionary and *l*ymphoid *i*nterest) is the human orthologue of Ups1 [43]. When expressed in *ups1Δ* yeast cells, PRELI was efficiently targeted to the mitochondria and functionally rescued the *ups1Δ* phenotype, indicating that the function of Ups1 is evolutionarily conserved among eukaryotes [42]. The mechanism of action of Ups1 will require further investigation.

Another protein, Mdm38 (*m*itochondrial *d*istribution and *mo*rphology 38), has been identified from genetic screens in yeast [44], and its mammalian orthologue is called LETM1 (*1*eucine zipper-, *EF*-hand-containing *trans*membrane protein 1) [45]. Mutations in LETM1 lead to Wolf–Hirschhorn disease, a devastating condition marked by severe growth retardation and epileptic seizures, generally leading to early death [46,47]. LETM1 is an inner membrane anchored protein containing two calcium-binding EF-hand motifs, a leucine zipper and several coiled-coil domains [46]. Loss of LETM1 leads to a fragmented and swollen mitochondrial phenotype. Recent experiments have shown that LETM1 plays a critical role in the exchange of K^+ for H^+ across the inner membrane, and therefore controls ion homeostasis [48,49]. The loss of this protein leads to an influx of water, which likely explains the swollen phenotype. Addition of nigericin, a K^+/H^+ ionophore, rescued the swelling and restored mitochondrial fusion in cells lacking LETM1 [49]. An alternative role as been proposed from a study showing that Mdm38 functions as a component of an insertion machinery in the inner membrane of certain mitochondrially encoded proteins [50]. Therefore, although Mdm38/LETM1 is required for the maintenance of mitochondrial morphology, the impact of this protein on the fusion machinery is likely indirect.

Factors regulating the core outer membrane fusion machinery have also been identified. Mdm30p is a yeast member of the Skp1-Cullin-F-box (SCF) ubiquitin ligase family, a family known to play a role in the regulated turnover and degradation of a number of cellular proteins. Consistent with this, Mdm30p was shown to play a role in mitochondrial fusion by controlling the turnover of Fzo1 in vegetative growing cells [51]. The loss of Mdm30 leads to mitochondrial aggregation and a failure to respire [51,52]. It was subsequently shown that Mdm30 is required to bind to Fzo1 and mediates its degradation [52,53]. Mdm30-dependent turnover of Fzo1 during vegetative growth was initially shown to occur even upon inactivation of the proteasome and/or the vacuole, indicating the presence of an unidentified mechanism for Mdm30-dependent mitochondrial outer membrane protein turnover [53]. However, more recently, it was shown that Mdm30 mediated degradation of Fzo1 during vegetative growth did depend upon the Skp1-Cullin-F-box (SCF) ubiquitin ligases, and the targeting of Fzo1 to the 26S proteasome [52]. Interestingly, Fzo1 mediated turnover through the proteasome

triggered by the addition of the mating pheromone α-factor is Mdm30 independent [54], suggesting more than one mechanism for the regulation of Fzo1 turnover depending on the state of cellular growth. The regulation of Mdm30 activity and the signals that drive Fzo1 ubiquitination will ultimately reveal important crosstalk between the triggers of cellular growth and mitochondrial morphology.

Interestingly, Mfb1 (*m*itochondria-associated *F-bo*x protein) is another yeast mitochondria-associated F-box protein, which is also required to maintain mitochondrial morphology [55,56]. Although yeast cells lacking Mfb1 are fusion competent, their mitochondria become slightly fragmented. During yeast cell division, Mfb1 remains within the mother cell and does not target the mitochondria within the bud, providing evidence of its asymmetric distribution during the cell cycle. The presence of two distinct F-box proteins on the mitochondria supports the idea that ubiquitination and mitochondrial protein turnover play important roles in the regulation of mitochondrial dynamics and cell cycle transition. The characterization of their targets will be an important goal of future experiments.

Recently Okamoto and co-workers reported that two of the TOM mitochondrial import receptors (*T*ranslocase of the *O*uter *M*embrane), Tom70 and Tom71, recruit Mfb1 to the mitochondrial surface [57]. Proteins of mitochondrial import machineries have already been implicated in the mitochondrial morphology and distribution (reviewed in [58]). This is perhaps not surprising given that impairment of mitochondrial import activity could have secondary effects on the import of components that regulate mitochondrial morphology. A typical example is the role of the mitochondrial import motor and the alternative topogenesis of Mgm1 [36]. Also in yeast, the import of the mitochondrial morphology protein Mdm35 requires the MIA pathway (*m*itochondria *i*mport and *a*ssembly), the specific machinery for import and assembly of intermembrane space proteins [59]. Nevertheless, it cannot be excluded that some factors of mitochondrial import may have a dual role and could influence the shape of the organelle. These factors would orchestrate the crosstalk between mitochondrial biogenesis and morphology by recruiting and modulating the activity of the morphology machinery.

In summary, there are a number of factors that have been identified as components of the mitochondrial fusion machinery. These represent the starting point to continue our exploration of the mitochondrial fusion reaction, including the molecular mechanisms that define mitochondrial docking, the formation of the fusion pore and bilayer mixing. The cellular regulation of fusion and the co-ordination of outer and inner membrane fusion are of critical importance for us to complete this very intricate cell biological puzzle.

2.2. Mitochondrial fission machinery

2.2.1. Outer membrane fission machinery

The central player that mediates mitochondrial fission is the mitochondrial *d*ynamin-*r*elated *p*rotein, Drp1, or Dnm1 in *S. cerevisiae* [2]. Drp1 is a cytosolic protein that is recruited to the mitochondrial outer membrane where it oligomerizes into ring-like structures that constrict the organelle in a GTP-dependent manner [60]. Drp1 forms punctate foci on the mitochondria but the mechanism of initial Drp1 recruitment is largely unknown. These punctate foci of Drp1 appear to migrate towards a site of mitochondrial fission, where they assemble into a functional scission complex. As predicted from its homology to Dynamin 1, the yeast Dnm1 has been shown directly to form oligomeric ring-like structures that require GTP hydrolysis for constriction and disassembly [60]. A second mitochondrial fission protein, called Fis1 (*Fis*sion protein *1*) was originally identified as an essential fission protein from yeast genetic screens [61]. Fis1 is a small, integral membrane protein containing a cytosolic exposed tetratrico-peptide repeat domain which mediates protein interactions. Fis1 is required for yeast Dnm1 recruitment to the mitochondria, but in mammalian systems, the human hFis1 is not essential for Drp1

recruitment [62,63]. However Fis1 is required for Dnm1 to assemble into the functional oligomers that drive the scission event [61,64,65]. The molecular link between hFis1 and Drp1 likely requires an adaptor protein with functional similarity to the yeast Mdv1 (*M*itochondrial *d*ivision *1*) and Caf4 (*Ca*ffeine-resistant *4*) [65–68]. Mdv1 is required for mitochondrial fission in yeast, and Caf4 is a very similar protein that appears to have an overlapping function. Both are soluble, cytosolic proteins that contain WD40 repeat domains and are known to form a complex with Dnm1 and Fis1. The human orthologue of these proteins remains elusive.

Other factors modulating mitochondrial division have also been reported. GDAP1 (*g*anglioside-induced *d*ifferentiation *a*ssociated *pro*tein *1*) is a mammalian mitochondrial outer membrane protein that regulates the mitochondrial network by promoting mitochondrial fission [69,70]. GDAP1 contains a characteristic Glutathione-*S*-Trans-ferase domain (GST) and a C-terminal hydrophobic signal-anchor sequence required for its activity. Given that GDAP1 contains a GST motif, it has been speculated that GDAP1 could provide a mechanism to regulate mitochondrial fusion based on the redox state of the mitochondria [70]. Interestingly, GDAP1 mutations have been associated with pathogenesis of Charcot-Marie-Tooth type 4A disease [71–73], supporting an essential role of GDAP1 in the control of mitochondrial dynamics and human physiology.

