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Molecular mechanisms and physiologic functions of mitochondrial dynamics

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Hidenori Otera and Katsuyoshi Mihara*

Department of Molecular Biology, Graduate School of Medical Science, Kyushu University, Fukuoka 812-8582, Japan

*Katsuyoshi Mihara, Department of Molecular Biology, Graduate School of Medical Science, Kyushu University, Fukuoka 812-8582, Japan. email: mihara@cell.med.kyushu-u.ac.jp

Mitochondria are highly dynamic organelles that continuously change their shape through frequent fusion, fission and movement throughout the cell, and these dynamics are crucial for the life and death of the cells as they have been linked to apoptosis, maintenance of cellular homeostasis, and ultimately to neurologic disorders and metabolic diseases. Over the past decade, a growing number of novel proteins that regulate mitochondrial dynamics have been discovered. Large GTPase family proteins and their regulators control these aspects of mitochondrial dynamics. In this review, we briefly summarize the current knowledge about molecular machineries regulating mitochondrial fusion/fission and the role of mitochondrial dynamics in cell pathophysiology.

Keywords: Mfn1/2/Opa1/Drp1/GTPase/ mitochondrial dynamics.

Abbreviations: AAA, ATPase associated with diverse cellular activities; ATP, adenosine triphosphate; CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; CMT2A, Charcot-Marie-Tooth disease type 2A; Drp1, Dynamin related protein1; ER, endoplasmic reticulum; ERMES, endoplasmic reticulum-mitochondria encounter structure; Fis1, Fission1; GDAP1, ganglioside-induced differentiation-associated protein 1; GED, GTPase effector domain; GTP, guanosine triphosphate; i-AAA, intermembrane space AAA protease; IMM, Inner mitochondrial membrane; IMS, mitochondrial intermembrane space; LC3, microtubule-associated protein1 light chain 3; m-AAA, matrix AAA protease; MAM, mitochondria-associated membrane; March5, membrane-associated ring finger 5; Mdm, mitochondrial distribution and morphology; Mdv1, mitochondrial division protein 1; Mff, Mitochondrial fission factor; Mfn1, Mitofusin1; Mfn2, Mitofusin2; MIB, mitofusin binding protein; Mmm, mitochondrial morphology maintenance; mtDNA, mitochondrial DNA; Oma1, overlapping activity with m-AAA protease; OMM, Outer mitochondrial membrane; Opa1, Optic atrophy1; RING, Really Interesting New Gene; RNAi, RNA interference; SOD, superoxide dismutase; SUMO, small ubiquitin-like modifier; TPR, tetratricopeptide repeat; VDAC1, voltage-dependent anion channel protein 1.

Mitochondria are double-membrane bound organelles that are essential for numerous cellular processes such as aerobic ATP generation, lipid biosynthesis and haem and iron-sulphur cluster biogenesis (*1*). They are now recognized as highly dynamic organelles that move within cells via microtubules or microfilaments and continuously fuse and divide in healthy cells, and these dynamic morphologic changes are essential not only for the maintenance of respiratory activity and mitochondrial DNA (mtDNA), but also for the control of cellular processes such as embryonic development, neuronal plasticity, apoptosis or calcium signalling (Fig. 1) (*2*). Mitochondrial fission contributes not only to the proper distribution of mitochondria in response to the local demand for ATP, but also to the elimination of damaged mitochondrial fragments through mitophagy (autophagy for mitochondria), whereas mitochondrial fusion facilitates the exchange of mtDNA and other vital components between mitochondria for the maintenance of functional mitochondria. Mitochondrial fusion and fission are controlled by four high molecular weight GTPases conserved from yeast to mammals: mitofusins Mfn1 and Mfn2 (Fzo1 in yeast) in mitochondrial outer membrane (OMM) fusion; Opa1 (a cause gene product of optic atrophy type I; Mgm1 in yeast) in mitochondrial inner membrane (IMM) fusion and cristae organization; and Drp1 (Dnm1 in yeast) in mitochondrial fission, indicating that the fundamental mechanisms controlling mitochondrial dynamics have been maintained during evolution. Abnormal mitochondrial dynamics often cause neuronal synaptic loss and cell death in several human neurologic diseases, such as Alzheimer's disease, Parkinson's disease and Huntington's disease (*3*). Furthermore, mitochondrial dynamics are suggested to be responsible for the pathologic conditions associated with oxidative stress and have also been linked to ageing.

