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Mitochondrial movers and shapers: Recent insights into regulators of fission, fusion and transport

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Abstract

Mitochondria are highly dynamic organelles that undergo rapid morphological adaptations influencing their number, transport, cellular distribution, and function, which in turn facilitate the integration of mitochondrial function with physiological changes in the cell. These mitochondrial dynamics are dependent on tightly regulated processes such as fission, fusion, and attachment to the cytoskeleton, and their defects are observed in various pathophysiological conditions including cancer, cardiovascular disease, and neurodegeneration. Various studies over the years have identified key molecular players and uncovered the mechanisms that mediate and regulate these processes and have highlighted their complexity and context-specificity. This review focuses on the recent studies that have contributed to the understanding of processes that influence mitochondrial morphology including fission, fusion, and transport in the cell.

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Mitochondrial dynamics, Mitochondrial fission, Mitochondrial fusion, Mitochondrial transport.

Introduction

Mitochondria in living cells exhibit a range of shapes and sizes. Healthy mitochondria typically appear tubular, whereas damaged mitochondria, which produce high amounts of reactive oxygen species (ROS) appear more spherical [1]. The mitochondrial morphology within a cell is a result of equilibrium between fission and fusion, which are active processes mediated by GTPases. On the other hand, the distribution of mitochondria within cells is dictated by their movement and anchoring on cytoskeleton filaments (F-actin and microtubules) effected by motor proteins myosins, kinesins and cytoplasmic dynein [2].

Mitochondrial fission

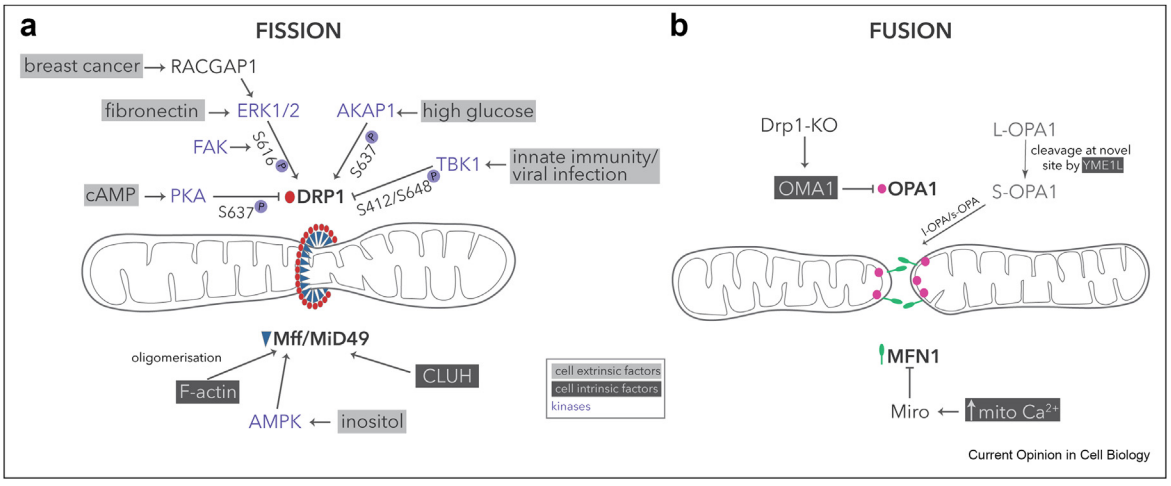
Fission is a process essential for the maintenance of mitochondrial morphology, and often precedes both partitioning at cell division, and mitophagy, where damaged mitochondrial are cleared in lysosomal compartments. The GTPase dynamin related protein 1 (DRP1) is the primary mitochondrial fission protein [3], whose recruitment to the outer mitochondrial membrane (OMM) is aided by the recruitment proteins Fis1, Mff, MiD49 and MiD51 in different cell types and contexts [4]. Mitochondrial fragmentation in diseases such as neurodegeneration, which is characterized by excessive mitochondrial ROS, but low ATP production is typically thought to result from uncontrolled fission [5]. However, a decline in fusion can also result in fragmented mitochondria [6].