Just as mitochondrial fusion depends upon a mitochondrial-anchored phospholipase D to modulate the lipid composition at the site of fusion, mitochondrial fission also requires at least one protein known to alter membrane properties. Endophilin B1 (Bif1/SH3GLB1), a protein related to endophilin1, is recruited to the mitochondria where it is required for mitochondrial fission [74]. Endophilin B1 cycles between the cytosol and the membrane where it binds through its *B*in-*A*mphiphysin-*R*vs (BAR) domain. This domain is thought to either sense, or generate membrane curvature that would help facilitate the membrane pinching that is required during fission [75]. Interestingly, the subcellular distribution of Endophilin B1 is governed by both autophagy [76] and apoptotic signals [77]. It has been shown to bind to Beclin1 and during nutrient depletion, it colocalizes with the autophagy related proteins, Atg9, LC3 and Atg5 within punctate foci [76]. This complex appears to assemble on small vesicles that fuse together to form the pre-autophagosome. Whether or not this function in autophagy requires mitochondrial membranes remains unclear. However, Drp1 was recently shown to be required for mitochondrial turnover through autophagy [78], hinting that Endophilin B1 may be important in regulating mitochondrial fission and Drp1 function during autophagy.

It seems likely that mitochondrial fission plays a specific and critical role during autophagy. Shirihai and co-workers reported that Drp1-dependent mitochondrial fission can result in an asymmetry within the two dividing mitochondria, where one of the two mitochondria has conserved a high electrochemical potential, and the other has a reduced membrane potential and low levels of the fusion GTPase OPA1. This loss of OPA1 could render some of these fragments with low $\Delta\Psi$ to become fusion incompetent, leading to their ultimate removal by the autophagosome [78]. It was similarly reported that mitochondrial fission induced by nitric oxide could be asymmetric [79], where one daughter mitochondria retained normal ultrastructure while the other one displayed disorganized cristae structure. The segregation of mitochondrial fragments that are fusion incompetent and instead somehow targeted for specific autophagic turnover is an emerging area of study.

2.2.2. Inner membrane fission machinery

Several observations support the model that both mitochondrial membranes utilize independent machinery that functionally sepa-rates the fission of the mitochondrial inner membrane and the outer membrane. However, we have not yet identified any absolute candidate proteins that play a core function to drive mitochondrial

inner membrane fission. Genetic screens in yeast have identified the inner membrane protein Mdm33 [44], which is anchored within the inner membrane with its N-termini exposed to the matrix and its C-termini domain containing coiled-coil motifs facing the intermembrane space. Biochemical analysis revealed that Mdm33 also forms higher order oligomers [80]. The loss of Mdm33 function led to highly fused mitochondria, with the resulting organelles displaying alterations within the internal cristae structure of mitochondria with elongated stretches of outer and inner membranes enclosing a very narrow matrix space [80]. From these data, Mdm33 has been proposed to play a specific role in fission of the mitochondrial inner membrane. The function of Mdm33 and potential interacting partners implicated in the maintenance of the inner membrane architecture and dynamics are still uncharacterized. To date, no homologue of Mdm33 has been reported in higher eukaryotes. However, in mammals, an intermembrane space protein anchored to the inner membrane, MTP18 (mitochondrial protein, 18 kDa), was shown to be required for mitochondrial fission [81,82]. Considering its localization, it is tempting to speculate that MTP18 could control the concerted fission of outer and inner membranes. The regulation of this component is remarkable since it has been first described as a transcriptional downstream target of phosphatidylinositol (PI) 3-kinase signaling and is essential for cell viability [81]. This protein could represent a new target for the modulation of mitochondrial dynamics in response to physiological changes in the cellular environment.

Finally, Dap3 (death associated protein 3) is a mitochondrial matrix protein containing a GTP-binding motif and was identified as another regulator of mitochondrial fragmentation [83]. Dap3 is a mitochondrial ribosomal protein that plays a role in mitochondrial protein synthesis in healthy cells [84]. Transient overexpression of Dap3 induced fragmentation of the mitochondria and this effect was dependent on its GTP-binding activity. However, Dap3 RNAi treated cells show a normal mitochondrial phenotype [83], indicating its role as a regulatory factor rather than a core component of any fission complexes. As with other members of the fission machinery, Dap3 was also shown to have a conserved role in apoptosis pathways [84,85]. Silencing of Dap3 did display a significant reduction of mitochondrial fragmentation induced by apoptosis [83], suggesting that Dap3 is likely activated during cell death to promote mitochondrial fragmentation. It has been proposed that the activation of Dap3 may involve the phosphorylation of residues around the GTP-binding motif [86], however future work is required to determine the potential kinases/phosphatases that regulate the function of Dap3 and its role in the regulation of mitochondrial fragmentation and cell death.

2.2.3. Regulation of mitochondrial fission

Recent discoveries have demonstrated that mitochondrial division is also regulated by ubiquitination, SUMOylation and phosphorylation [87–91]. MARCHV/MITOL is a mitochondrial ubiquitin ligase that spans the outer membrane of mitochondria four times, with the conserved RING-finger domain exposed to the cytosol [87,92,93]. This ligase associates with both hFis1 and Drp1 and mediates the ubiquitination of these fission components to promote mitochondrial fission. In addition to these interactions with the fission machinery, MARCHV was also shown to bind Mfn2, but not Mfn1 [87]. Although biochemical experiments have shown these interactions with both the fusion and fission machinery, the silencing of MARCHV led to an inhibition of mitochondrial fission, resulting in highly elongated mitochondria. This suggests a functional requirement for MARCHV in the activity of Drp1 and hFis1, rather than Mfn2. Experiments overexpressing MARCHV or mutants of the protein did not lead to changes in the protein levels of its potential targets, suggesting that the ubiquitination activity of MARCHV does not lead to changes in protein turnover, instead playing a significant role in the regulation of fission complex assembly or disassembly.

Drp1 cycling between the cytosol and the membrane is also dependent on SUMOylation, [63,88,89]. SUMO (Small ubiquitin-like modifier) is a small protein that, like ubiquitin, becomes covalently conjugated to target substrate proteins through a series of enzymatic reactions involving a heterodimeric E1 enzyme, an E2 SUMO ligase called UBC9, and a SUMO E3 ligase, which provides substrate specificity. The consequence of SUMOylation upon its target varies, but generally leads to a change in protein conformation resulting in altered complex assembly, localization or function (for review see [94]). Initially discovered through a yeast two-hybrid interaction screen, the SUMOylation of Drp1 is a transient, sub-stoicheometric modification [88]. Overexpression of SUMO1 [88], or silencing of the specific SUMO/Sentrin protease 5 (SenP5) [89], both lead to an increase in the stability of Drp1 from degradation, and increase the membrane association of Drp1 and mitochondrial fragmentation. Importantly, there are a number of unidentified mitochondrial proteins that are a target of SUMOylation [88,89] indicating that the identification of the enzymes that regulate these modifications will lend important insights into the physiological importance of these processes.

Finally, Drp1 is also phosphorylated by cyclin B1, cAMP and calcium signals [90,91,95]. The functional consequence of Drp1 phosphorylation depends upon which residue is modified. The regulation of mitochondrial fission through post-translational modifications is providing new insights into how this process can be responsive to intracellular signals. These links are highlighted in the later sections of this review.

2.3. Cristae architecture

The mitochondrial inner membrane is highly folded to form cristae emerging from the membrane through tubular connections called crista junctions [96]. This internal structure is highly plastic and is altered depending on the metabolic state of the organelle [97]. Cristae morphology has been shown to vary between two extreme morphologies, namely the condensed and the orthodox state [98,99]. The orthodox state shows narrow cristae presenting few junctions with the inner membrane space together with an expanded matrix. Conversely, the condensed state is characterized by large cristae with multiple cristae junctions and a condensed matrix. It appears that the conversion between these two states requires the fusion and fission of the inner membrane, suggesting that these internal morphological changes are partially dependent on the machineries governing membrane remodeling of the organelle. This is in agreement with the role of OPA1 in maintaining cristae structure [100,101]. In OPA1 RNAi treated cells, the mitochondria display disorganized cristae and enlarged cristae junctions, while in cells overexpressing OPA1, narrow cristae and cristae junctions are observed {Olichon, 2003 #84; Frezza, 2006 #679}. The role of OPA1 in regulating the mitochondrial cristae structure remains to be clarified. Nevertheless, it appears that the function of OPA1 in cristae structure can be dissociated from its function in mitochondrial fusion [101]. Another important element controlling the cristae structure is the oligomerization of ATP synthase [102,103]. In yeast, oligomerization of ATP synthase is dependent on the dimerization of the complex and is required to generate mitochondrial cristae [102]. Dimerization of ATP synthase is mediated by the interface composed of subunits e and g [104–106]. Recently, phosphorylation of a serine residue of subunit g has been shown to regulate the dimerization of the complex, describing a new mechanism in the regulation of mitochondrial inner membrane dynamics [107]. The kinase, phosphatase and general signaling cascades responsible for regulating subunit g phosphorylation are unknown. Moreover, a link between Mgm1 and the ATP synthase has been proposed where Mgm1 could act as an upstream regulator of the subunit e stability [108].