Mitochondrial fusion machinery and regulation

Mitochondrial fusion with closely apposed mitochondria is a complex regulatory process involving multiple proteins that fuse both the OMM and IMM of each mitochondrion. Although fusion reaction between OMMs of apposed mitochondria and the subsequent fusion between IMMs are normally highly synchronized, the two processes can be functionally uncoupled (*4*, *5*). In cultured cells, the lack of mitochondrial fusion leads to a defect in oxidative phosphorylation, because fusion-deficient mitochondria cannot exchange contents, they are unable to restore and/or maintain the mtDNA-encoded proteins required for

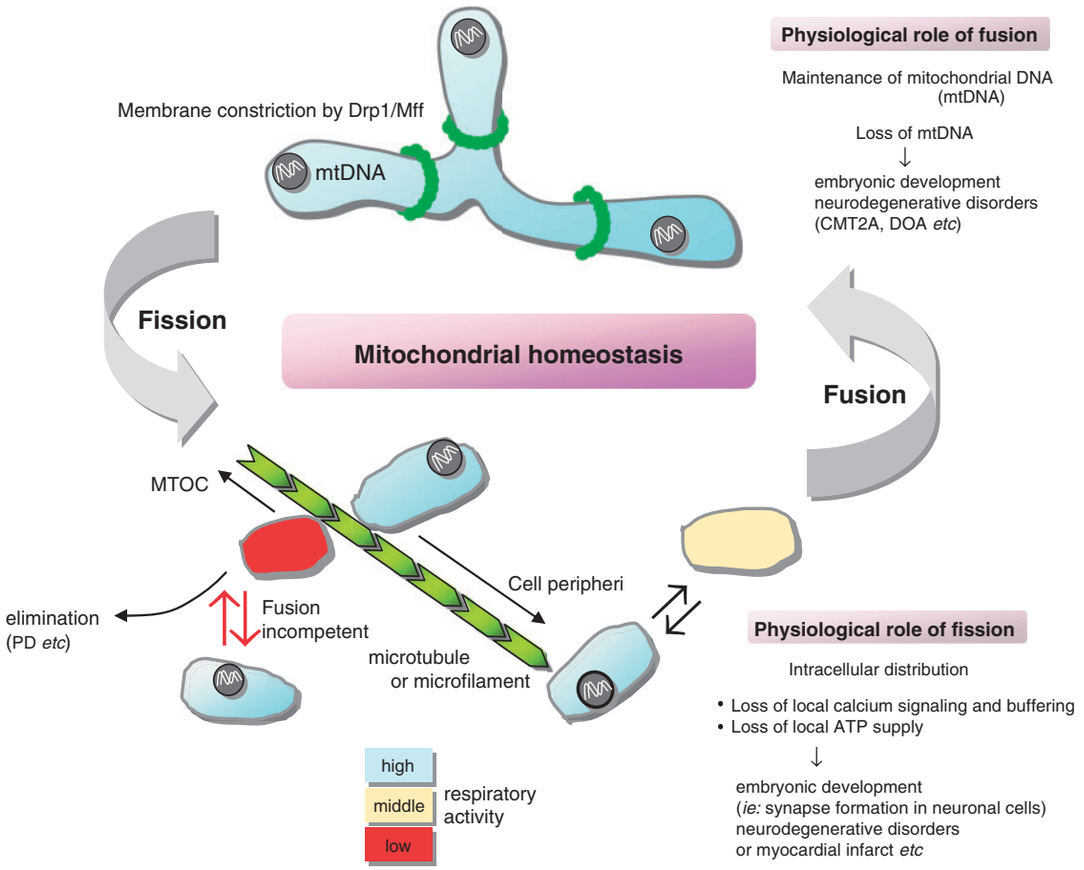

Fig. 1 Overview of mitochondrial dynamics and homeostasis. Mitochondrial morphology is maintained by fusion and fission. Excessive mitochondrial fission often causes the generation of depolarized (respiratory inactive) mitochondria. Although the mildly depolarized mitochondrial fragments fuse back with the active mitochondria and recover the respiratory activity, severely depolarized mitochondrial fragments cannot fuse back with the reticulum. Therefore, mitochondrial fusion prevents the loss of mtDNA nucleoids and contributes to maintain mitochondrial respiratory activity. Dysfunctional mitochondria return to the cell soma and are eliminated by the autophagy system, named mitophagy. Disruption of the mitochondrial dynamics or mitochondrial quality control system leads to the accumulation of dysfunctional mitochondria and causes a collapse of the cellular environment followed by cell death. CMT2A: Charcot–Marie-Tooth disease type 2A, DOA: Optic Dominant Atrophy, PD: Parkinson's disease.

electron transport, thus becoming respiration-defective (Fig. 1) (*6*).

