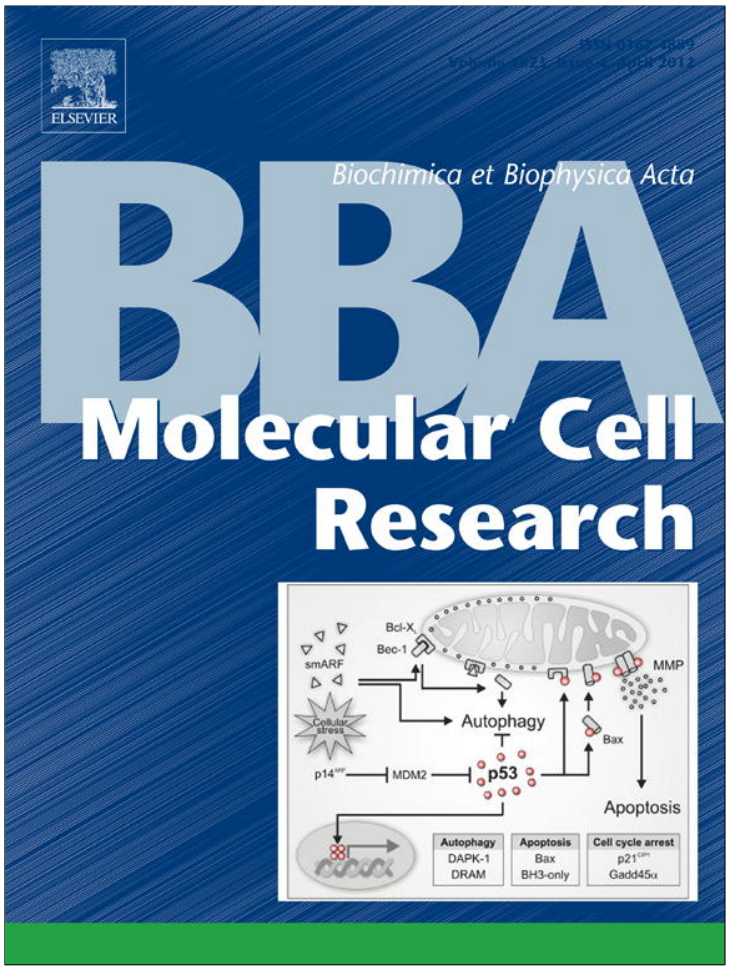

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Review

New insights into the function and regulation of mitochondrial ssion

Hidenori Otera [a], Naotada Ishihara [b], Katsuyoshi Mihara [a,b,]

[a] Department of Molecular Biology, Graduate School of Medical Science, Kyushu University, Fukuoka 812-8582, Japan
[b] Department of Protein Biochemistry, Institute of Life Science, Kurume University, Kurume 839-0864, Japan

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ABSTRACT

Mitochondrial morphology changes dynamically by coordinated fusion and ssion and cytoskeleton-based transport. Cycles of outer and inner membrane fusion and ssion are required for the exchange of damaged mitochondrial genome DNA, proteins, and lipids with those of healthy mitochondria to maintain robust mitochondrial structure and function. These dynamics are crucial for cellular life and death, because they are essential for cellular development and homeostasis, as well as apoptosis. Disruption of these functions leads to cellular dysfunction, resulting in neurologic disorders and metabolic diseases. The cytoplasmic dynamin-related GTPase Drp1 plays a key role in mitochondrial ssion, while Mfn1, Mfn2 and Opa1 are involved in fusion reaction. Here, we review current knowledge regarding the regulation and physiologic relevance of Drp1-dependent mitochondrial ssion: the initial recruitment and assembly of Drp1 on the mitochondrial ssion foci, regulation of Drp1 activity by post-translational modi cations, and the role of mitochondrial ssion in cell pathophysiology.

1. Introduction

Mitochondria are essential organelles for the life and death of eukaryotic cells and participate in oxidative phosphorylation; biogenesis of iron-sulfur clusters, heme, certain lipids, and amino acids; calcium signaling; and regulation of apoptosis [1 9]. Mitochondria move along cytoskeletal tracks to sites of high-energy demand, and change their overall morphology by fusion and ssion in response to the cellular environment and differentiation [10 13]. Mitochondria proliferate by growth and division, thus their fusion and ssion are important for maintaining mitochondrial number and function. High-molecular weight GTPases are key components involved in regulating the mitochondrial morphologic dynamics [4 8]; in vertebrates, mitofusin proteins (Mfn1 and Mfn2) of the mitochondrial outer membrane (MOM) regulate MOM fusion [14 18] and mitochondrial inner membrane (MIM) protein Opa1 is involved in MIM fusion, probably coupled with MOM fusion and cristae remodeling [19 21], whereas dynamin-related protein Drp1 (or Dlp1) mostly localizes to the cytoplasm and is recruited to mitochondria to regulate mitochondrial ssion [22 25]. The balance of these opposing events is precisely controlled to maintain the overall architecture and metabolic stability of the mitochondria [26].

During mitochondrial ssion, cytoplasm-localized Drp1 as small oligomers is recruited to the MOM and assembles into ssion foci, occasionally at points of tubule constriction and ssion [4,6,8,22 25]. Binding of Drp1 to the MOM-anchored receptor(s) and subsequent formation of the functional ssion complex (ssion foci) are thought to be essential for the initial step of mitochondrial ssion. Although several MOM proteins have been identi ed as Drp1 receptor candidates in mammals, their mechanistic roles and relative importance in mitochondrial ssion are unclear [8,27 29]. Mitochondrial ssion is regulated by post-translational modi cations of Drp1, including phosphorylation, *S*-nitrosylation, SUMO(small ubiquitin-like modi er)ylation, ubiquitination, and O-GlcNAcylation, in response to diverse cellular stimuli [30]. Defective regulation of mitochondrial morphology is linked to a number of human disorders, including neurodegenerative diseases, myopathies, obesity, diabetes, and cancer [3,7,31,32]. This review provides a comprehensive overview of the current knowledge regarding mitochondrial ssion. We rst address recent ndings concerning the key components of mitochondrial ssion machinery and discuss their interplay, and then focus on the physiologic roles of mitochondrial ssion in mammals.