In addition to metabolic transitions, changes in the mitochondrial cristae are known to accompany programmed cell death. It has been observed that the cristae junctions are disassembled during cell death, leading to an increased accessibility of proteins like cytochrome *c* to the outer membrane permeability transition pore [109–111]. The reorganization of the cristae structures during cell death also involves OPA1, but Drp1 appears to lie upstream of cristae remodeling during cell death [110]. This is likely due to the recruitment of factors like Endophilin B1 that may change the fluidity or curvature of the outer mitochondrial membrane [74,77]. One could imagine these changes affecting the stability of the contact sites and cristae junctions, leading to inner membrane remodeling. However, the current list of proteins that modulate inner membrane dynamics is insufficient to provide any detailed mechanistic insights into the true nature of the dynamic changes in the cristae. Understanding the link between cristae plasticity and the metabolic or apoptotic status of the mitochondria will be essential to explain the functional relevance of the mitochondrial membrane architecture.

2.4. Motility and mitochondrial positioning

Whether mitochondria are migrating into the yeast bud during cell division, or are transported to the axon in primary human neurons, mitochondrial motility is an essential aspect of mitochondrial dynamics. The ability to position the mitochondria within the cell is crucial for the development of many polarized cells both during, and after cellular differentiation [7,112]. The primary function for mitochondrial positioning is likely to provide local ATP and buffer calcium transients [113,114]. However, some more recent studies are beginning to indicate that the mitochondria may also function as a unique and essential signaling platform (Fig. 2). In this capacity, the positioning of the mitochondria within polarized cells may be important for signaling cascades in addition to its role in maintaining energy capacity [113,115]. Mitochondrial motility in neurons has been an intensively studied area of research and represents a model for directed mitochondrial movement. In mammalian cells, mitochondria are transported along microtubules in an anterograde movement from

the cell body to the distal portion and in retrograde movement in the opposite direction. Interestingly, mitochondria with high membrane potential preferentially migrate in the anterograde direction and conversely, mitochondria with low membrane potential progress in the retrograde direction [116]. Thus, mitochondrial motility and energy state seem to be coupled although the mechanisms controlling this selection are unknown.

Mitochondrial distribution in neurons involves the molecular motors kinesin-1 (KIF1B) and kinesin-3 (KIF5B), along with the cytoplasmic dynein [117]. The nature of the interactions between mitochondria and these factors is unclear and can involve lipid binding or the presence of adaptors. In *Drosophila*, the two proteins Milton and Miro have been identified as adaptor complex that anchors kinesin-1 to mitochondria [118,119]. Miro is a Rho-like mitochondrial outer membrane GTPase containing calcium-binding motifs. In yeast, Gem1, the Miro orthologue, also contains Rho GTPase and EF-hand domains essential for mitochondrial distribution and morphology [120]. The presence of these conserved domains within Miro strongly suggests that the movement of mitochondria is regulated by both GTPase switch mechanisms and calcium signaling pathways. Miro also functions as an adaptor for Milton, which binds directly to the kinesin heavy chain [121]. The binding of Milton to the heavy chain competes for the light chain, and leads to the formation of a Miro/Milton/Kinesin complex. The reason why Milton appears to have replaced the kinesin light chain is unclear, but it has been proposed that Milton may not be the only protein that functionally replaces the kinesin light chain in the regulation of microtubule based movement [121]. This complex is essential for the transport of mitochondria within the *Drosophila* oocyte [122], and axonal transport of mitochondria in neurons [119]. Milton does not appear to be conserved in yeast, perhaps reflecting the primary dependence of yeast mitochondria on the actin cytoskeleton rather than microtubules. Syntabulin is another adaptor molecule initially identified as a syntaxin-binding protein linking syntaxin-containing vesicles to kinesin heavy chain [123]. Syntabulin is also a linker between mitochondria and motor protein KIF5B and participates in the anterograde trafficking [124,125]. Syntabulin directly binds the kinesin heavy chain and its carboxyl-terminal tail is
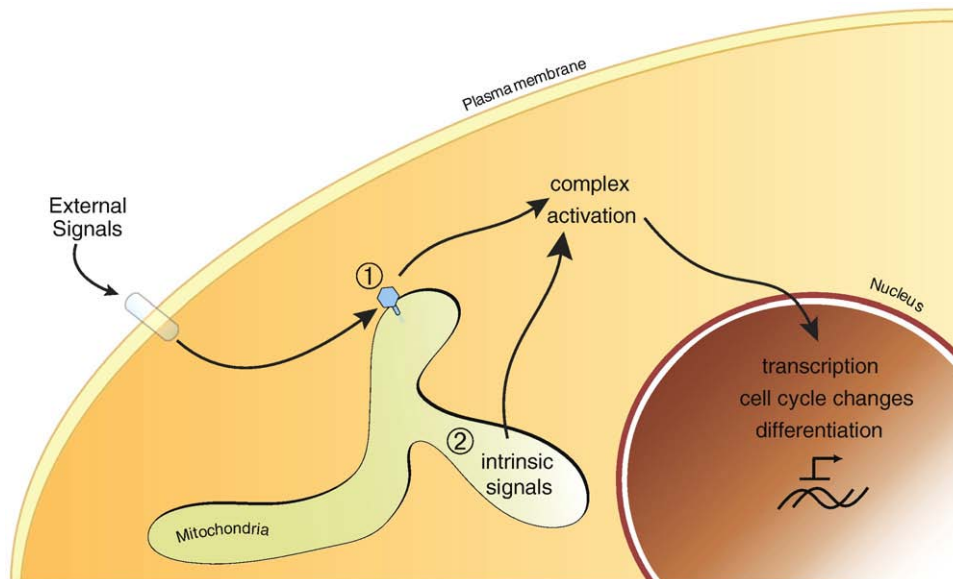

Fig. 2. Mitochondria as critical signaling platforms. The mitochondria are important cellular platforms that both propagate and initiate intracellular signals that lead to overall cellular and metabolic responses. In (1), external signals, like signals for growth, or viral infection are sensed by novel proteins anchored in the mitochondrial outer membrane, including MAVS and FKBP38. The engagement of these mitochondrial proteins leads to the activation of downstream signaling complexes that ultimately affect cellular transcription and growth programs. In (2), signals can be initiated from within the mitochondria, including the intuitive changes in the production of ROS or ATP levels. These metabolites affect many cellular programs, including cell cycle progression from G1 to S and apoptosis. The identification of many new mitochondrial kinases, phosphatases and GTPases like PINK1 suggests that additional mechanisms may play a prominent role in transducing mitochondrial signals within the cell. See text for details.

associated with mitochondria. A number of additional studies have identified cellular requirements for mitochondrial movement, including *c*yclin-*d*ependent *k*inase 5 (CDK5), *p*rotein *p*hosphatase 1 (PP1) and *g*lycogen *s*ynthase *k*inase 3 *b*eta (GSK3beta) [126–128]. However, the mechanisms and regulation of these pathways are also unclear.