As mentioned above, three large GTPase proteins, Mfn1, Mfn2 and Opa1 mediate mammalian mitochondrial fusion (*7–10*). Mfn1 and Mfn2 are anchored to the OMM with both a large N-terminal GTPase domain and C-terminal coiled-coil domain exposed to the cytosol, and mediate OMM fusion in a GTPase-dependent manner. Mutations in Mfn2 cause Charcot–Marie-Tooth disease type 2A (CMT2A) (*11–13*). The mechanism by which Mfn2 mutations cause CMT2A is not fully elucidated, but there are many loss of function Mfn2 mutations found in CMT2A within the GTPase domain. Both Mfn1 and Mfn2 form homo- or hetero-protein complexes (*7*, *14*, *15*). Such physical interactions between mitofusins on opposing mitochondria serve to tether and possibly to fuse the OMMs. Knockout of Mfn1 results in small fragmented mitochondria broadly dispersed within the cell, whereas knockout of Mfn2 leads to large fragmented mitochondria concentrated near the nucleus (*7*). Consistent with these distinct knockout phenotypes, purified recombinant Mfn1 exhibits higher GTPase activity than Mfn2 (*14*). Thus, the two mitofusins seemed to have distinct roles in mitochondrial OMM fusion; Mfn1 is thought to be responsible for the initial GTP-dependent OMM tethering. It is not clear how Mfn1 and Mfn2 promote OMM fusion beyond the initial tethering step. Mfn2 is also enriched in the mitochondria-associated membranes (MAM) of the endoplasmic reticulum (ER), where it interacts with Mfn1 and Mfn2 on the mitochondria to form interorganellar bridges (*16*, *17*). Close contacts between mitochondria and the ER are physiologically important for Ca^{2+} signalling, metabolite exchange, and, therefore, the regulation of mitochondrial metabolism and apoptosis. Because the ER–mitochondria contacts are still formed in mitofusin-deficient cells, however, other proteins may also participate in the ER–mitochondria contacts. In yeast, in this relation, the ER–mitochondria tethering complex, the ERMES complex comprising Mdm34, Mdm10, Mdm12 and Mmm1, is reported to form the contacts involved in phospholipid exchange (*18*, *19*). The role of Mfn2 in the formation of the ER–mitochondria contacts and their functional significance remain to be further

elucidated. In addition to these two players, a 55-kDa mitofusin binding protein (MIB) was identified as a fusion regulator. MIB is a member of the medium-chain dehydrogenase/reductase protein superfamily and has a conserved coenzyme-binding domain. MIB overexpression in HeLa cells induces mitochondrial fragmentation, whereas MIB silencing by RNA interference (RNAi) leads to the formation of elongated mitochondria (*20*). Therefore, MIB functions as a negative regulator of mitofusin proteins. To understand the molecular mechanisms of OMM fusion in detail, further identification of the Mfn1/Mfn2-interacting proteins is required.

Opa1 is another key molecule essential for mitochondrial IMM fusion and cristae remodelling. Mutations in Opa1 cause autosomal dominant optic atrophy, a degenerative disease of the optic nerve (*3*, *8–10*). There are eight Opa1 splice variants, which are all synthesized as precursor proteins with the mitochondrial localization sequence in the N-termini and the following hydrophobic stretches that are responsible for sorting the protein into the IMM (*21–23*). During mitochondrial import, the mitochondrial localization sequence of Opa 1 precursors is removed by mitochondrial processing peptidase to form L-forms (*21*). They are anchored to the IMM with the GTPase domain exposed to the mitochondrial intermembrane space (IMS), and are subsequently processed either in the IMS to produce S-forms by the intermembrane space AAA protease (i-AAA protease) YME1L or in the matrix by m-AAA protease Afg3L1, Afg3L2 or Paraplegin of mice mitochondria (Afg3L2 and Paraplegin for human mitochondria) depending on whether the process site localizes in the IMS (for splice variants carried the exon 5b-encoded region) or in the matrix (for the other splice variants), respectively (*24–28*). Prohibitins (PHB1 and PHB2), the evolutionally conserved IMM proteins that function as the protein- and lipid-scaffolds and are essential for cell proliferation and development, regulate Opa1 processing (*29–30*). Interestingly, defective phenotypes of PHB knockout cells (growth defect, mitochondrial fragmentation, susceptibility to apoptosis and defective cristae morphogenesis) are all complemented by exogenous expression of the Opa1 L-form, indicating that the PHB complex is epistatic to Opa1 processing (*29*). Mutations in the GTPase domain lead to the fragmented mitochondria, indicating that GTP hydrolysis is essential for mitochondrial fusion activities (*31*). Many Opa1 mutations found in the dominant optic atrophy are detected in the GTPase domain. The IMM-exposed region locating next to the GTPase domain is involved in the tetramerization and higher order self-assembly of Opa1 (*32*). Under normal conditions, L- and S-forms are both essential for sufficient mitochondrial fusion (*27*, *33*). It is demonstrated in yeast that l-Mgm1 functions as the membrane anchor for s-Mgm1 and the GTPase-defective l-Mgm1 mutant is functional (*34*). In this relation, other report demonstrated that l- and s-Mgm1 both exist as inactive GTPase monomers in the absence of membrane, but together in *trans* they form a functional dimer in a cardiolipin-dependent manner that is the building block for higher order assemblies (*35*). Importance of cardiolipin in stimulating GTPase and assembly of Mgm1 and Opa1 is also reported (*36*, *37*).