2. Mitochondrial ssion machinery in mammals

Mitochondrial ssion is important for maintaining cellular function, and mitochondrial dysfunction causes aging, neuronal synaptic

Abbreviations: AKAP1, A kinase anchoring protein 1; Endo B1, endophilin B1; ER, endoplasmic reticulum; GDAP1, ganglioside-induced differentiation-associated protein 1; GED, GTPase effector domain; GFP, green uorescent protein; KO, knockout; LRRK2, leucine-rich repeat kinase 2; MEF, mouse embryonic broblast; Mff, mitochondrial s-sion factor; MiD51, mitochondrial dynamics 51; MIEF1, mitochondrial elongation factor 1; MIM, mitochondrial inner membrane; Mfn2, mitofusin 2; MOM, mitochondrial outer membrane; NO, nitric oxide; PKA, cAMP-dependent protein kinase; RNAi, RNA interference; SUMO, small ubiquitin-like modi er; VD, variable domain

Corresponding author at: Department of Molecular Biology, Graduate School of Medical Science, Kyushu University, Fukuoka 812-8582, Japan. Tel.: +81 92 642 4709; fax: +81 92 642 6768.
E-mail address: mihara@cell.med.kyushu-u.ac.jp (K. Mihara).

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loss, and cell death in several human neurologic diseases. At the cellular level, mitochondrial ssion contributes to ensuring the proper distribution and quality control of mitochondria. A largely cytosolic member of the dynamin family of GTPases, Drp1 (Dnm1 in yeast), is the major player in mitochondrial ssion in mammals [22 25]. Genetic and biochemical studies in yeast revealed that Dnm1-mediated mitochondrial ssion requires a MOM protein, Fis1, and a soluble protein, Mdv1, or its paralogue, Caf4 [4,6,33 40]. During mitochondrial ssion, Fis1 transiently interacts via cytosolic adaptor proteins Mdv1/Caf4 with Dnm1 by its tetratricopeptide-repeat motif, indicating that Fis1 functions as the mitochondrial Dnm1 receptor. Mdv1 comprises an N-terminal extension containing a helix loop helix motif that interacts with Fis1, a central coiled-coil region that mediates Mdv1 homo-oligomerization, and a C-terminal WD40 domain to form a multibladed β-propeller that interacts with a variable domain (VD or insert B) of Dnm1 [41 44]. After targeting Dnm1 to the mitochondrial membrane, Fis1 Mdv1 nucleates the Dnm1 assembly at mitochondrial ssion sites, where it stimulates Dnm1 oligomerization and subsequent constriction of the mitochondrial membrane in a GTP-dependent manner [25,40,45]. Dnm1 is also reported to interact with cortical Num1, which is facilitated by MOM protein Mdm36 for mitochondrial ssion. The Dnm1 Mdm36 Num1 system also mediates anchoring mitochondria to the cell cortex and may be required for equal inheritance of mitochondria during cell division [46,47]. Unlike Fis1, homologues of Mdv1, Caf4, Num1, and Mdm36 have not been found in mammalian cells. Although several ssion-related proteins have been identi ed in mammals, their mechanistic roles in mitochondrial ssion remain obscure.

2.1. Structure and function of mitochondrial fission GTPase Drp1

Drp1 (Dnm1 in yeast) is a conserved dynamin GTPase superfamily protein that mediates membrane remodeling in a variety of cellular membranes. 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 (Fig. 3) [4,44 49]. Unlike Dynamin, Drp1/Dnm1 lacks the lipid-interacting pleckstrin homology domain, but contains an uncharacterized variable domain (VD; also called insert-B) between the middle domain and the GED [49 51]. Based on the crystal structure of near full-length Dynamin, Drp1 is predicted to exist as a T-shaped dimer or tetramer that contains a head (GTPase domain), leg (VD), and stalk (middle and GED domains) [52 56]. GTP induces the rearrangement of the head and stalk, which generates a force ultimately resulting in membrane constriction [54 56]. During mitochondrial ssion, Drp1 existing as dimer or tetramer in the cytoplasm assembles into larger oligomeric structures as foci at the mitochondrial ssion sites depending on GTP binding, wraps around the mitochondria, and then severs the mitochondrial membrane by GTP hydrolysis [23 25] (Fig. 1). Time-lapse imaging of the green uorescent protein (GFP)-tagged Drp1 (GFP-Drp1) shows that mitochondrial tubules divide at the sites of these foci [23,57]. Approximately 3% of total Drp1 is localized to mitochondria as foci and ~5% of the foci are involved in division each hour [23]. A recent report demonstrated that mitochondrial division occurs at points where endoplasmic reticulum (ER) tubules wrap around the mitochondria and mark mitochondrial division sites [58 60]. The GTP-hydrolysis defective mutant (K38A) sequesters endogenous Drp1 into uncharacterized aggregated or dotted structures consisting of membrane tubules, thus inhibiting its localization to the mitochondrial ssion sites and acting as a dominant negative mutant [24]. Intermolecular interactions between the N-terminal GTPase domain and C-terminal GED are also important for Drp1 self-assembly and functional regulation [52,61,62].