Hollenbeck and colleagues demonstrated that the movement of mitochondria along the axon can be regulated by the local addition of *n*erve *g*rowth *f*actor, NGF [112,129]. Using NGF-coated beads, they showed that the activation of the TrkA receptor led to an accumulation of mitochondria in the vicinity of the bead. This relocalization depended upon PI(3)kinase activity, and was due to a shift of mitochondria from a microtubule association to their more stable association with actin filaments [129]. The specific role of Miro or Milton has not yet been examined within this experimental model. A more recent study has identified WAVE1, or Wiskott–Aldrich syndrome protein (*WA*SP)-family *ve*rprolin homologous protein 1, as an important factor that triggers the movement of mitochondria into dendritic spines in response to NMDA (*N*-*m*ethyl-*D*-*a*spartic acid) receptor activation [130]. NMDA receptor activation triggers the formation of new filopodia and dendritic spines, leading to long term potentiation. The examination of actin based changes responsible for filopodia formation led to a careful examination of WAVE1, since it is known to activate the *a*ctin-*r*elated *p*rotein, Arp2/3 complex, leading to the robust formation of actin filaments. WAVE1 has been previously localized to the mitochondria, however its function there had been unclear [131]. Using isolated primary cortical neurons, it has now been shown that the activation of NMDA receptors leads to transient neuronal depolarization, and the downregulation of the regulatory p35 subunit of Cdk5. Cdk5 is the kinase that maintains WAVE1 in a phosphorylated, inactive state. Upon the downregulation of p35, WAVE1 becomes activated and facilitates the recruitment of mitochondria into the dendritic spines. It had previously been shown that only small fragments of mitochondria can become enriched at the base, or within, these spines, suggesting that there may also be some link between WAVE1 activation and the shift to more fragmented mitochondria during neuronal depolarization. The switch to an actin based motility from long-range microtubule based movement of mitochondria along the axonal shaft may also be explained by the recruitment of the Arp2/3 complex to the mitochondrial surface.

It has long been observed that mitochondria are highly enriched within the synapse, yet it has been unclear how this asymmetric distribution is maintained. A recent study has shed new light on this question through the identification of Syntaphilin as a docking receptor for mitochondria targeted to the axon [132]. Syntaphilin is a direct docking receptor that it is associated with the outer membrane of mitochondria by its hydrophobic carboxyl-terminal tail and interacts with the cytoskeleton through its microtubule-binding domain. This protein represents a new target for the regulation of mitochondrial stationary phase in neurons. Interestingly, phosphorylation of syntaphilin by cAMP-dependent *p*rotein *k*inase *A* (PKA) decreases its interaction with syntaxin-1 [133] and it can be hypothesized that cAMP-dependent transduction pathway may similarly affect the role of syntaphilin in mitochondrial docking. Further investigations will determine whether cellular signals and synaptic activity can modulate its function.

In addition to neuronal synapses, mitochondrial positioning has recently been shown to play a critical role in the formation of immunological synapses as well [134]. In this study, the mitochondria within the T-helper cells were shown to move rapidly towards the immunological synapse formed with the antigen presenting cell. Once there, the mitochondria sit within 200 nm from the plasma membrane where their presence was required to maintain the calcium-dependent T-cell activation. The mitochondria recruited to the synapse showed significantly higher calcium transients relative to those located further away, suggesting a specific role in modulating local calcium-dependent signaling complexes. Within migrating T-

lymphocytes, it was also shown that the movement of fragmented mitochondria into the uropod was required for the propagation of the cell [135]. If the mitochondrial reticulum was highly fused, lymphocyte migration was significantly reduced, providing evidence that the machinery governing mitochondrial position and morphology must be integrated into the signals that drive cellular polarization and migration. Indeed, the movement of mitochondria depended on G-protein signaling and microtubule dynamics, as relocalization was inhibited by pertussis toxin and nocodazole [135]. Based on additional experiments manipulating the ATP levels in the cell, the authors concluded that the requirement for mitochondrial localization within the uropod helped to ensure a high capacity of ATP to drive the myosin motors responsible for the cellular migration.

Finally, components of the protein import machinery have also been shown to have a dual function in import and in the regulation of mitochondrial motility and position. In yeast, mitochondrial morphology proteins Mmm1, Mdm10 and Mdm12 have a major role in the assembly of β-barrel proteins in the outer mitochondrial membrane [136–138], where Mdm10 plays a specific role in the final stages of assembly of the TOM complex [136]. In addition to these established roles, Mmm1 and Mdm10 may control the interaction of the organelle with actin, pointing to a possible regulation of mitochondria motility [139]. Finally, the outer membrane Mmm1, Mdm10, Mmm2 and Mdm12 [139,140] and the inner membrane proteins Mdm31 and Mdm32 [141] have been shown to colocalize with nucleoids, and they may play a direct role in their assembly and/or function. In summary, the regulation of mitochondrial movement is highly coordinated through multiple signaling pathways that require the participation of a large number of proteins. Many of these appear to have dual functions and future work will continue to uncover the relationships between these players.

2.5. Mitochondrial derived vesicles

Recently, a new aspect of mitochondrial dynamics has been revealed with the identification of carrier vesicles emerging from mitochondria [142]. These *m*itochondrial *d*erived *v*esicles (MDVs) are uniform in size, between 70–100 nm, and contain selected mitochondrial cargo. Moreover, they are formed by a lateral segregation and enrichment of the cargo and the budding process does not require the dynamin Drp1, suggesting the existence of a machinery independent of the fission apparatus. These profiles contained either one or both mitochondrial membranes present as concentric rings, with cristae rarely observed. Their discovery came with the identification and characterization of a new outer membrane *m*itochondria-*a*nchored *p*rotein *li*gase called MAPL. Overexpression of MAPL leads to the Drp1-dependent mitochondrial fragmentation, suggesting that this mitochondrial RING-finger containing protein may play a role in the regulation of mitochondrial fission. Whether the activation of Drp1 is through potential ubiquitin or SUMO E3 ligase activity within the RING-finger remains to be clarified [143]. Regardless of its role in stimulating mitochondrial fission, the surprise came when examining cells where this fragmented phenotype was rescued by the inhibition of Drp1. In these cells, the mitochondria were highly interconnected, as is the expected phenotype upon inactivation of Drp1. However, although fission was inhibited, EM and confocal imaging demonstrated that MAPL was still found in vesicular mitochondrial structures, termed mitochondrial derived vesicles (MDVs). Remarkably, MAPL-containing vesicles selectively excluded mitochondrial protein Tom20 and vice-versa demonstrating that MDVs contain selected cargo. Furthermore, MAPL-containing vesicles were shown to fuse with a subset of peroxisomes [142]. Since the distinct vesicle population labeled with Tom20 does not fuse with peroxisomes, it appears that the cargo specificity is a primary determinant of MDV fate [142]. Further investigations are required to uncover the function and fates of the vesicle populations that are not addressed to the

peroxisomes. Regardless of other potential fates, the transport of mitochondrial cargo to the peroxisomes offers the first molecular mechanism for the traffic between these two organelles. The exact nature of these MAPL-containing vesicles and their role in peroxisomes physiology remain to be determined. Nevertheless, the description of machineries controlling the recruitment of the cargo and the budding process promises to be a very exciting field.

The process of MDV formation may provide explanations for two unique aspects of mitochondrial phenomenology; first that some mitochondrial proteins or complexes are found at extra-mitochondrial sites [144,145], and second, that asymmetric mitochondrial fission is becoming much more commonly observed [78,79]. The unexpected ectopic localization of mitochondrial proteins is best exemplified by the presence of mitochondrial ATP synthase complex at the cell surface of endothelial cells where it plays an essential role in lipid metabolism [145–147]. Although some proteins are dually targeted to different organelles, the ATP synthase complex contains 2 proteins encoded by the mitochondrial genome. Therefore, it is obligate that this complex assembles within the inner membrane prior to its appearance on the cell surface. Currently, there is no described mechanism able to explain the transport of this complex necessarily assembled in the mitochondria to another cell compartment. It is reasonable to hypothesize that the MDVs could fulfill the role of carrier vesicles to transport mitochondrial proteins to other destination. Second, the establishment that MDVs incorporate only selected mitochondrial cargo provides further support for the previous evidence that the mitochondria, like other organelles, have the capacity to laterally enrich selected proteins for various purposes. At least two reports suggest that the fission of fusion-incompetent mitochondria allow for selective degradation of damaged fragments [33,78]. This concept presupposes that damaged mitochondrial cargo and lipid must reside preferentially within the fusion incompetent half of the segregated fragment. Interestingly, there has also been a series of new studies indicating that mutated mtDNA is somehow selected against during early embryogenesis [148,149]. Although the mechanisms that underlie this process remain obscure, it is likely that there is some form of selective autophagy of mitochondrial fragments containing the mutant mtDNA. Therefore, the discovery that the mitochondria are able to bud vesicles containing selected cargo is perhaps not wholly unexpected and may provide a new framework to study the mechanisms that govern selective mitochondrial traffic. The challenge is to understand the regulation of Drp1-independent vesicle formation and how this process may functionally differ from Drp1-dependent asymmetric mitochondrial fission.