Loss of the mitochondrial membrane potential by a protonophore carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) induces significant fragmentation of mitochondria concomitant with a rapid conversion of L-Opa1 to S-Opa1. The loss of mtDNA, ATP deficiency or apoptosis primes similar processing, and this 'induced' Opa1 processing is mediated by Oma1 (for overlapping activity with m-AAA protease), a protease with multiple membrane-spanning segments and a zinc-binding motif (*38–40*). Neither knockdown nor overexpression of Oma1 affects mitochondrial morphology in mouse embryonic fibroblast cells, suggesting that Oma1 is dispensable for the balanced formation of L-forms and S-forms by constitutive cleavage under normal conditions. The regulation of Opa1 activities mediated by Oma1 will likely affect tissue development or the progression of neurodegenerative diseases. Opa1 is also reported to be involved in maintenance of the cristae structure; knockdown of Opa1 induces disintegration of the cristae structure concomitant with cytochrome *c* release and apoptosis induction (*25*, *41*, *42*).

Molecular insights into Drp1 actions during mitochondrial fission

Drp1 is a member of the conserved dynamin GTPase superfamily, which includes a broad range of membrane fission proteins. It is a cytosolic protein with an N-terminal GTPase domain thought to provide mechanical force, a dynamin-like middle domain and a GTPase effector domain (GED) located in the C-terminal region. A dominant-negative middle domain mutation (A395D) in Drp1 has been reported in a lethal disorder with microcephaly, abnormal brain development, optic atrophy and hypoplasia (*43*). The cells derived from this patient showed aberrant mitochondrial elongation (*43*). Drp1 mainly localizes in the cytosol, and during mitochondrial fission, translocates from the cytosol to prospective fission sites on the mitochondria (Fig. 2A) (*44–46*). *In vitro* studies as well as studies of yeast Dnm1 revealed that Dnm1 or Drp1 assembles, like dynamin, into self-assembled higher-order structures that wrap around the mitochondrial tubule (*44*, *47*, *48*). These spiral higher-order structures are thought to constrict and eventually sever the mitochondrial membrane by a GTP hydrolysis-dependent mechanism. Time-lapse imaging of the GFP-tagged Drp1 shows that mitochondrial tubules divide at sites where these punctate structures are found (*44*, *49*). The GTP-binding defective mutant (K38A) sequesters endogenous Drp1 into uncharacterized aggregated or dotted structures, thus inhibiting its localization on the mitochondrial fission sites to act as a dominant negative mutant (*44*, *47*). Intermolecular interactions between the N-terminal GTPase domain and C-terminal GED are also important for Drp1 self-assembly and functional regulation (*50*, *51*). The regulation of IMM scission as well as the mechanisms synchronizing scissions of the IMM and the OMM are