Cryo-electron microscopy revealed that, upon GTP hydrolysis, Dnm1 constricts liposomes and subsequently dissociates from the lipid bilayer by undergoing larger conformational changes [48]. Unlike the pleckstrin homology domain of dynamin, the VD of Dnm1 does not integrate into the lipid bilayer, suggesting a weak interaction between the lipid and Dnm1 [48,63 65]. As described above, Janet Shaw and collaborators recently demonstrated that the VD domain of Dnm1 is essential for binding to the Mdv1 β-propeller domain [54]. In contrast, interestingly, Strack and Cribbs reported that the VD of Drp1 is dispensable for recruitment to the mitochondrial membrane and mitochondrial ssion in live cells [66]. Indeed, several ΔVD mutants constitutively localize to the MOM surface and fragment mitochondria more ef ciently than wild-type Drp1. There is a 3- to 4 nm gap between Dnm1 and the lipid in the 3D structure of the Dnm1-liposome tube [48], which easily accommodates the protein cofactors required for mitochondrial ssion (see below). In vivo, additional Dnm1-associated factors might regulate and facilitate membrane constriction and scission events. F-actin is reported to be involved in mitochondrial ssion by facilitating mitochondrial recruitment of Drp1 [67]. In this context, Tau inhibits Drp1 recruitment by stabilizing actin, which leads to mitochondrial elongation and neurotoxicity; a direct consequence of tau toxicity in neurons in Alzheimer's disease and related neurodegenerative disorders [68]. Furthermore, the Dynein/dynactin system is reported to control mitochondrial morphology by regulating the mitochondrial recruitment of Drp1 via microtubules [69]. The functional division of these mechanisms is not known.

2.2. Mff is important for Drp1 recruitment to mitochondrial fission sites

At the cellular level, almost all Drp1 is cytosolic with only a small fraction of the total Drp1 residing at the mitochondrial membrane. Extrapolation from the mitochondrial ssion process in yeast suggests that mammalian Fis1 (hFis1 for humans) could be a receptor for Drp1 recruitment. Depletion of hFis1, however, does not affect mitochondrial recruitment of Drp1 [70]. Thus, the mechanisms by which cytoplasmically-localized Drp1 is activated and recruited to the prospective mitochondrial ssion sites have remained unclear. Mitochondrial ssion factor (Mff) is a C-tail anchored protein recently identi ed in a *Drosophila* RNA interference (RNAi) library search for mitochondrial morphology alterations [71]. Mammalian mitochondria contain an Mff orthologue and silencing this factor by RNAi induces mitochondrial elongation in mammalian cells, suggesting its involvement in mitochondrial ssion [71,72]. To better elucidate its role, we rst 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. Conversely, Mff overexpression induced mitochondrial fragmentation with increased Drp1 recruitment to the mitochondria [8,27,72]. Consistent with these observations, both in vitro and in vivo experiments demonstrated that Mff transiently interacts with Drp1 through its N-terminal cytoplasmic region. Furthermore, Mff mostly colocalizes with the Drp1 foci on the MOM, in marked contrast to the uniform localization of hFis1 in the MOM [72]. Interestingly, dotted structure of Mff disperses throughout the MOM in the absence of Drp1 (Otera and Mihara; unpublished results), suggesting that Drp1 affects oligomerization of Mff. These observations indicate that Mff functions as a Drp1 receptor to mediate mitochondrial ssion (Fig. 1). Drp1 might self-assemble via its ability to homo-oligomerize on the Mff of MOM [71], probably forming spiral structures around the mitochondrial tubules. Unidenti ed Mff-interacting proteins might affect the assembly of the ssion machinery, leading to membrane constrictions or lipid remodeling and eventually to membrane scission (see below). As mentioned above, it is also possible that ER tubules wrapping around mitochondria mediate constriction and de ne the mitochondrial division sites prior to Mff-dependent Drp1 assembly [58 60].

In contrast to the conservation of Fis1 through various species, there are no obvious homologues of Mdv1/Caf4 in metazoans, and

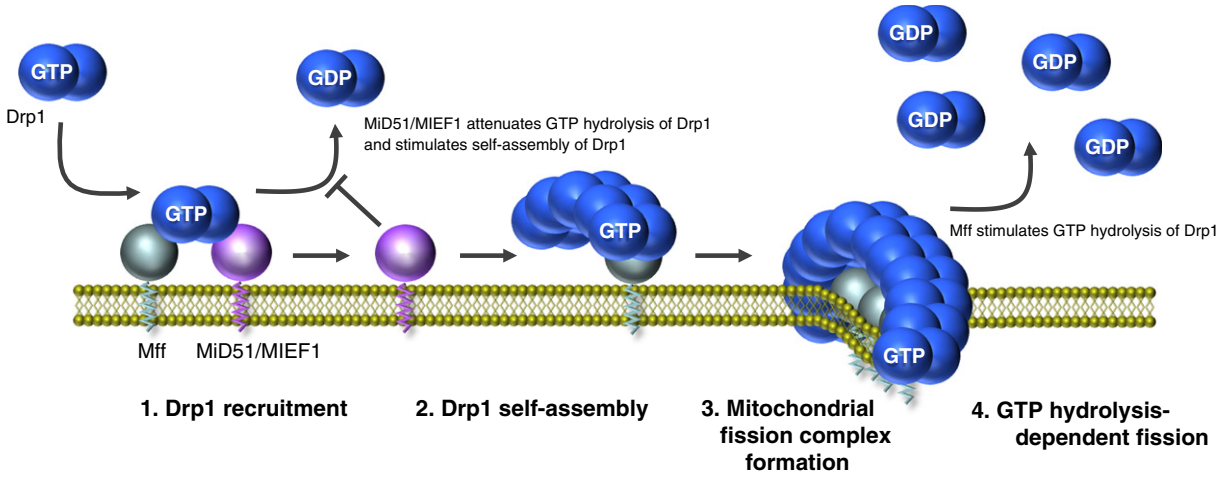

Fig. 1. Hypothetical model for the assembly of mitochondrial ssion complexes. Mff functions as a dominant Drp1 receptor. MiD/MIEF proteins inhibit GTPase activity of Drp1 and mediate subsequent oligomerization of Drp1 in the $Drp1^{GTP}$ form (Fig. 1). $Drp1^{GTP}$ multimers then promote the oligomerization of Mff with unknown factors into ssion complexes, leading to the stimulation of Drp1 GTPase to induce mitochondrial ssion. Under conditions of MiD/MIEF1 overexpression, $Drp1^{GTP}$ self-assembles into large inactive structures on mitochondria and results in the arrest of subsequent mitochondrial membrane ssion.