3. Integration of mitochondrial dynamics within intracellular signaling cascades

The machineries described above provide the mechanisms to control the positioning of the mitochondria within the cell, the ultrastructure of the cristae membranes and the interconnectivity of the mitochondrial reticulum. Together these elements ensure that the metabolic engine is well positioned for the cell to function properly. Recent work by a number of laboratories has shed light on the mechanisms by which the cellular signals can influence the mitochondrial morphology machinery under distinct cellular conditions, from apoptosis to cell division (Fig.1). The flow of information from the cell to the mitochondria allows the exquisite regulation of mitochondrial function. The importance of this flow is becoming more evident as a number of disease conditions like Parkinson's, Charcot-Marie Tooth Type 2A, and many others appear to progress because of a direct effect on mitochondrial dynamics [1]. Therefore the regulation of mitochondrial morphology in cellular physiology has a profound impact on human health and development.

In addition to signaling from the cell to the mitochondria, the mitochondria also have a strong capacity to initiate signaling cascades that can directly influence cellular behavior. Pioneering work on retrograde signaling was done by Ron Butow, to whom this review is dedicated, who focused on the stimulation of mitochondrial biogenesis due to accumulating mitochondrial dysfunction [150–152]. As predicted by the work of Butow, exciting new studies are continuing to expand these ideas. Critical signaling pathways including those involved in immunity and cellular proliferation are transduced in a manner dependent upon the activities of novel mitochondrial-anchored signaling complexes. The requirement for the mitochondria within these pathways is unexpected and it is not yet clear what the functional implications might be. However, with the molecular identity of mitochondrial signaling complexes, we now have a starting point to explore the significance of these findings. One might predict that the activity of these new complexes in transducing retrograde mitochondrial signaling will depend upon the precise positioning of the mitochondria, the co-ordination of membrane microdomains, and the regulation of metabolic outputs. Therefore, the machinery that regulates mitochondrial morphology must be incorporated within mitochondrial signaling pathways. These concepts will be expanded upon in the sections below.

3.1. Anterograde signaling

3.1.1. Metabolism and morphology

One of the first correlates that emerged from the examination of mitochondrial dynamics was that the loss of mitochondrial fusion led to mitochondrial depolarization, loss of mtDNA, and a general inability to respire [7,153–156]. These data suggested that the process of ongoing mitochondrial fusion was essential to maintain mitochondrial function. The loss of the mitofusins or OPA1 in cultured cells led to a nearly complete loss in respiration [156], and the loss of these proteins in animal models led to early embryonic lethality [7,157]. In general, the ability to share metabolites within the mitochondrial reticulum has been considered an important factor for metabolic stability. Metabolites within the mitochondria may become concentrated within the intercristal membranes, allowing their efficient use by the respiratory chain [97,103,158]. However, should the regulation of cristae assembly be compromised, then these stores may become depleted. Therefore it has been proposed that the shape-shifting machinery ensures that the cristae remain both properly assembled and dynamic, thereby allowing a regulated distribution of metabolites within an organelle, and the reticulum. This may appear intuitive in some ways, however it is still not clear why the shape of the mitochondria should so profoundly affect the enzymatic activity of the respiratory chain. Nevertheless, since the plasticity of the mitochondria is essential for metabolism, then the machinery that modulates mitochondrial dynamics represent an excellent axis for metabolic regulation.

There is some evidence that the fusion/fission machineries are modulated in response to changes in the metabolic conditions of the cell. For example, in conditions of high glucose, where the mitochondria must catabolise pyruvate very quickly, the mitochondria were seen to undergo a Drp1-dependent fragmentation event that occurred in the first 10–15 min of glucose addition [159]. This morphological change was reversible, and within 30–60 min, mitochondria had returned to their previous, tubular state. Surprisingly, inactivation of Drp1 dramatically inhibited pyruvate uptake into the mitochondria, the respiration increase and associated ROS production, emphasizing the control of mitochondrial metabolism by the morphological state of the organelle. It remains unclear how Drp1 activity is regulated in response to the influx of glucose, but given the multiple post-translational modifications encountered by Drp1, it is likely to be a direct target of the signaling activities linked to glucose uptake. In general, it appears that mitochondrial fission may be required to induce inner membrane remodeling in order to adapt mitochondrial metabolism for optimal metabolic output. This observation highlights

an important point, that the appearance of fragmented mitochondria alone cannot be interpreted as a sign of respiratory incompetence. Instead, mitochondrial plasticity — or the ability of the mitochondria to fuse and divide in a coordinated manner, appears to be the critical element determining mitochondrial function.

Given that Mfn2 overexpression or silencing affects metabolism it has been considered that mitochondrial fusion may directly or indirectly play a role in relaying cellular cues to metabolic outputs [156,160,161]. It has not yet been determined whether or how the fusion machinery becomes activated in response to cellular signaling. One potential clue may be that mutations within the intermembrane space domain of Mfn2 completely inhibit mitochondrial fusion, even though the GTPase and cytosolic heptad repeat domains are intact [10]. These intermembrane space residues are not themselves required for the fusion reaction, since an Mfn2 construct harboring both the IMS mutation, and the dominant active mutation within the GTPase domain, fused in a highly stimulated manner. These data suggest that the intermembrane space region of Mfn2 may be required to activate the protein and drive mitochondrial fusion, suggesting that the impetus for mitochondria to fuse may be derived from their individual metabolic status. This does not exclude the additional possibility that cytosolic factors may also activate the mitofusins through their GTPase domains and trigger organelle fusion based on metabolic cues. The potential upstream factors that regulate Mfn2 activation and Drp1 recruitment to the mitochondria will be important to identify in the future.

3.1.2. Apoptosis

The requirement for the fission machinery, including Drp1, endophilin B1 and hFis1 in the efficient progression of cell death has been demonstrated in most cellular model systems studied, from cultured cells to primary neurons [77,162–164]. In addition, Drp1 has been implicated in the progression of apoptosis in a number of genetic and developmental models, including *C. elegans*, *Drosophila* and yeast [165–169]. In all three cases, the loss of Drp1 (or Dnm1) led to an increase in interconnected mitochondria and inhibition of developmentally regulated, or age-related, cell death. In contrast, a conditional knock-out mouse where Mfn2 loss was initiated within the cerebellum following birth, revealed a nearly complete loss of Purkinje cells, strongly indicating that the fusion machinery plays a critical role in the protection of cells against mitochondrial dysfunction and cell death [11]. Similarly, the loss of Mfn2 using lentiviral delivered siRNA within cultured primary cortical neurons led to the induction of cell death, whereas infection of the dominant active form of Mfn2 provided significant protection against a number of apoptotic stimuli [170]. These and many other studies have provided a solid foundation for the examination of mitochondrial dynamics during apoptosis.

The genetic links between cell death and mitochondrial morphology are important, but the molecular details of these processes are also rapidly emerging. It has been observed for some time that the mitochondria undergo dramatic changes in their morphology upon apoptotic stimuli, including an increase in fragmentation [162], inhibition of mitochondrial fusion [171], opening and remodeling of the cristae [109,110] and release of apoptotic proteins like cytochrome *c*, *a*poptosis-*i*nducing *f*actor (AIF), HTrA2/Omi (*H*igh *t*emperature *re*quirement *A2*), Endonuclease G, OPA1 and Smac/Diablo [172]. Although there is still some debate whether mitochondrial fragmentation is essential for cell death [173,174], it is generally accepted that the mitochondrial fragmentation that accompanies apoptosis is an early, Drp1-dependent event that requires the presence of active Bax/Bak. The precise nature of the molecular intersection between Drp1 and the pro-apoptotic proteins is unclear, however they are known to coalesce into distinct foci on the mitochondrial membrane [63,175], indicating a local assembly of macromolecular networks. These Bax/Bak-dependent apoptotic complexes are extremely stable as deter-

mined by the lack of recovery of Drp1-YFP (*yellow fluorescent protein*) within these foci after photobleaching [63]. Interestingly, the formation of such stable complexes of Drp1-YFP on the mitochondrial surface coincides with the stable, Bax/Bak-dependent SUMOylation of Drp1, implicating this modification as a part of the mechanism for the modulation of Drp1 function during apoptosis.