Recent discoveries summarised below have shed light on how fission is controlled under various cellular contexts via differential DRP1 phosphorylation, by regulation of recruitment or localization of fission machinery. Importantly, these findings have also established a role of mitochondrial membrane tension in fission and mapped out the fate of the fission products.

Differential phosphorylation of DRP1 provides a handle to tune fission

DRP1 is typically cytosolic, but is recruited to the OMM prior to fission, undergoes oligomerisation to form rings around the mitochondrion, and constricts and eventually splits mitochondria in GTPase-dependent manner [7]. Phosphorylation of DRP1 to alter mitochondrial form and function is a strategy commonly employed by cells to quickly respond to intracellular and extracellular cues [8]. Indeed, cyclic stretching in neonatal rat ventricular myocytes was observed to activate DRP1 by the focal adhesion kinase (FAK)-mediated phosphorylation at S616 (Figure 1a). FAK is an environmental biosensor that integrates and relays multiple extracellular cues. Similarly, extracellular fibronectin activated DRP1 via an extracellular signal-regulated kinase 1/2 (ERK1/2) pathway, leading to increased mitochondrial fission,

Figure 1



(a) A range of cell extrinsic factors impact mitochondrial fission by phosphorylation of DRP1 at specific serine residues to promote or inhibit its activity. Fission is also promoted by CLUH by regulation of Mff and MID49 mRNA levels, phosphorylating activation of Mff by AMPK in response to inositol, and by F-actin-mediated oligomerization of Mff, **(b)** Cleavage of l-OP1 by proteases OMA1 and YME1L to s-OPA1 regulates mitochondrial fusion. Cells employ Miro-induced MFN1 inhibition to prevent fusion upon increased mitochondrial calcium levels.

oxygen consumption rate (OCR) and ATP production [9] (Figure 1a).

Further, the Rac GTPase activation protein 1 (RACGAP1) is a component of the centralspindlin complex that typically regulates cytokinesis. RACGAP1 was recently observed to be overexpressed in breast cancer [10] and to promote breast cancer metastasis by an ERK1/2-dependent enhancement of DRP1-S616 phosphorylation and subsequent mitochondrial fission (Figure 1a). This RACGAP1-triggered fission in turn induced mitochondrial turnover via mitophagy, and mitochondrial biogenesis in a PGC1a-dependent fashion. However, the mechanism of regulation of the primary controller of biogenesis, PGC1a by RACGAP1 remains to be understood.

In another study, the presence of high glucose in diabetic rats led to A-kinase anchoring protein 1 (AKAP1)-mediated S637 phosphorylation of DRP1, direct recruitment of DRP1 to mitochondria, high ROS and low ATP production [12] (Figure 1a). Phosphorylation of DRP1 at S637 via cyclic AMP-dependent protein kinase A has been documented to lead to detachment of DRP1 from human mitochondria [11], and therefore inhibition of fission. Cyclic AMP (cAMP) is an important second messenger involved in several signal transduction pathways, including metabolism, and often acts through activation of protein kinase A (PKA, Figure 1) [11]. AKAP1 is a scaffolding protein that recruits PKA to mitochondria during cAMP signalling [11]. However, under conditions of high glucose in diabetic rats, mitochondria were found to undergo more fission in the presence of S637-DRP1 [12]; as a result, podocytes (specialized epithelial cells involved in filtration of blood in the kidneys) underwent increased apoptosis. Cultured human podocytes exhibited similar enhancement of mitochondrial fission and apoptosis in the presence of high glucose. These studies highlight the contrasting, context-specific function of mitochondria upon increased fission. In future, it would be interesting to disentangle the AKAP1-PKA axis of DRP1's S637 phosphorylation, and the effect this has on mitochondrial morphology.