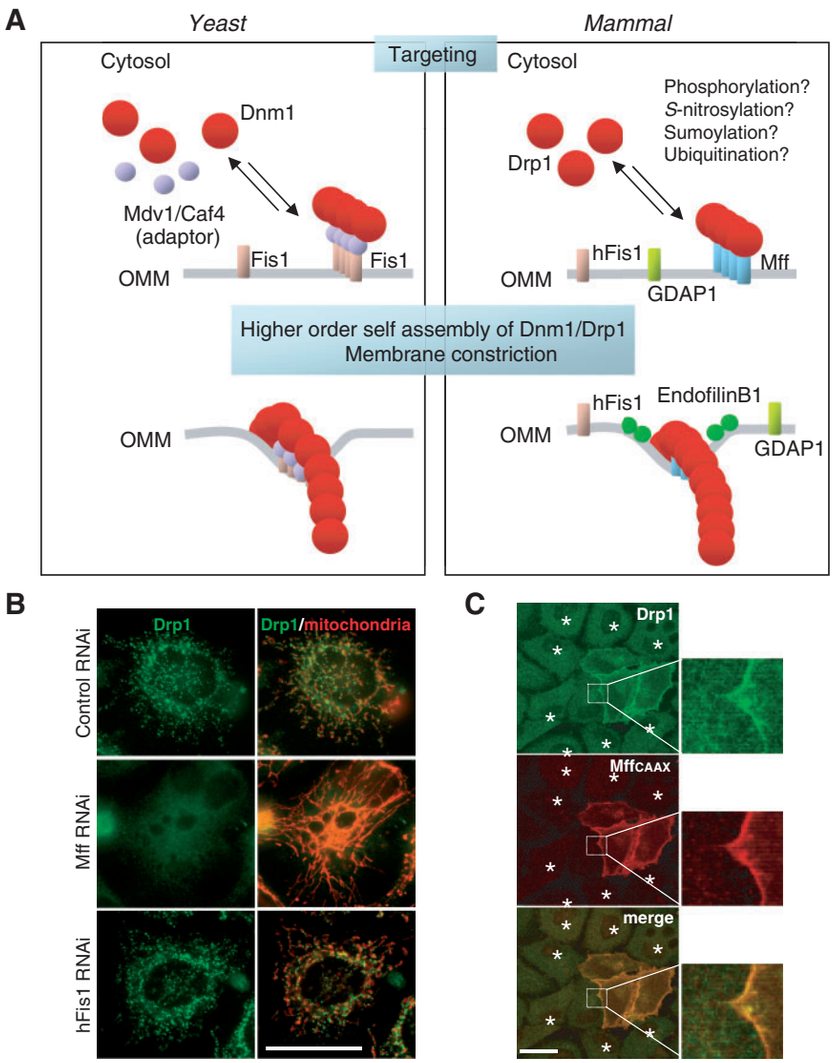

Fig. 2 Drp1/Dmn1 assembly on the OMM in yeast and mammals. (A—left panel) Mitochondrial fission in yeast involves the interaction of Dnm1 with Fis1 via the soluble adaptor proteins Mdv1/Caf4. (A—right panel) Mitochondrial fission in mammals requires the tail-anchored protein Mff. Cytosolic Drp1 is recruited to the OMM by Mff. Although homologues of yeast Mdv1/Caf4 have not been identified in higher eukaryotes, Mff seems to function simultaneously as an adaptor and a receptor for the mitochondrial recruitment of Drp1. The function of hFis1 and Gdap1 in mammals remains to be elucidated. (B) Control, Mff, or hFis1 RNAi HeLa cells were stained with antibodies against Drp1 (green) and mitofilin (red). Magnified images are shown. Scale bar indicates 20 μm. (C) HeLa cells were transfected with Mff-siRNA and further transfected with FLAG-Mff-CAAX, an Mff mutant in which the C-terminal transmembrane domain (TMD) of Mff was replaced with the plasma membrane-targeted CAAX motif. The cells were stained with antibodies against Drp1 (green) and FLAG (red). FLAG-Mff-CAAX directs Drp1 to the cell surface membrane. 'Asterisks' denotes FLAG-Mff-CAAX non-expressing cells. Scale bar indicates 10 μm.

not yet known. The IMM-localized MTP18 is identified as a transcriptionally regulated target of the phosphatidylinositol 3-kinase signalling and regulates mitochondrial fission coupled with the action of Drp1 at the OMM (*52*, *53*). Although the mechanism of MTP18 in the IMM fission is unclear at present, the identification of its interacting partners will help understanding the molecular mechanisms that synchronize scissions of the IMM and OMM.

Fis1 is a C-tail anchored OMM protein with its N-terminal multiple tetratricopeptide repeat (TPR) motif exposed to the cytoplasm (*54*, *55*). In yeast, Fis1 is required for Dnm1 recruitment (Fig. 2A). During mitochondrial fission, Fis1 transiently interacts via cytosolic adaptor proteins Mdv1/Caf4 with Dnm1 by its TPR motif, suggesting its function as the mitochondrial Dnm1 receptor (*56*, *57*). In mammals, Fis1 has also been identified in mitochondria (hFis1 for human Fis1) and is thought to be involved in recruiting Drp1 to mitochondria as in yeast through direct or indirect interactions (*55*). The actual function of hFis1, however, remains enigmatic, because the Mdv1/Caf4-like adaptor proteins have not been identified, hFis1 localizes throughout the OMM in contrast to the punctate localization of Drp1 and mitochondrial recruitment of Drp1 is not affected by hFis1-knockdown (Fig. 2B) (*58*). Whether or not hFis1 induces mitochondrial fission is even controversial. Yeast Fis1 is well established to mediate mitochondrial fission, and similarly, plant Fis1 is required for

mitochondrial fission (*59*). The deletion of Fis1 and Fis2 in *Caenorhabditis elegans*, however, does not result in any detectable mitochondrial defects (*60*). Moreover, hFis1 cannot rescue the phenotype of yeast *fis1Δ* cells, indicating that the two proteins are structurally divergent or act through different mechanisms (*54*). Recently, we reported that the mitochondrial morphology and the mitochondrial recruitment of Drp1 remain unaffected in hFis1-knockout cells (*61*). Although previous studies showing mitochondrial fission used hFis1 overexpression or RNAi for hFis1 in living cells, these manipulations sometimes induce non-physiologic stress in the cells, which seemed to lead to mitochondrial morphology changes. Therefore, caution should be taken when interpreting data from overexpressing membrane proteins or RNAi used in previous studies. Further studies are needed to clarify the functional relevance of hFis1 in Drp1-dependent mitochondrial morphology regulation.