In addition to SUMOylation, the phosphorylation of Drp1 also plays a role in apoptosis. It was recently demonstrated that the regulation of Drp1 GTPase activity is dependent on phosphorylation within the GTPase effector domain (GED) by protein kinase A (PKA) [91,95]. Phosphorylation of Ser-637 within human Drp1 by the cAMP-dependent protein kinase causes a significant reduction of the GTPase activity, and since hydrolysis of GTP is requisite to drive mitochondrial fission, the phosphorylation led to an inhibition of mitochondrial division [91]. Mechanistically, the use of a phosphomimetic mutant provided evidence that the interaction between the GED and the GTP-binding domain was disturbed upon phosphorylation, providing an explanation for the inhibition of GTP hydrolysis within Drp1. Although this precise mechanism was challenged by another study reporting that the same phosphomimetic mutant did not affect GTPase activity [95], the fission activity of Drp1 was also reduced upon phosphorylation. Moreover, the dephosphorylation of this site is mediated by calcineurin also known as *protein phosphatase 2B* (PP2B), possibly providing a molecular explanation for the role of calcium signaling in the activation of Drp1-dependent mitochondrial fission [95,110,176]. These studies further proposed that this regulatory network of phosphorylation can play an important role in the control of apoptosis. Indeed, the pseudo-phosphorylation of the Ser residue led to a fused mitochondrial reticulum that was resistant to cell death, whereas the Serine to Alanine mutation led to an increased sensitivity to apoptosis [95]. It is important to note that cAMP and calcium regulate multiple physiological programs, therefore this regulatory network is likely to have a more fundamental role in the adaptation of mitochondria to changes in cellular ATP requirements. For example, during short-term adaptation of mitochondria to the cellular energy demand, Ca^{2+} signaling induces the activation of mitochondrial ATP production [177,178]. As described earlier, the transient activation of Drp1 can facilitate pyruvate uptake and increased respiration [159], perhaps indicating that the dephosphorylation of Drp1 by calcineurin upon calcium based signaling may lead to its increased recruitment and fission activity.

Although Drp1 initially received the most attention as an apoptotic regulator, recent studies have provided more direct molecular links between the pro-apoptotic proteins and Mfn2. It was first shown that the activation or overexpression of the mitofusin proteins is protective against apoptosis, suggesting that ongoing fusion, or the fusion machinery itself, could interfere with the progression of cell death [142,179]. Later, the use of a photoactivatable GFP based mitochondrial fusion assay revealed that fusion was indeed inhibited during the early stages of apoptosis, which was an event distinct from the activation of mitochondrial fission and the involvement of Drp1 [171]. Richard Youle and colleagues further showed that the pro-apoptotic machinery of Bax and Bak were required for the normally high levels of mitochondrial fusion in steady state, non-apoptotic conditions [180]. More specifically, Bax and Bak play a role in the control of mitochondrial fusion by modulating the distribution and motility of Mfn2 into foci and promoting its assembly into large complexes. The Bax/Bak-mediated modulation of Mfn2 localization was also shown to depend on the nucleotide state of the Mfn2, with the GTP-bound form losing responsiveness to the pro-apoptotic proteins. This is consistent with the observation that the GTP-bound form of Mfn2 provides significant protection against Bax-mediated cell death.

The studies described above made use of the Bax/Bak double knock-out cell lines, however it was recently demonstrated that much of the regulation of mitochondrial morphology may be through the actions of Bak rather than Bax [181]. The introduction of Bak alone into

the DKO cell lines rescued mitochondrial fusion, and Bak was shown to bind both Mfn1 and Mfn2. Interestingly, upon cell death, Bak releases Mfn2 and preferentially binds to Mfn1. Mutations in the BH3 domain of Bak which are essential for the stimulation of cell death did not affect the interaction with Mfn1, and still bound to Mfn2 under non-apoptotic conditions. However, upon apoptotic treatment, mutated Bak did not release Mfn2, suggesting that the release of Mfn2 from this complex is requisite for the progression of cell death [181]. Finally, it was also reported that the anti-apoptotic Bcl-XL, but not Bcl-2, binds specifically to Mfn2, and expression of Bcl-XL led to an increased mitochondrial fusion [182]. More work is needed to dissect the mechanisms that define these complexes and their apoptotic transitions, but it is evident that both the pro- and anti-apoptotic machinery plays a direct role in the regulation of the fusion GTPases. Given the emerging links between the pro-apoptotic machinery and their role as sentinels of the cellular metabolic state [183,184], these links may eventually provide new insights into the metabolic regulation of mitochondrial fusion.

3.1.3. Cell cycle

One of the more curious aspects of mitochondrial biology is whether, and if yes, how mitochondrial biogenesis and mtDNA replication may be coupled to the stages of cell cycle progression. Early evidence indicated that mtDNA did not increase during the S phase of the cell cycle, indicating that the replication of the two genomes is not temporally linked [185,186]. Instead, mitochondrial mass appeared to increase most during the growth phase of the cell cycle, as might be anticipated due to increased energy demands. Therefore the most obvious link between regulated mitochondrial behavior/morphology and the cell cycle was considered to be the directed transport of yeast mitochondria into the emerging bud. To address the molecular requirements for mitochondrial partitioning during mitosis in yeast, a number of groups initiated various genetic screens to identify candidate proteins involved in this process [22,44,61,187,188]. These screens led to the identification of most of the proteins described in the first section of this review, but provided very limited insight into how the mitochondria may be integrated within the cell cycle machinery, resulting in an uncertain picture of how the morphology machinery is modulated in response to cell cycle triggers. Although they did not identify any mutants that resulted in an absolute block of mitochondrial inheritance, they did identify at least one mutant that caused a delay. The loss of the type 2C serine/threonine phosphatase PTC1 (*mdm28-1*) in yeast results in a delay of mitochondrial transport into the bud [189], however the mechanisms and targets of this process have not yet been determined.

There was a recent exception, where Jensen and colleagues provided a novel link between a Dnm1 and Num1 [190]. Num1 is a protein associated with the cell cortex initially described to participate in nuclear migration into the yeast bud [191]. However, the new study determined that the absence of Num1 also resulted in a defect in mitochondrial division, showing that Num1 helps facilitate mitochondrial fission [190]. In addition, Num1 interacts with Dnm1, supporting its role in mitochondrial fission. The interaction between Dnm1 and the cortical anchored Num1 also provided a molecular explanation for the observation that most mitochondria-associated Dnm1 is localized along the mitochondrial tubules in a polarized manner, on the cell cortex exposed side [65]. Therefore, this new study determined that Num1 plays a role both in mitochondrial fragmentation and positioning under the cell cortex. Given its established role in mitosis, the authors also examined the role of Num1 in mitochondrial migration into the bud. In yeast mitosis, mitochondria must move along actin cables in order to be delivered into the growing bud. The authors showed that together Dnm1 and Num1 were required for the retention of mitochondria within the mother cell. In their absence, the entire mitochondrial population was found within the daughter bud in a highly fused state for about 20% of the cells. These data

indicate that the positioning of the Dnm1 fission machinery at the bud tip allowed ongoing fission events to return a population of mitochondria back to the mother cell, which appears to ensure the equal distribution of organelles between the mother and daughter cell [192]. These studies provide a molecular basis for the cell cycle regulated changes in mitochondrial morphology and position.