Finally, cytosolic viral RNA sensing by the innate immunity system was recently observed to inhibit mitochondrial fission [13] (Figure 1a). Upon Sendai virus infection, DRP1 was recruited by the mitochondrial antiviral signalling protein (MAVS) into the MAVS signalling complex and the kinase TBK1 subsequently mediated inactivation of DRP1 by phosphorylation at S412 and S684. Together, these prevented the oligomerisation and mitochondrial fragmentation by DRP1, resulting in massive tubulation and elongation of the organelle, which eventually enhanced host antiviral defense [13]. While excessive mitochondrial fusion was observed to aid host anitviral response against the Sendai virus, fusion of mitochondria serves to evade innate immunity and enhance proliferation of Dengue and Zika viruses [14].

DRP1 recruitment factors provide additional points of regulation of mitochondrial fission

Clueless (Clu) in *Drosophila* [15] and its orthologue CLUH in mammalian cells [16] are RNA-binding

proteins that typically associate with nuclear encoded mRNA of proteins involved in mitochondrial function including those responsible for OXPHOS and fatty acid metabolism. In recent studies, CLUH was found to regulate the levels of Mff and MID49 mRNA, and thereby alter the recruitment of DRP1 to mitochondria [17] (Figure 1a). The *clu null* phenotype is rescued by DRP1 overexpression, indicating a role for Clu/CLUH in regulating mitochondrial fission upstream of DRP1.

Furthermore, mitochondrial fission factor Mff has been proposed to both activate and oligomerise DRP1 [18]. *In vitro* reconstitution of Mff with DRP1 and F-actin revealed that Mff binds to DRP1-bundled actin and that oligomerised Mff are required for fission of mitochondria and peroxisomes in U2OS cells [19] (Figure 1a).

In another study, reduction in the levels of inositol (precursor to phosphoinositides and a product of the TCA cycle) was found to lead to activation of the cellular energy sensor, AMP-activated protein kinase (AMPK) in human cell lines [20]. AMPK in turn induced an activating phosphorylation of Mff, and thereby increased mitochondrial fission (Figure 1a). Thus, AMPK was proposed to act as an inositol sensor, which reined in mitochondrial fission in the presence of adequate inositol and led to uninhibited fission in limiting conditions of inositol (e.g., stress).

Insights on localisation of fission machinery, and mitochondrial fate upon fission

ER tubules act to pre-constrict mitochondria in yeast and mammalian cells and thereby facilitate oligomerisation of DRP1 at these constricted sites on the mitochondrial membrane [21,22]. Membrane contact sites between ER and mitochondria have recently been visualised to act as hotspots separating polarised and depolarised regions of the mitochondrial membrane [23], with the mitochondrial outer membrane fusion proteins mitofusins, and the mitochondrial fission protein DRP1 localising to the same hotspot on ER tubules. While this is an attractive model for quick cellular responses to changing metabolic cues, it remains to be seen how fission and fusion might be coordinated and regulated at these hotspots, and whether these ER hotspots of fission and fusion machinery are essential for mitochondrial homeostasis in cells.

In other work, by following the fate of live mitochondria using super-resolution microscopy in a variety of cells, Kleele et al. [24] demonstrated that mitochondrial fission leads to distinct outcomes depending on the location of the fission event. Mitochondria that split near the centre were more likely to participate in biogenesis thereafter, whereas the smaller mitochondria that resulted from a split near one of the mitochondrial ends ('endzone' fission) underwent degradation. Accordingly, these two distinct fission patterns resulted in differences in membrane polarity, pH, ER-actin contact (all lower in endzone fission) and lysosomal contact (higher in endzone fission).