Ganglioside-induced differentiation-associated protein 1 (GDAP1) is another mitochondrial fission factor located at the OMM (*62*). GDAP1 is involved in the maturation of gangliosides. GDAP1 mutation leads to the peripheral neuropathy Charcot–Marie-Tooth disease (CMT), affecting Schwann cells, the myelinating glia of the peripheral nervous system, and neurons (*63*). Although it is not clear how GDAP1 is involved in mitochondrial fission, these data suggest the importance of membrane lipid compositions such as gangliosides in mitochondrial fission.

Mff, a key player in Drp1-dependent mitochondrial fission

Despite extensive studies, the mechanisms by which cytoplasmically localized Drp1 is activated and recruited to the prospective mitochondrial fission sites have remained unclear. Mitochondrial fission factor (Mff) is a C-tail anchored protein on the OMM that recently identified by the *Drosophila* RNAi library search for mitochondrial morphology alterations (*64*). Mammalian mitochondria contain an Mff orthologue and silencing this factor by RNAi induces mitochondrial elongation in mammalian cells. The specific role of Mff in mitochondrial fission, however, remains unknown. To better elucidate its role, we first examined whether Mff RNAi affects the mitochondrial recruitment of Drp1. Endogenous Drp1, observed as dotted structures on mitochondria, was clearly decreased and was dispersed in the cytoplasm in Mff RNAi cells concomitant with mitochondrial network extension (Fig. 2B). In contrast, Mff overexpression induced mitochondrial fragmentation, concomitant with increased Drp1 recruitment to the mitochondria (*61*). Consistent with these observations, both *in vitro* and *in vivo* experiments demonstrated that Mff transiently interacts with Drp1 through its N-terminal cytoplasmic region (Fig. 2C). Furthermore, Mff mostly co-localizes with the Drp1 foci on the OMM in marked contrast to the uniform localization of hFis1 in the OMM (*61*). Furthermore, conditional knockout of hFis1 in colon carcinoma cells revealed that it is dispensable for mitochondrial fission (*61*). These observations indicate that Mff functions as a Drp1 receptor to mediate mitochondrial fission. Drp1 might self-assemble via its ability to homo-oligomerize at the Mff-containing foci on the mitochondrial surface, forming spiral structures around the mitochondrial tubules. After this process, hFis1 or unidentified Mff-interacting proteins might affect the assembly of the fission machinery, leading to membrane constrictions or lipid remodelling and eventually to membrane scission. In contrast to the conservation of Fis1 through various species, there are no obvious homologues of Mdv1/Caf4 in metazoans, and Mff appears to be restricted to metazoans. Mammalian mitochondria seem to adopt fission mechanisms that are distinct from those of yeast or plants (Fig. 2A). The mechanistic details of these processes and their GTP-dependence remain key questions to be analysed in the future.

Regulation of Drp1 by posttranslational modifications

Various stressors outside or inside cells induce mitochondrial fission to remodel mitochondria and cellular function. During apoptosis, cytoplasmic Drp1 is translocated to the mitochondria and induces mitochondrial fragmentation prior to caspase activation by the release of cytochrome *c* (*65*, *66*). Such increased fission events are also important for autophagic clearance of depolarized (or dysfunctional) mitochondria or for proper segregation of mitochondria into daughter cells during mitosis (*67*, *68*). Overexpression of wild-type Drp1 does not lead to mitochondrial fragmentation, suggesting that a simple alteration of Drp1 levels does not change mitochondrial fission but regulation of Drp1 properties, such as mitochondrial translocation, higher order assembly or GTPase activity is rather critical. In this context, posttranslational modifications are implicated as regulatory mechanisms during mitochondrial fission (Fig. 2A).

During mitosis, rat Drp1 is activated by the Cdk1/cyclin B-mediated phosphorylation of Ser585 (Ser616 for human Drp1) in the GED domain. This mitotic phosphorylation promotes Drp1-dependent mitochondrial fission and facilitates the proper distribution and segregation of mitochondria into daughter cells (*69*). The exact mechanisms linking Drp1 phosphorylation at Ser585 to increased fission activity remain to be determined. A serine residue within the GED domain (Ser637 for human Drp1; Ser656 for rat Drp1) is phosphorylated by protein kinase A in HeLa and PC12 cells. This phosphorylation inhibits mitochondrial fission through the inhibition of intra-molecular interaction between GTPase and GED domains, GTPase activity and eventually mitochondrial recruitment of Drp1 (*70*). In this context, calcineurin dephosphorylates Ser637 and stimulates the translocation of Drp1 to the mitochondria (*71*, *72*). In contrast, Ca^{2+} signalling activates Ca^{2+}/calmodulin-dependent protein

kinase Iα to phosphorylate Ser637 and increases mitochondrial translocation of Drp1 through increasing the Drp1 binding affinity for hFis1 in hippocampal neurons (*73*). Thus, phosphorylation of Drp1 at the same GED domain residues is likely to have opposite effects on the mitochondrial fission activity in different cells or tissues.