In the mammalian system it hasn't been quite as apparent whether specific machinery would be required to ensure the equal inheritance of mitochondria during mitosis. Given the large number of mitochondria in most cell types, it is possible that the process may simply be stochastic. However, microscopy studies reveal that mitochondria do undergo distinct morphological transitions during yeast meiosis and sporulation [193,194], processes that do not involve polarized transport into an emerging bud. In addition, more recent studies report that the mammalian mitochondria are highly fused during G1, and shift towards a fragmented phenotype during G2 and into mitosis [90]. These phenomena suggest that the machinery governing mitochondrial shape may also be linked to the cell cycle machinery. To support this idea, one novel mechanism has shed insight into the dependence of mitochondrial morphology on the cell cycle phase. During mitosis, rat Drp1 is phosphorylated on Ser-585 by Cdk1/cyclin B to promote its fission activity [90]. Although this site is also within the GED domain of Drp1, it is a different serine residue than that identified as a target of PKA, described earlier [91,95]. When expressing the phosphorylation-deficient mutant S585A, mitotic cells show a dramatic decrease of the mitochondrial division {Taguchi, 2007 #775}. The presence of this phosphorylation site in the GTPase effector domain of Drp1 suggested a possible effect on its GTP hydrolysis activity. However, no change in the GTPase activity of Drp1 was observed in the phosphorylation-negative mutant or in a phosphomimetic mutant S585D. Thus, the stimulation of Drp1 fission activity by phosphorylation of Ser585 could be mediated by an enhanced interaction with other fission factors or affect Drp1 self-assembly rate. Interestingly, this is in contrast to the effects of PKA-mediated phosphorylation of Drp1 in the GED domain that inhibited its fission activity, suggesting that the use of multiple post-translational modifications within Drp1 can provide a powerful means to specifically modulate its activity.

In summary, many new insights have been generated recently that illustrate how well integrated the mitochondria must be with the onset and process of cell division. As may be expected, many of these mechanisms rely on regulated post-translational modifications of proteins like Drp1. As we uncover more of the details that govern mitochondrial dynamics, it is clear that there will be many more links to be found between cell cycle and mitochondrial activity. In addition to the established role of mitochondria in regulating cell death, these new data may point to exciting possibilities for the use of alternative mitochondrial targets in drug therapies related to cellular proliferation in cancer.

3.2. Retrograde signaling

Recent data from a number of groups studying varied topics in biology, from immunology to development and disease, have found unexpected links between established signaling pathways and the mitochondria. So far, the understanding of these links is limited, where it is often just the identification of a signaling protein on the mitochondrial surface [195,196]. In other examples, the signals originate from within, through the activity of mitochondrial kinases [197]. We have included a few examples of recently identified signaling axis where it appears that the signal is initiated from the mitochondrial surface, or utilizes the mitochondria to propagate a signal. To date, the role of mitochondrial morphology, positioning and function remains largely unknown with respect to these signaling pathways. However, it is likely that mitochondrial morphology and

positioning will play an important role in the ability of the mitochondria to participate and propagate these signaling events.

3.2.1. Metabolic check points

In addition to the integration of the mitochondria into cellular signaling cascades, it has also been evident for a number of years that the mitochondria can also initiate distinct signaling cascades in a retrograde fashion. One of the most intuitive examples of this is the observation that low levels of ATP within a cell can inhibit processes like cell cycle progression. Recently this idea has been taken from an intuitive phenomenon to a process with a defined molecular pathway. More specifically, two new signaling pathways were described in *Drosophila* that link mitochondrial dysfunction and cell cycle checkpoint. A mutation in the cytochrome *c* oxidase Va (CoVa) causes a 60% decrease in the cellular ATP production and led to an arrest in cellular proliferation [198]. Interestingly, this ATP level was still sufficient to maintain cell survival and differentiation. This difference in functional requirements for ATP was explained through the specific role of AMPK activation. Loss of CoVa led to an increase in AMP level, resulting in the activation of AMPK and p53. This activated pathway triggers the selected degradation of Cyclin E, blocking the cell cycle at the G1/S transition point. This highlights the specificity of the AMP signal towards an inhibition of cell cycle progression while not affecting cellular differentiation and survival.

The same group recently described a second mutation affecting cell cycle progression, this time in the complex I protein Pdsw [199]. This mutation impaired efficient oxidative phosphorylation, leading to a significant increase in reactive oxygen species (ROS) production. Importantly, this mutation did not affect the cellular levels of ATP. The high ROS concentration resulted in the upregulated expression of Dacapo, an inhibitor of the Cyclin E–CDK2 complex, again blocking the cell cycle at the G1/S transition point. These data indicate that mitochondrial metabolism exerts a control on cell cycle progression through both AMP and ROS levels. These data provide a much more precise picture of how the metabolic status of the mitochondria contributes a critical cell cycle check point at the G1/S transition.

3.2.2. Oxidative signaling

The role of mitochondrial ROS as a signal to block cell cycle progression is consistent with many previous studies that focus on ROS as a signaling messenger derived from within the mitochondria [200]. Mitochondria are the major source of intracellular reactive oxygen species (ROS). Respiration generates ROS through the oxidative phosphorylation and ROS are especially produced in conditions of mitochondrial dysfunction or altered respiration. Hence, mitochondria are the primary site of oxidative damage in the cell, a process essential in several human diseases and aging. For example, the use of a complex I inhibitor rotenone in a mouse model of Parkinson's disease was an early indicator of how a local increase in ROS production can lead to disease [201,202]. In addition to steady state ROS production that may accumulate over time and contribute to age-related diseases, there are also defined mechanisms that stimulate more acute ROS production within the mitochondria. The best example of this involves the activation of the p66Shc protein, which sensitizes cells to apoptotic stimuli [203]. The current model for this pathway suggests that cytosolic PKCβ can be activated by oxidative conditions, which in turn directly phosphorylates cytosolic p66Shc. Phosphorylated p66Shc is subjected to a conformational change catalyzed by the peptidyl-prolyl-isomerase Pin1. Isomerized p66Shc will be translocated into the mitochondrial intermembrane space where it acts as an oxidoreductase, transferring electrons directly from cytochrome *c* to oxygen to generate H_2O_2. This will ultimately induce the opening of the mitochondrial permeability transition pore to finally promote mitochondrial fragmentation and cell death [204,205]. This complex mechanism provides an explana-

tion for how the generation of an oxidative environment through mitochondrial ROS signaling can feed back to induce apoptosis and may help explain the role for p66Shc in the determination of life span [206].

3.2.3. Mitochondria in immunity

One unanticipated role of mitochondria in cellular signaling was provided by the discovery that proteins involved in viral immunity were targeted and anchored within the mitochondrial outer membrane. A number of independent studies identified a new protein anchored within the mitochondrial outer membrane, now termed MAVS (mitochondrial antiviral signaling) [195,207–209]. Together, these studies revealed that MAVS functions downstream of RIG-I, an intracellular receptor for viral RNA, and is essential for the activation of NF-κB and IRF3 pathways in response to viral infection. The N-terminal CARD-like domain of MAVS likely interacts with CARD-like domain containing proteins RIG-I and MDA-5. Moreover, additional data suggested that MAVS enters into a detergent-resistant membrane domain following viral infection, suggesting the modulation of mitochondrial membrane properties in the transduction of the signal [195]. Recent studies identified NLRX1 as another mitochondrial outer membrane protein contributing to this signaling pathway [210]. NLRX1 is a member of the nucleotide-binding domain proteins containing leucine rich repeats, a family known to play a critical role in the cellular surveillance for pathogens. NLRX1 is the only member of this family to target the mitochondria and was found to interact with MAVS, where it specifically inhibits the intracellular antiviral response. A second study also determined that NLRX1 overexpression stimulates the production of reactive oxygen species, which may play a role in its function at the mitochondria [211]. This likely plays a critical role in the downregulation of the immune response. Importantly, the reason why MAVS and NLXR1 must be anchored within the mitochondrial membrane remains unclear. It may reflect some metabolic or functional check point in this signaling pathway, or a unique property of the mitochondrial membranes to assemble specific complexes. Regardless, we can conclude that the intersection of the mitochondria along the pathway for NF-κB activation in immunity is likely to attract a significant amount of attention in the future.