So too, the membrane tension of mitochondria was found to modulate mitochondrial fission, with higher membrane tension enhancing fission in mammalian cells *in vitro* [25]. In this work, the fluorescence lifetime of a mitochondrial-targeted mechanosensitive FliptR probe was used to estimate membrane tension, with lower lifetime indicating lower membrane tension. Reduction of mitochondrial membrane tension using a myosin II inhibitor (ML-7 and blebbistatin) or microtubule depolymerizing drug (nocodazole) resulted in lower mitochondrial fission. However, the drop in mitochondrial membrane tension following nocodazole treatment was lower than that following ML-7/blebbistatin treatment, likely indicating that microtubules were not the primary contributors to maintenance of mitochondrial membrane tension. Further, while washing out ML-7/blebbistatin restored mitochondrial membrane tension, nocodazole wash-out did not re-establish mitochondrial membrane tension even after several hours. These findings are also in contrast to results from fission yeast which implicated microtubule attachment in reduced mitochondrial fission; depolymerization of microtubules thus led to enhanced mitochondrial fission [26]. Microtubule depolymerization in mammalian cells enhances myosin II contractility via the GEF-H1 pathway [27]. While this would lead to overall enhancement in cellular tension, it could also confound microtubule-specific mitochondrial membrane tension measurements and downstream effects. Future studies using targeted approaches to microtubule depolymerization such as optogenetics will help clarify the specific role of the microtubule-mitochondrion link in mediating mitochondrial dynamics.

Mitochondrial fusion

Mitochondrial fusion is a multi-step process involving the outer and inner mitochondrial membrane (OMM and IMM) interfaces following the tethering of adjacent mitochondria. Mechanistically, membrane GTPases belonging to the dynamin related protein (DRP) superfamily-mitofusin 1 (MFN1) and mitofusin 2 (MFN2) facilitate adjacent mitochondrial tethering and fusion of the OMM [28], and optic atrophy protein 1 (OPA1) enables fusion of the IMM [29]. The process of fusion enhances mitochondrial respiratory function through exchange of gene products and metabolites.

The papers described below illustrate additional important roles for mitochondrial calcium, and proteolytic cleavage of the fusion protein OPA1 in regulating mitochondrial fusion.

Calcium as a regulator of fusion

Fatiga et al. [30], recently reported increased mitochondrial calcium as a novel mechanism regulating MFN-mediated fusion. Inducing membrane tethering between mitochondria by expressing MFN1 or MFN2 in corresponding knockout (KO) backgrounds did not result in mitochondrial fusion. Mitochondria in these cells remained fragmented, with elevated mitochondrial calcium levels, which were sensed by the calcium sensing OMM protein Miro1/2, that regulates the transport of mitochondria on microtubules [2]. Apex2-mediated proximity labelling, and co-immunoprecipitation data suggested that calcium-bound Miro1/2 interacts and thereby inhibits MFN function (Figure 1b). Interestingly, Miro1/2-mediated abrogation of fusion was independent of its function of mitochondrial transport. While the molecular and structural mechanisms of the Miro1/2-MFN interaction remains to be elucidated, this study highlights the multifaceted functions of Miro and the need to develop functional modulators for the mechanistic understanding of these processes.

OPA1 cleavage enables control of fusion

Alternate splicing and posttranslational modifications give rise to multiple OPA1 isoforms. Long l-OPA1 is proteolytically processed to form short s-OPA1 by proteases OMA1 and YME1L [2]. Wang et al. [31] recently identified an additional site of l-OPA1 that was cleaved by YME1L (Figure 1b). This s-OPA1 collaborated with l-OPA1 to regulate IMM fusion, and interestingly the ratio of l-to s- OPA1 was a critical criterion in determining level of fusion. This property was dependent on s-OPA1's GTP hydrolysis activity, however, it remains unclear if the different s-OPA1s resulting from unique cleavage sites show distinct functionality [31].