S-Nitrosylation is a ubiquitous protein modification in redox-based signalling. β-Amyloid protein, a key mediator of Alzheimer's disease, stimulates nitric oxide production to cause *S*-nitrosylation of human Drp1 at Cys644 within the GED domain, which enhances GTPase activity and Drp1 oligomer formation in association with excessive mitochondrial fission in neurons, leading to synaptic loss and neuronal damage in the brains of Alzheimer's disease patients (*74*). A mutation of Cys644 prevents mitochondrial fragmentation and blocks the neurotoxicity induced by nitric oxide or β-amyloid protein (*74*).

The small ubiquitin-like modifier (SUMO) protein also affects Drp1 activity. Overexpression of SUMO1 stabilizes Drp1 in a Bax/Bak-dependent manner on the mitochondrial membrane and induces mitochondrial fission, suggesting that sumoylation is a step in the regulation of Drp1 during early apoptosis progression (*46*). Mitochondrial SUMO E3 ligase (MAPL) has been identified as SUMO E3 ligase for Drp1 (*75*). Conversely, overexpression of the Sumo protease SENP5 decreases Drp1 sumoylation and rescues SUMO1-induced mitochondrial division (*76*). Neuspiel *et al*. (*77*) reported that MAPL is incorporated in previously uncharacterized mitochondria-derived vesicles that bud from mitochondria and are transported to peroxisomes. Communication with peroxisomal membranes might thus influence mitochondria morphology or lipid biosynthesis (*78*).

In addition to sumoylation, ubiquitination regulates Drp1 activity. March5 (also known as MITOL), a mitochondria-associated RING-finger E3 ubiquitin ligase, ubiquitinates Drp1 on the OMM, although the effect of March5/MITOL-dependent ubiquitination of Drp1 on mitochondrial dynamics remains controversial. March5/MITOL silencing or overexpression of the March5/MITOL mutant lacking ubiquitin ligase activity induced mitochondrial fragmentation in previous studies (*79*, *80*). Karbowski *et al*. (*45*), however, demonstrated later that March5/MITOL silencing, as well as overexpression of the RING-inactive March5/MITOL mutant, induces abnormal mitochondrial accumulation of Drp1 in association with abnormal mitochondrial elongation and their interconnections. In addition, March5/MITOL might play a more general role in the quality control of mitochondria by ubiquitinating mutated, damaged or misfolded protein accumulated in the OMM, as was observed for a mutated version of SOD or expanded poly-Q proteins (*81*, *82*). Thus, whether and how March5/MITOL contributes to mitochondrial dynamics is an important issue for future studies.

Different effects induced by the same modification might depend on circumstances such as where and when the effects occur within the cells or the cell type. Although the exact mechanism of Drp1 regulation via phosphorylation, *S*-nitrosylation, ubiquitination or sumoylation is unclear, it is likely that posttranslational modifications of Drp1 both positively and negatively regulate its function in mitochondrial dynamics.

Physiologic role of mitochondrial dynamics

Mitochondria accumulate in sites where high amounts of energy are required or where Ca^{2+} buffering is required (Fig. 1). The proper regulation of mitochondrial dynamics is therefore particularly important in highly polarized cells such as neurons, due to their high demand for energy at the synapses as well as axonal transport and calcium homeostasis for normal synaptic activities; functional mitochondria are supplied to the synaptic area along microtubules by kinesin (anterograde transport), whereas dysfunctional mitochondria are returned to the soma by dynein (retrograde transport) (*83*–*85*) to restore activity by fusion with the respiratory active mitochondria or to be eliminated by mitophagy (mitochondria-specific autophagy) (Fig. 1). Changes in mitochondrial morphology are thus important for transport to the appropriate destination. Milton 1 and 2 localize on the mitochondrial surface and interact with kinesin heavy chain to mediate the axonal transport of mitochondria to the synapse along microtubules, acting as an adaptor or regulator of mitochondrial transport (*86*, *87*). The mitochondrial Rho-GTPase protein Miro is a protein that interacts with Milton, based on studies using a yeast two-hybrid system (*88*, *89*). Miro is an OMM protein with two cytoplasmic GTPase domains and two Ca^{2+} binding EF hand motifs. Loss of Miro in flies results in a lack of axonal mitochondrial transport and the accumulation of mitochondria within the neuronal cell bodies (*88*). Based on its motif structures, Miro is a potential regulator of mitochondrial motility; a high concentration of Ca^{2+} decreases mitochondrial motility, leading to the accumulation of mitochondria near areas of high energy demand such as active synapses. Thus, Miro is another essential component of the machinery for mitochondrial transport and has a crucial role in neuronal function.