Mff appears to be restricted to metazoans. Mammalian mitochondria seem to have adopted ssion mechanisms distinct from those of yeast or plants [4,6,33 40,73 75]. The mechanistic details of these processes and their GTP-dependence remain key questions for future studies.

2.3. Role of MiD/MIEF1 proteins in Drp1 assembly and mitochondrial morphology

Mitochondrial dynamics 51 (MiD51), also called mitochondrial elongation factor 1 (MIEF1), and the variant MiD49 are MOM proteins identi ed by a random cellular localization screen of uncharacterized proteins whose expression causes unique distribution and changes in mitochondrial morphology [76,77]. Shortly after the induction of MiD51/MIEF1 in cultured cells, Drp1 is initially recruited to mitochondria where it induces mitochondrial ssion. At later stages, the mitochondrial network becomes more fused, concomitant with Drp1 accumulation at the mitochondrial surface [76]. Palmer et al. suggested that Drp1 becomes sequestered at the mitochondria in a non-functional form, thereby blocking ssion and shifting the balance towards fusion [76]. Opposite knockdown phenotypes, however, are also reported; Palmer et al. found that knockdown of MiD49 or MiD51 alone does not affect mitochondrial morphology, whereas the knockdown of both causes mitochondrial elongation [76]. On the other hand, Zhao et al. claimed that the knockdown of MIEF1/MiD51 induces mitochondrial fragmentation. They concluded that MIEF1 recruits Drp1 and inhibits the GTPase-dependent ssion activity of Drp1, but instead it has fusion activity independent of Mfn2 in the fusion pathway [77]. The reason for these discrepancies between two groups remains to be clari ed, although our experiments indicated that knockdown of MiD51/MIEF1 reproducibly inhibits Drp1-dependent mitochondrial s-sion and induces mitochondrial elongation, con rming the results of Palmer et al. Interestingly, MiD51/MIEF1 interacts with recombinant Drp1 to inhibit its GTPase activity accompanied by Drp1 oligomerization. In contrast, Mff competes with MiD51/MIEF1 to stimulate Drp1 GTPase activity (Otera and Mihara; unpublished results); MiD51/MIEF1 seems to bind oligomerized Drp1 and stabilize them at the surface of the mitochondrial membrane in the GTP-locked state to inhibit mitochondrial ssion. What might be the functional relation of Mff and MiD/MIEF proteins in the Drp1-dependent mitochondrial ssion process? Based on common denominator of the reports of two groups [76,77] and our ndings that Drp1 foci are ef ciently removed by Mff RNAi even in the presence of endogenous MiD/MIEF proteins [72], we speculate that Mff functions as a dominant Drp1 receptor and MiD/MIEF proteins are involved in subsequent Drp1 oligomerization in the $Drp1^{GTP}$ form (Fig. 1). Mff collaborating with unknown factor(s) then stimulates Drp1 GTPase activity to induce mitochondrial ssion. In support of this hypothesis, a dominant-negative GTPase mutant, Drp1-K38A, is also recruited to the mitochondria following MiD51/MIEF1 expression, whereas dominant-negative mutants containing the middle domain mutations Drp1-A395D and G363D, which have defects in higher-ordered assembly, are not recruited to the mitochondria [76]. However, the possibility that Mff and MiD/MIEF proteins function independently in distinct Drp1-dependent mitochondrial morphology regulation pathways cannot be ruled out.

2.4. Enigmatic roles of hFis1 in mitochondria dynamics in mammals

Fis1 is a C-tail anchored MOM protein with its N-terminal multiple tetratricopeptide repeat motif exposed to the cytoplasm. Fis1 has also been identi ed in mammalian mitochondria (hFis1 for human Fis1) and is thought to be involved in recruiting Drp1 to the mitochondria through direct or indirect interactions as in yeast [78,79]. The actual function of hFis1, however, remains unclear, because the Mdv1/Caf4-like adaptor proteins have not been identi ed. hFis1 evenly localizes throughout the MOM [80,81] in contrast to the punctate localization of Drp1 and Mff [72], and mitochondrial recruitment of Drp1 is not or only marginally affected by hFis1-knockdown or exogenous expression [70,72,81]. Thus, whether or not hFis1 induces mitochondrial ssion is controversial. Yeast Fis1 is well established to mediate mitochondrial ssion, and similarly, plant Fis1 is required for mitochondrial ssion [73,74,82]. Deletion of Fis1 and Fis2 in *Caenorhabditis elegans*, however, does not result in any detectable mitochondrial defects [83]. Moreover, hFis1 cannot rescue the phenotype of yeast *fis1* cells [79], indicating that the two proteins are structurally divergent or act through different mechanisms. Moreover, conditional knockout of hFis1 in colon carcinoma cells revealed that it is dispensable for mitochondrial ssion [72]. Thus, the actual function of hFis1 in mitochondrial ssion in mammals remains a mystery.