In a recently identified stress response pathway in mammalian systems described as 'mitochondrial safeguard', short pulses of membrane depolarization (defined by the authors as 'flickering') activated OMA1-mediated l-OPA1 cleavage in cells with hyperfused mitochondria (DRP1 KO background, Figure 1b). In the absence of mitochondrial fission, this OPA1 cleavage, along with the degradation of OMM fusion protein MFN1, protected cells against bioenergetic defects when mitochondria transitioned from hyper to extreme fusion states. While calcium signalling and uncoupling protein activity are suggested to not be involved, it will be interesting to uncover the mechanism underlying localized membrane depolarization that enables the flickering process, and subsequently modulates the stress response [32].

Co-regulation of mitochondrial fission and fusion

Mitochondrial morphology is determined by the two opposing processes, fission and fusion, with an equilibrium between the two central to mitochondrial functionality, energy generation, and intracellular transport [33]. Indeed, several new studies highlight how both fission and fusion are simultaneously altered under different physiological contexts of mechanical stress, aging and cancer.

Mechanical stress-mitochondrial dynamics axis

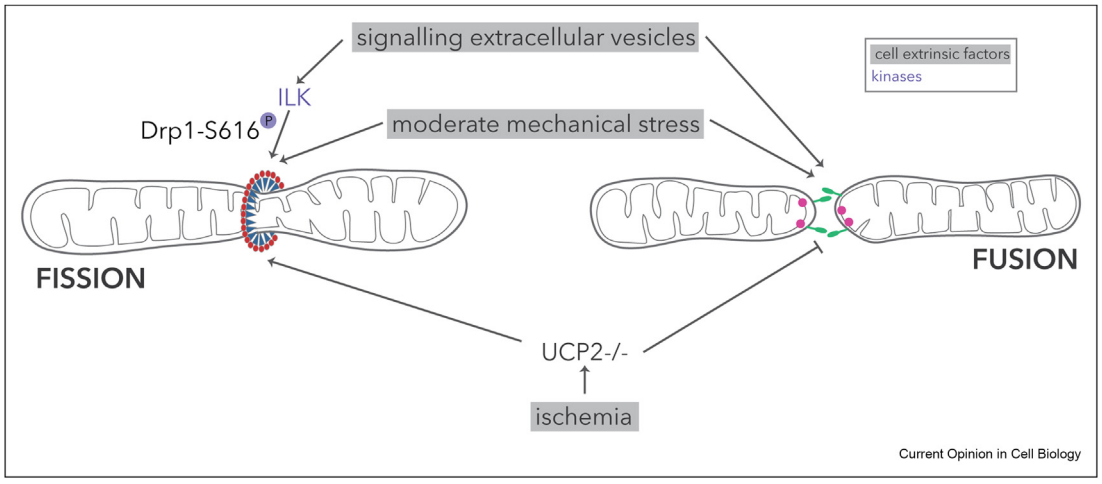
Mechanical forces applied at right angles to the long axis of mitochondria, for instance via an atomic force microscope tip, have been demonstrated to result in DRP1-mediated fission [34]. This mechanical stress is proposed to induce constriction of mitochondria, facilitating recruitment of Mff, and further downstream, DRP1 to effect fission. While this pertains to local mechanical stress on individual mitochondria, moderate mechanical stress applied at the level of whole cells in rat chondrocytes using a four-point bending system [35] treated with the anti-inflammatory cytokine IL-1β was recently identified to promote mitochondrial dynamics and protect against apoptosis [36]. Under such conditions, fusion proteins MFN1/2 and OPA1 exhibited enhanced expression, and DRP1 translocated from the cytoplasm to assemble on mitochondria (Figure 2). Interestingly, while mild stress imparted a protective response, excessive stress led to uncoupling of fission and fusion, resulting in highly fragmented mitochondria and mitochondrial dysfunction [36]. Therefore, the level of stress experienced by the cell and mitochondria within could dictate the fission-fusion response.