Key proteins of mitochondrial fusion and fission play important roles in the regulation of apoptosis, and mutations or abnormal expression of these proteins are associated with neurodegenerative disorders (*3*, *90*). We and others previously reported that Drp1 deficiency results in an irrelevant mitochondrial distribution within neuronal cells by inducing the aggregation of enlarged mitochondria around the nucleus, which may prevent neurite and synapse formation probably due to an inefficient energy supply, inefficient buffering of local Ca^{2+} or inefficient formation of synaptic vesicle pools (*91*, *92*). Mitochondrial fission likely contributes to the proper distribution of the mitochondria along cytoskeletal tracks, as well as to facilitate the equal segregation of mitochondria into daughter cells during cell division.

Because mitochondrial fusion leads to the exchange of contents including mtDNA to complement damaged contents, mitochondrial fusion contributes

to maintain the functional oxidative phosphorylation system (Fig. 1) (*6*). In contrast to fusion, the role of fission in mitochondrial function is not well elucidated. Mitochondrial fission segregates depolarized mitochondrial segments and the dysfunctional mitochondria are sequestered from respiration-active mitochondria within the cells and are then eliminated by mitophagy (Fig. 1) (*67*, *68*, *93*).

It was recently demonstrated that cause gene products of Parkinson's disease, PTEN-induced mitochondrial protein kinase 1 (PINK1) and cytoplasmic ubiquitin E3 ligase Parkin mediate mitophagy (*94*, *95*, *96*). Loss of the mitochondrial membrane potential compromises the degradation of otherwise unstable PINK1 on the OMM, which leads to the recruitment of Parkin to initiate an autophagy cascade. In addition, a recent report demonstrated that Mfn1 and Mfn2 are ubiquitinated by Parkin upon membrane depolarization and degraded by proteasome through a AAA^+ ATPase p97, thereby preventing fusion of depolarized mitochondria and promoting mitophagy (*97*). In this relation, dissipation of mitochondrial membrane potential causes the conversion of L-OPA1 to the S-form, which prevents damaged mitochondria from further fusion reactions (*21*). Thus, the Parkin-PINK1 system is a mitochondrial quality control system to monitor damaged or uncoupled mitochondria generating excessive reactive oxygen species (*3*, *98*, *99*) and dysfunction of this mechanism is a possible cause of Parkinson's disease (*100*, *101*, *102*). It is reported that polyubiquitination of VDAC1 is required for the recognition by LC3 of autophagosomal membrane through adaptor protein p62 (*103*), although the significance of this reaction remains to be confirmed. As it is thought that mitochondrial fission is related to the progression of mitophagy, inhibition of mitochondrial fission by the dominant negative mutant of Drp1 or specific inhibitor of Drp1-GTPase mdivi-1 compromises Parkin-PINK1 dependent mitophagy (*104*). Together, mitochondrial fusion and fission are more likely to be involved in mitochondrial quality control in healthy cells. Further experiments are necessary to elucidate precise molecular mechanism of the Parkin-PINK1-dependent mitophagy.

Perspectives

Recent advances in live cell imaging revealed that mitochondria are highly dynamic through continuous fusion and fission as well as movement along the cytoskeleton within the cells. Mitochondrial fusion and fission have important roles not only in the modulation of mitochondrial morphology but also in other biologic processes, including bioenergetics, cellular metabolism, mitochondrial maintenance, synaptic integrity and neuronal cell death. Over the past decade, although a significant amount of relevant data has accumulated regarding the identification of proteins modulating mitochondrial dynamics and their molecular function, characterization of the coordination and regulation of these proteins is only preliminary. Several fundamental questions, such as the exact role of the mitochondrial shape proteins and the coordination of fusion and fission, remain unsolved. A number of studies have demonstrated that mitochondrial morphology and its physiologic function are different based on the cell-type or tissue. These variations and the cell-type specificity of mitochondrial dynamics might be related to specific cellular functions, such as in neuronal cells. Therefore, learning how and the degree to which mitochondrial dynamics influence cell-specific functions in various tissues continues to be a challenging task. Future research to investigate the complex crosstalk between mitochondrial dynamics and their physiologic function are likely to provide exciting breakthroughs in the fields of cell biology and for clinical medicine.

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Conflict of interest

None declared.

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