What then is the physiologic function of hFis1? Iwasawa et al. recently demonstrated that hFis1 transmits an apoptosis signal from the mitochondria to the ER by interacting with Bap31 at the ER to facilitate

its cleavage into the pro-apoptotic processed form (p20Bap31). p20Bap31 causes rapid transmission of ER calcium signals to the mitochondria at close ER-mitochondria junctions [84]. This calcium influx into the mitochondria stimulates Drp1-dependent mitochondrial fission and cytochrome c release [85]. Although the exact mechanism of apoptosis regulation by hFis1 is unclear, hFis1 might function as the ER gateway for ER-mediated apoptosis in mammals rather than in mitochondrial division [86]. Gomes and Scorrano reported that overexpression of hFis1 induces mitochondrial fragmentation and dysfunction, and the mitochondria are targeted to autophagosomes [87], suggesting a functional link to autophagy [88,89], although the artificial effect of membrane protein overexpression cannot be ruled out. Recently, we identified TBC domain family member 15 (TBC1D15) as a novel hFis1-interacting protein. TBC1D15 is thought to be a GTPase-activating protein for small GTPase Rab7 and Rab11, which promotes fusion events between late endosomes and lysosomes [90,91]. At the cellular level, the majority of TBC1D15 is cytosolic with only a small amount residing in the mitochondria. hFis1 directly interacts with TBC1D15 and its overexpression stimulates mitochondrial recruitment of TBC1D15 [92]. TBC1D15 RNAi cells display an elongated and protonophore carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) sensitive mitochondrial network without alterations in the Drp1, suggesting the presence of hFis1-dependent, but Drp1-independent, mitochondrial morphology regulation.

2.5. Other fission-related proteins

2.5.1. Ganglioside-induced differentiation-associated protein (GDAP) 1

Ganglioside-induced differentiation-associated protein 1 (GDAP1) is another mitochondrial fission-related factor located on the MOM through C-terminal hydrophobic transmembrane domain extruding the bulk N-terminal domain to the cytoplasm [93]. It is expressed in myelinating Schwann cells and motor and sensory neurons. GDAP1 mutations lead to the peripheral neuropathy Charcot Marie Tooth disease, either with primary axonal loss or with primary demyelination of peripheral nerves [94]. GDAP1 mutants found in patients with Charcot Marie Tooth disease are not targeted to the mitochondria and lack mitochondrial fragmentation activity [93]. GDAP1-induced mitochondrial fragmentation is inhibited by Drp1 knockdown or expression of the dominant-negative Drp1-K38A mutant, indicating that GDAP1 is a Drp1-dependent regulator of mitochondrial fission [93]. GDAP1 has two glutathione S-transferase family domains, although the functional relation to mitochondrial morphology regulation remains unknown [95]. Gangliosides, sialic acid-containing glycosphingolipids, are suggested to play important roles in neural differentiation through the signal transduction pathway [96,97]. Although it is not clear how GDAP1 is involved in mitochondrial fission, these findings suggest the importance of membrane lipid components such as gangliosides in mitochondrial fission. Upon apoptotic stimulation, for example, Drp1 and hFis1 associate with the raft-like microdomains, glycosphingolipid-enriched structures in the MOM [98]. Further, in lymphoid cells from patients with Huntington's disease, Drp1 and mutated huntingtin are efficiently targeted to the raft-like microdomains of MOM, causing increased production of reactive oxygen species and apoptosis vulnerability [99]. Disruption of these rafts by treatment with chemical inhibitors of ceramide synthesis, Fumonisin B1, and [D]-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol, or by cyclodextrin results in decreased mitochondrial fission and apoptosis. These raft-like microdomains might function as platforms for Drp1 recruitment and to secure apoptotic signal transduction. The link between mitochondrial fission and peroxisomal fission also raises the possibility that GDAP1 regulates the division of peroxisomes. It would be interesting to test whether the cells of patients with Charcot Marie Tooth disease contain elongated peroxisomes.

2.5.2. Endophilin B 1

Endophilins, fatty acyl transferases, are suggested to mediate changes in membrane curvature and to participate in membrane scission during endocytosis and intracellular organelle biogenesis; they possess an N-terminal Bar domain that interacts with membranes and a C-terminal SH3 domain that mediates protein binding [100 103]. Endophilin B1 (Endo B1, also called Bif-1) was identified by yeast two-hybrid protein screening to bind a proapoptotic Bcl-2 family member Bax, and is reported to be involved in apoptosis [104,105], mitochondrial morphogenesis [106], and autophagosome formation [107]. Karbowski et al. demonstrated that Endo B1 dynamically cycles between the cytosol and mitochondria and only a small fraction resides on the tips and at the MOM [106]. During apoptosis, Endo B1 translocates from the cytosol to the mitochondria as clusters. Its knockdown or overexpression of the truncated form induces striking mitochondrial morphology alterations; the dissociation of MOM and MIM, and the formation of MOM vesicles and tubules [106]. Interestingly, these morphologic phenotypes are suppressed by Drp1 knockdown or expression of a dominant-negative Drp1-K38A mutant, indicating that Drp1 acts upstream of Endo B1 to maintain the mitochondrial network dynamics [106].