Altered fission-fusion dynamics in aging and disease

Uncoupling protein 2 (UCP2) is an anion carrier protein that uncouples mitochondrial oxygen consumption from ATP production. In aging ischemic mice, deletion of UCP2 was found to shift the balance towards fission by enhancing activity of fission proteins DRP1 and Fis1 and reducing levels of fusion proteins OPA1 and MFN2 [37] (Figure 2). Further, these UCP2−/− mice produced excess ROS, and exhibited mitochondrial ultrastructure damage, including mitochondrial swelling and disarrayed cristae, collectively resulting in aggravation of cerebral ischemic damage [37].

Additionally, cancer progression is correlated with tight interaction between cancer cells and surrounding non-cancerous epithelial cells, particularly via release of extracellular vesicles [38]. Signalling extracellular vesicles released by breast cancer cells under hypoxic conditions were identified to activate DRP1 by phosphorylation of SER616 and caused increased expression of MFN1 and MFN2, all of which culminated in increased fusion and fission [39] (Figure 2) in the recipient normal mammary epithelium. A key regulator of this response was the integrin linked kinase (ILK) packaged in the extracellular vesicles which activated the serine-threonine kinase Akt in recipient cells by an unknown mechanism. Akt has been shown to be able to

Figure 2



Balance of mitochondrial fission and fusion is altered in specific contexts by cell extrinsic factors. Moderate mechanical stress and signalling extracellular vesicles from hypoxic breast cancer cells promote cycles of fission and fusion in the recipient chondrocytes and mammary epithelial cells respectively. UCP2 mutation in ischemic mice induces mitochondrial fission by activation of DRP1 and Fis1 and opposes fusion by inhibiting MFNs.

integrate external stimuli to regulate cell proliferation, and ILK, as its name suggests, is typically involved in integrin signalling and has been linked to cellular functions including proliferation, adhesion and migration [40]. Interestingly, in addition to the activation of cycles of fission and fusion, the mobility and intracellular positioning of mitochondria were also altered, with mitochondria accumulating at the cortical cytoskeleton in hypoxic conditions. This finding could have interesting implications in linking the mechanobiology of breast cancer, its progression (proliferation/adhesion/migration) and concomitant changes in mitochondrial morphology and function in these cells.

Mitochondrial transport

Mitochondrial position within cells is orchestrated by microtubule- and actin-based machinery, including plus-end directed kinesins, minus-end directed cytoplasmic dynein (dynein henceforth) and processive myosin motors [2]. In metazoans, mitochondria associate with microtubule-based motor proteins via the OMM proteins Miro1/Miro2 and the motor adaptors TRAK1/TRAK2 for long-range movement. Active intracellular transport of mitochondria is essential for isotropic distribution of mitochondrial function, particularly in highly polar cells or those with high energy demands, such as neurons and cardiomyocytes [2].

Summarized below are some new studies that have provided additional insights into control of mitochondrial transport in the presence of high glucose and mechanical forces, by the motor adaptors for microtubule-based transport, and by the myosin Myo19 for actin-based transport.