3.2.4. mTOR signaling

The most established retrograde signaling pathway between the mitochondria and the nucleus is the regulation of mitochondrial biogenesis in response to nutrient sensing, and the sensing of mitochondrial dysfunction. The study of this pathway in yeast identified a number of retrograde genes (RTG), which are linked to TOR (target-of-rapamycin) signaling, aging and nutrient sensing [152]. Although many of these mechanisms are rather well established in yeast model systems, the signaling links between cellular growth and mitochondrial biogenesis are less well understood in higher eukaryotes. It is clear in both systems that the metabolic requirements for cellular proliferation and growth are extreme. The assembly of new proteins, lipids, nucleotides and structural elements demands increased amino acid production and numerous other building blocks. One of the central regulators of these processes in the context of cellular growth are the components of the serine-threonine kinase mTOR (mammalian target of rapamycin) pathway [212,213]. This pathway is more complex in the mammalian system as it is activated in the presence of growth factors like insulin. In addition to the increase in glucose uptake, the PI(3)kinase-dependent activation of Akt leads to the inhibition of GTP activating proteins (Tsc1 and Tsc2) for the small GTPase Rheb [214]. This allows the switching of Rheb into the GTP-bound state, which can then activate mTOR. mTOR then facilitates amino acid uptake into the cell and can activate protein synthesis, providing a gateway for the global increase in cellular nutrients. In this way, the mammalian signals that activate mTOR are

initiated primarily from extracellular cues rather than mitochondrial retrograde signaling.

However, recent evidence may provide a unique intersection between these two models. FKBP38 is a member of the peptidyl-prolyl *cis/trans* isomerase (PPIase) family and possesses a unique C-terminal transmembrane domain anchored in the mitochondrial outer membrane [215,216]. In an unexpected twist, mitochondrial-anchored FKBP38 interacts directly with mTOR, resulting in the inhibition of its kinase activity [196]. Upon activation of Rheb to its GTP-bound form, Rheb competes for mTOR binding with FKBP38, thereby releasing mTOR from the mitochondrial surface, allowing its cellular activation. Interestingly, mTOR has just been found to control mitochondrial oxidative phosphorylation through the expression of the mitochondrial transcriptional regulator PGC-1alpha, indicating a complex axis of signaling that may include the mitochondria at multiple levels [217]. These studies raise a number of important questions, first among them the curious recruitment of these players to the mitochondrial surface. Most signaling pathways have depicted these proteins at the plasma membrane, where the initial receptors are localized. However, given that a number of the building blocks required for cell growth are generated within the TCA cycle of the mitochondria, and the strong evidence for retrograde signaling in yeast, it may actually make considerable sense to include this organelle in the signaling pathways. It has been unclear how the enzymes within the mitochondria are regulated in order to shift their focus on complete catabolism of glucose to CO2, and move towards the production of the intermediates required for nucleotide and amino acid synthesis, including oxaloacetate, β-ketoglutarate, etc [213]. Perhaps the positioning of this signaling cascade on the membrane helps to facilitate these types of metabolic shifts. Alternatively, the functional status of the TCA cycle may represent another check point for the successful propagation of the mTOR signal, similar to the metabolic check points that function at the G1/S transition. Future work will continue to explore these complex links and help to position both anterograde and retrograde mitochondrial signaling within multiple cellular pathways.

3.2.5. Signaling from within: PINK1

Retrograde signaling, by definition, implies the involvement of mitochondrial kinases and other regulatory enzymes capable of initiating the propagation of a signal from within the mitochondria to the cell. Instead the examples given above highlight signaling pathways that have an essential requirement for the mitochondria to propagate their signals, thereby expanding the definition of retrograde signaling. The presence of a number of uncharacterized regulatory enzymes associated with the mitochondria suggests that we have only just begun to understand the extent to which the mitochondria functions in a signaling capacity, and more importantly, what these pathways might mean to health and disease. One important new mitochondrial kinase is the Parkinson-related protein kinase PINK1 (PTEN-induced kinase 1) [218]. PINK1 is primarily localized to the mitochondrial inner membrane where its kinase domain is exposed to the intermembrane space [197]. There is a consistent cytosolic pool of PINK1 that also has functional importance [219], however the regulation of these two pools has not yet been established. To date, two potential mitochondrial targets for PINK1 have emerged, the heat shock protein TRAP1 [220], and the serine protease Omi/HtrA2 [221]. Interestingly, Omi/HtrA2 is also a Parkinson's disease (PD) gene providing support that these proteins may function on the same pathway [222]. From these recent data, a model is emerging that posits that the activation of PINK1 leads to the phosphorylation of TRAP1, which helps to fold any unfolded intermembrane space proteins, facilitating the mitochondria to recover from damage. Similarly, the potential phosphorylation of Omi1/HtrA2 activates the protease activity of this enzyme, leading to the degradation and increased turnover of unfolded proteins.

Although the complete mechanisms remain somewhat speculative, the emerging picture is that PINK1 helps to maintain mitochondrial integrity during periods of stress. Consistent with a requirement in mitochondrial integrity, the loss of PINK1 in mammalian cells leads to a fragmented mitochondrial reticulum with altered cristae structure [223]. In an interesting twist, the loss of PINK1 in *Drosophila* leads to the opposite morphological phenotype, where the mitochondria instead become highly fused and enlarged [223–226]. Flies deficient in PINK1, or another PD related gene that functions downstream of PINK1, called Parkin, show the degradation of dopaminergic neurons, harbor a wing muscle defect and cannot fly. Both PINK1 and Parkin mutant flies show the same morphological change in their mitochondria. Importantly, the inhibition of mitochondrial fusion by the loss of OPA1 or Mfn rescued the phenotype, demonstrating that the shift towards the fused state was functionally detrimental for the fly [226]. Furthermore, crossing these flies with a Drp1+/− heterozygote reduced the residual levels of mitochondrial fission even further, which led to complete embryonic lethality [226]. Together, these data indicated that the residual fission that did occur in the PINK1 or Parkin mutants was critical to the survival of the embryo and highlight the surprising sensitivity of the organism towards changes in the balance of mitochondrial fission and fusion.

Why do the fly and human phenotypes in PINK1 deficient cells appear so dramatically different? One potential explanation may lie in an upstream regulator of mitochondrial dynamics called PARL. As described earlier, PARL is a mitochondrial inner membrane rhomboid protease [227]. A recent study showed that mammalian PARL is phosphorylated on at least three sites within the matrix exposed N-terminus [228]. The kinases and phosphatases that regulate this event are unknown, however the phosphorylation of PARL regulates a specific cleavage event, termed the β-cleavage. Overexpression of the wild type PARL leads to an increase in mitochondrial fragmentation, however a mutant form of PARL where the β-cleavage site is removed does not lead to any change in mitochondrial morphology. The molecular explanation for this effect on mitochondrial morphology is not yet clear. Interestingly, the beta-cleavage site is not conserved within the *Drosophila* form of PARL, called Rhomboid-7 [227,228]. Depending on how β-cleavage of PARL is regulated under physiological conditions, it is possible that the stress induced by PINK1 deficiency may lead to β-cleavage of PARL and the inhibition of fusion in mammalian cells. In contrast, the *Drosophila* cells lacking PINK1 would not be affected by PARL and instead continue to fuse in an attempt to rescue the damaged organelles. This speculation serves only to give an example of how a potentially simple evolutionary change could lead to alternative outcomes in different genetic models. Interestingly, the PINK1 target Omi/HtrA2 is also a substrate of PARL protease activity [229], placing another layer of complexity into the system. There is an emerging functional triad between PINK1, Omi/HtrA2 and PARL that will likely have a profound impact on our understanding of PINK1 function and Parkinson's disease progression.

4. Conclusions

This review has attempted to provide some new insights into how the plasticity of the mitochondria plays a central role in the function of the organelle, and in relaying diverse cellular signals. We hypothesize that the enzymes controlling mitochondrial morphology provide a platform through which cellular signals are transduced within the cell in order to affect mitochondrial function. These functions include metabolic regulation, changes in the position of the mitochondria, partitioning of mitochondria during cell division, and the control of cell death. The mechanisms that integrate mitochondrial function and morphology with intracellular demands are rapidly emerging, and this review has highlighted only a few of them. We are now armed with an increasing list of new mitochondrial kinases, phosphatases, ligases and GTPases that may play a role in these pathways, providing

exciting new perspectives within the field. More importantly, these signaling links can provide new paradigms and drug targets relevant for the study of many human diseases, including neurodegenerative, proliferative and immunological, among many others. From its humble origins as an ancient bacterium, it is now clear that the mitochondria have evolved into a central player in all aspects of cellular behavior. The future will undoubtedly provide us with many more unexpected discoveries that will continue to fuel the current renaissance in mitochondrial research.

Acknowledgements

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