2.5.3. Sacsin

Sacsin is a 4579-amino acid multi-domain protein encoded by the SACS gene, whose mutations cause childhood-onset autosomal recessive spastic ataxia of Charlevoix-Saguenay [108]. It is highly expressed in the central nervous system, and is also found in the skin, skeletal muscles, and at low levels in the pancreas. Recent work indicates that sacsin is localized to the mitochondria, partially overlapping with the Drp1 foci [109]. Exogenously expressed sacsin (1 1368 residue segment) interacts with endogenous Drp1. Interestingly, mitochondria in cells of sacsin knockout (KO) mice and patients with autosomal recessive spastic ataxia of Charlevoix-Saguenay display a hyperfused balloon-like mitochondrial morphology, mimicking the mitochondrial phenotype of Drp1-depleted cells; a characteristic structure observed in Drp1- or Mff-knockdown cells or dominant-negative Drp1-K38A-expressing cells. In sacsin-deficient neuronal cells, enlarged mitochondria are clustered and accumulate in the soma and proximal dendrites, and display fewer dendrites than control cells, a morphologic response observed in the neuronal cells of Drp1 KO mice (see below). Importantly, sacsin KO mice display age-dependent loss of cerebellar Purkinje cells, probably due to disturbances in the mitochondrial delivery within neurites. Interaction of sacsin with Drp1 was demonstrated by immunoprecipitation [109]. These results suggest that sacsin regulates Drp1 activity.

2.5.4. LRRK2

The leucine-rich repeat kinase 2 (LRRK2) is a large multi-domain kinase (2527 amino acids) including the C-terminal WD40 domain and its mutations are linked with autosomal dominant Parkinson's disease (PARK8-type) [110]. It is found in the cytoplasm and is associated with the mitochondrial membrane. Exogenous expression of LRRK2 stimulates mitochondrial recruitment of Drp1 by direct interaction and induces mitochondrial fission, mitochondrial dysfunction, and cell susceptibility to stress, probably through its kinase activity [111,112]. The mechanism by which LRRK2 kinase mediates mitochondrial recruitment of Drp1 to induce mitochondrial fission remains to be analyzed. LRRK2 might interact with Drp1 through its C-terminal WD40 motif as is the case for the Mdv1 Dnm1 interaction [41,42].

2.5.5. Mutant huntingtin (Htt)

Huntington's disease (HD) is an autosomal dominant disease caused by abnormal polyglutamine (polyQ) expansion within huntingtin (Htt), leading to the progressive loss of striatal and cortical neurons, cognitive and motor impairment, and eventually death. Bossy-Wetzel and

collaborators demonstrated that mutant Htts (Q46 and Q97) directly interact with Drp1 to stimulate Drp1 GTPase activity, which triggers mitochondrial fragmentation, leading to the inhibition of axonal mitochondrial transport and neural cell death. All these defects were rescued by reducing Drp1 GTPase activity by the dominant-negative Drp1-K38A mutant [113]. In addition to this effect, as discussed below (Section 6.1. Phosphorylation), mutant Htts hyperactivate phosphatase calcineurin to activate Drp1 by dephosphorylation of inactive Drp1-Ser637(P), leading to mitochondrial fragmentation, cristae disruption, and increased apoptosis susceptibility in HD cells [158,159,165]. How these direct and indirect action modes of mutant Htts are coordinated in HD cells remain to be clari ed.

3. Functional relevance of Drp1-dependent mitochondrial ssion

In an immortalized Drp1 KO broblast cell line that we established (Drp1 KO mouse embryonic broblasts [MEFs]), the growth rate, respiratory activity, cellular ATP levels, and mitochondrial DNA levels are comparable with those of control MEFs, indicating that Drp1 is dispensable for cell viability and the maintenance of active mitochondria in MEFs [114]. Another group, however, reported that primary Drp1 KO MEFs are viable but grow slower than control cells, despite normal cellular ATP levels [115]. In Drp1 KO cells, mitochondria are segregated, although unequally, to daughter cells by forced ssion at the midbody (cell ssion site) during cytokinesis, suggesting that mitochondrial ssion is not essential for mitosis but facilitates stochastic distribution of the mitochondria to daughter cells.

To elucidate the detailed physiologic roles of mitochondrial ssion in vivo, we and another group generated tissue-speci c Drp1 KO mice using the Cre and loxP system [114,115]. Although Drp1 is dispensable for MEF viability, as described above, Drp1 KO mice die at around embryonic day 12.5 with developmental abnormalities, particularly in the forebrain. In addition, a missense mutation in mouse Drp1 in the middle domain that is essential for intramolecular interactions (Python mice; C452F mutation) leads to cardiomyopathy [116]. The main developmental abnormalities observed in Drp1 KO mice are defects in forebrain and synapse development, poorly developed livers, and compromised cardiac formation or function [114,115], while heterozygous Python mice exhibit depletion of cardiac ATP and cardiomyopathy [116]. To date, only a dominant-negative middle domain mutation (A395D) in Drp1 has been reported in a lethal disorder with microcephaly, abnormal brain development, optic atrophy, hypoplasia, persistent lactic acidemia, and a mildly elevated plasma concentration of very-long-chain fatty acids [117]. Immuno- uorescence microscopy analysis revealed abnormal elongation of the mitochondria and peroxisomes in cultured broblasts from the patient. Surprisingly, histologic and histochemical analyses of the muscle-biopsy sample from the patient did not reveal mitochondrial abnormalities. Despite its severe clinical phenotype, activity of the respiratory-chain enzymes was normal in muscle and in cultured broblasts derived from the patient. Furthermore, biochemical investigations of skin broblasts revealed no abnormalities in the peroxisomal functions, such as β-oxidation of cerotic acid and pristanic acid, making it dif cult to determine the *bona fide* roles of mitochondrial ssion based on studies of the patient [117].