Transport of mitochondria in response to chemical and physical cues

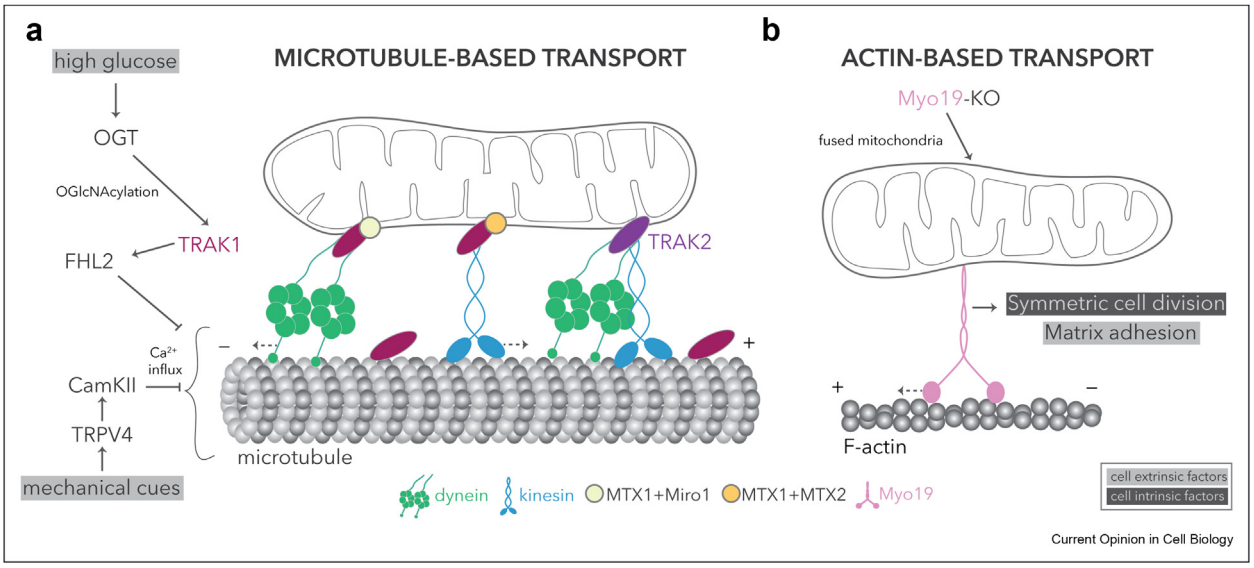
Recent studies have confirmed that mitochondrial positioning is tuned to cellular cues by showing that the protein FHL2 (four and a half LIM domains) enhanced the formation of an actin cluster around mitochondria when exposed to high glucose in a process dependent on OGlcNAcyl transferase (OGT). OGT transferred GlcNAc to serine and threonine residues of TRAK1, which recruited FHL2 and thereby impeded mitochondrial movement [41] (Figure 3a).

In another study that links mechanotransduction to mitochondrial movement in flies, a neuropathogenic mutation (R296C) of TRPV4 (mechanosensitive Ca2+ channel that is activated by temperature and stretch [42]) induced activation of TRPV4, increased CaMKII-mediated Ca2+ influx and therefore perturbed mitochondrial transport in axons [43] (Figure 3a). The mutation led to an increase in the stationary mitochondrial population and reduction in run lengths of dynein-mediated mitochondrial movement. Interestingly, a Miro GTP-bound mutant phenocopied the TRPV4 mutant, but there was no enhancement of the mitochondrial movement phenotype in a double mutant of Miro-GTP and TRPV4 (R296C).

Characterisation of known motor adaptors and discovery of novel adaptors

Metaxins are a class of proteins typically involved in protein import into mitochondria [44]. Zhao et al. [45] discovered that metaxins 1 and 2 (MTX1 and MTX2) also contribute to mitochondrial movement in both axons and dendrites of *C. elegans* neurons. Loss of

Figure 3



(a) Mitochondrial transport on microtubules is inhibited by high glucose levels and mechanical cues which modulate this response by OGlyNACylation of TRAK1 and calcium influx respectively. TRAK1 can bind to microtubules independently, and TRAK2 co-ordinates movement of mitochondria by antagonistic motors, kinesin-1 and dynein. Metaxins MTX1 and MTX2 regulate mitochondrial transport in neurons. **(b)** Myo19 KO cells exhibit increased mitochondrial fusion, and Myo-19 based mitochondrial motility promotes symmetric cell division and matrix adhesion.

metaxin-dependent mitochondrial transport in dendrites correlated with age-dependent neurodegeneration. While the MTX1/MTX2/Miro1 complex was found to link mitochondria to kinesin motors, MTX2/Miro1/TRAK1 was required to bind to dynein to effect transport within neurons (Figure 3a). While similar observations were made in human iPSC-derived cortical neurons, it will be interesting to explore if metaxins act to form the core mitochondrial adaptor complex for motor-driven transport in other cell types.