Neurons are particularly vulnerable to mitochondrial dysfunction (Fig. 2). Neuron-speci c Drp1 KO mice are viable at birth, but quickly die due to neurodegeneration [114,115]. In primary cultured neural cells from Drp1 KO embryos, enlarged and aggregated mitochondria are sparsely distributed in the neurites, and the synaptic structures are lost [114,115] (Fig. 2). These ndings suggest that Drp1-de ciency causes an abnormal distribution of enlarged mitochondria in extremely polarized cells such as neurites; these spatiotemporal defects may inhibit the ATP supply and Ca^{2+} signaling, eventually preventing synapse formation. Taken together, these results suggest that Drp1 de ciency results in unusually shaped, large mitochondria with compromised intracellular movement, which leads to neuronal cell death (Fig. 2). In this context, the Parkinson's disease factors PINK and Parkin (see Mitochondrial ssion and mitophagy section) target Miro, a Rho-like GTPase that plays an important role in the mitochondrial transport in neurons, for phosphorylation and degradation to halt the movement of damaged mitochondria in neurites [118]. The importance of mitochondrial movement within cells is also observed in T-cell differentiation. Drp1 de ciency or inactivation affects the delivery of mitochondria to the immune synapse and affects T-cell receptor signaling at the immune synapse [119]. When Drp1 is deleted in postmitotic Purkinje cells in the cerebellum using the L7-Cre system, the mitochondria elongate due to excess fusion, then become swollen by increased reactive oxygen species accumulation through compromised respiratory activity, leading to age-dependent neurodegeneration. Thus mitochondrial ssion ensures the survival of neurons [120]. In contrast, Grohm et al. recently reported that enhanced mitochondrial ssion, loss of membrane potential, and apoptosis induced by glutamate toxicity or oxygen-glucose deprivation in mouse hippocampal neuronal cells, or transient focal ischemia in a mouse model are protected by Drp1 knockdown or the inhibition of Drp1 by a chemical inhibitor mdivi-1 [121]. In addition to the role of Drp1 in mitochondrial distribution, these ndings suggest that mitochondrial ssion also functions as a quality control system to suppress age-dependent oxidative damage and thus promote neuronal survival [89,122]. Determining the physiologic relevance of Drp1 in other tissues that might underlie various human diseases remains a challenge to be addressed.

4. Mitochondrial ssion and apoptosis

It is generally accepted that the mitochondrial network collapses into small spherical structures in response to apoptotic stimuli, and that pro-apoptotic and anti-apoptotic Bcl-2 family member proteins play important roles in regulating mitochondrial morphology [123]. During apoptosis, cytosolic Bax targets the MOM and colocalizes with Drp1 and Mfn2 at mitochondrial sites where ssion subsequently occurs [124]. Bak, which initially localizes uniformly on the MOM, also coalesces into discrete foci at mitochondrial ssion sites during apoptosis. tBid-triggered Bax/Bak activation correlates with a reduction in mitochondrial fusion, possibly through the inhibition of Mfn2, and eventually leads to mitochondrial fragmentation [125,126]. Upon Bax activation, Drp1 stably associates with the MOM through Bax/Bak-dependent SUMO modi cations of Drp1 [127]. This mitochondrial fragmentation is caspase-independent and occurs concomitantly with permeabilization of the MOM, cristae disorganization, and subsequent cytochrome *c* release [70,128]. Increased mitochondrial ssion in apoptotic cells apparently parallels the release of cytochrome *c*, and the inhibition of ssion by Drp1 knockdown compromises the release of cytochrome *c*, suggesting that the release of cytochrome *c* from the inter membrane space is intimately involved in mitochondrial ssion [70]. Consistent with these data, Mff depletion by RNAi results in extensive mitochondrial elongation, delayed cytochrome *c* release, and retardation of apoptosis [71,72]. Similarly, MEFs from Drp1 KO mice exhibit a delay in cytochrome *c* release, caspase activation, and nuclear DNA fragmentation [114,115]. Notably, mitochondria with network structures that are subtly different from the structures observed prior to cytochrome *c* release are frequently detected in Drp1 KO cells after the release of cytochrome *c*, and seem to undergo fragmentation in the advanced stage of apoptosis, suggesting that Drp1-independent mitochondrial fragmentation likely occurs late after the release of cytochrome *c* [114]. This suggests that Drp1-independent ssion might participate in mitochondrial ssion during apoptosis. In this context, it is reported that *Drosophila* PMI and its human homologue TEMEM11 of the MIM regulate mitochondrial ssion in a Drp1- and Mfn-independent manner [129]. Nakamura et al. recently reported that α-synuclein, which plays a central role in Parkinson's disease, promotes mitochondrial ssion through a Drp1-independent pathway [130]. In