In vitro reconstitution has been invaluable in investigating motor-driven processes. Henrichs et al. employed this technique to characterise the mitochondrial adaptor TRAK1 [46]. TRAK1 was discovered to attach directly to microtubules and serve as an activating adaptor for the kinesin-1 family motor KIF5B (Figure 3a). A constitutively active KIF5B mutant bound to TRAK1 had higher run lengths and interaction times on the microtubule that had roadblocks than KIF5B alone. These findings suggest that mitochondrial transport through crowded environments may be enhanced by attachment to TRAK1. However, it remains to be seen how tug-of-war situations between kinesin-1 and dynein motors bound to mitochondria via TRAK1 are resolved. TRAK2 on the other hand has been thought to regulate minus-end directed movement of mitochondria, due to its ability to enhance dynein-mediated movement upon overexpression [47]. However, using single-molecule imaging of TRAK2 in COS7 cell lysates, Fenton et al. demonstrated that TRAK2 is also a potent activator of kinesin-1 [48]. The dynein regulator LIS1 increased the frequency and processivity of minus-end directed movement of TRAK2, but on its own, TRAK2 only mildly activated dynein. Interestingly, TRAK2 was found in a complex with both dynein and kinesin-1 and is therefore proposed to co-ordinate minus- and plus-end directed movement of mitochondria within cells (Figure 3a). Future studies will reveal precisely how the movement of a single mitochondrion bound to antagonistic motors dynein and kinesin is regulated. So too, *in cellulo*/*in vivo* experiments will be essential to establish how the cues from multiple motor-adaptor complexes translates to movement in a crowded cellular context.

Myo19 at the fore of actin-based mitochondrial movement

Myo19 is an unconventional vertebrate myosin that is capable of short-range transport of mitochondria towards the plus (barbed) ends of actin filaments [49]. Single-molecule imaging of purified Myo19 on de-membraned cells was employed to confirm this function of the motor [50]. Myo19 KO in human cells further revealed an important role for the motor in mediating cellular processes including symmetric cell division and matrix adhesion [51] (Figure 3b). Cells without Myo19 also exhibited highly fused mitochondria (Figure 3b), and mitochondrial enrichment of TRAK1 and the dynactin subunit p150. Dynactin is an essential component of the tripartite complex that is required for dynein to engage in minus-end directed transport [52]. However, dynein inhibition failed to rescue to the mitochondria or cell-level

phenotypes [51], and it remains unclear how mitochondrial transport is coordinated between the actin-based and microtubule-based motors.

Outlook

While the field of mitochondrial research continues to move at a fantastic pace, there are areas which remain to be addressed. First, cell- and context-specific mitochondrial behaviour have been probed in detail, yet the underlying unifying principles are yet to be described. Second, standardized methods to visualise and characterise mitochondrial form need to be agreed upon by the field so as to enable comparison of results from different labs.

This review has covered studies that combined traditional biochemical and molecular biology approaches with cutting-edge techniques including super-resolution live-cell microscopy and *in vitro* reconstitution with cell lysates, to deliver insights into mitochondrial dynamics. However, to amalgamate the complex and diverse datasets that become available with these techniques, the future of research in this area will require an influx of interdisciplinary researchers: biologists, chemists, computer scientists and physicists will integrate cell and developmental biology, chemistry, deep learning, and soft matter physics to uncover novel mitochondrial phenomena in health and disease.

Conflict of interest statement

Nothing declared.

Data availability

No data was used for the research described in the article.

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* of special interest
** of outstanding interest

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This study identifies two types of fission that are functionally and mechanistically distinct. While both are mediated by DRP1, division at the periphery termed endzone fission segregates regions of damage within the mitochondrion and destines them for degradation. In contrast mitochondria that divide at the midzone are more likely to participate in biogenesis. Interestingly, lysosomal contact and FIS1 regulation are associated with endzone fission whereas pre-constriction mediated by endoplasmic reticulum and F-actin, and MFF determine fission at the midzone.

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