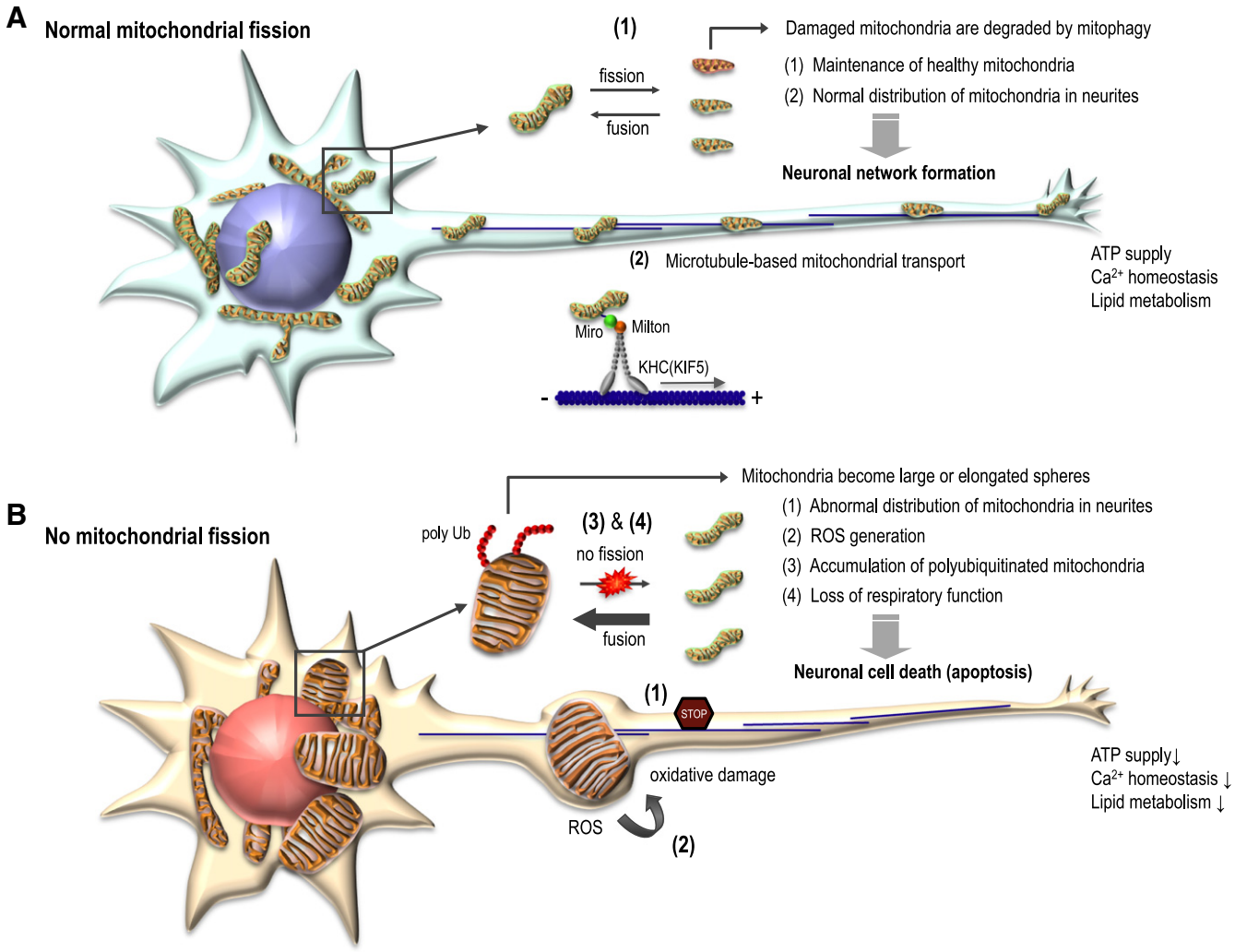

Fig. 2. Physiologic function of mitochondrial ssion in neuronal cells. (A) Mitochondria are usually distributed throughout the soma and neurites of normal neuronal cells by microtubule-based mitochondrial transport. (B) Fission-de cient mitochondria form aggregates in the soma and neurites, which inhibit ef cient mitochondrial distribution within the neurites, leading to the synaptic structure loss. In normal cells, mitochondrial respiratory activity is maintained by the PINK/Parkin-dependent quality control system called mitophagy. In ssion-de cient cells, mitochondria elongate due to excessive fusion, become large spheres due to oxidative damage, accumulate ubiquitinated MOM proteins, and lose respiratory function, leading to neurodegenerative diseases. Parkin/PINK1 system also targets Miro on damaged mitochondria for phosphorylation and degradation to halt the movement of damaged mitochondria in neurites.

vitro, recombinant oligomeric α-synuclein directly interacts and fragments liposomes containing the mitochondrial lipid cardiolipin [130]. Taken together, these ndings indicate that delayed cytochrome *c* release in these cells is relatively modest, which suggests that although the Drp1-Mff system is dispensable, it facilitates the normal progression of apoptosis [114,115]. Conversely, the inhibition of mitochondrial fragmentation by the activation of fusion-related proteins, such as Mfn1, Mfn2, or Opa1 antagonizes apoptosis progression. The role of Drp1-dependent mitochondrial ssion in apoptosis progression, however, remains controversial. In contrast to the apparent Drp1 function in apoptosis described above, the inhibition of mitochondrial ssion by Drp1 RNAi results in spontaneous apoptosis in both human lung and colon cancer cells [131].

A pharmacologic inhibitor of Drp1-GTPase, mdivi-1, inhibits tBid-dependent cytochrome *c* release from isolated mitochondria that are incapable of undergoing ssion in vitro [132,133]. These ndings suggest either that mdivi-1 inhibits Drp1 functions other than mediating mitochondrial ssion or that it inhibits molecules other than Drp1 that regulate cytochrome *c* release [132]. Martinou and coworkers demonstrated that Drp1 promotes the formation of a nonbilayer hemi ssion intermediate in which the activated and oligomerized Bax forms a hole, leading to MOM permeabilization [134]. A recent study demonstrated that mdivi-1 reduces cell death and protects adult murine cardiomyocytes against ischemia/reperfusion injury and reduces myocardial infarct size in the murine heart and hyperproliferation of vascular smooth muscle cells in pulmonary arterial hypertension [135]. These observations suggest that the inhibition of Drp1 by mdivi-1 provides an ef cient pharmacologic strategy for human diseases including cancer, and cardiac and brain damage [121,133,136]. Of note, although mitochondrial fragmentation is indeed associated with apoptosis, excessive mitochondrial fragmentation can occur in a variety of conditions independently of apoptotic processes, such as reversible fragmentation when exposed to CCCP. Thus, the input of additional death signals on Drp1 function is required for the cells to cross the point of no return.

5. Mitochondrial ssion and mitophagy

During mitochondrial ssion, a single mitochondrion divides into functionally uneven daughter mitochondria; the daughter mitochondria with a high membrane potential and a high probability for subsequent fusion, and those with low respiration activity, decreased membrane potential and a reduced probability for fusion, and the

latter fractions are rapidly eliminated by autophagy (termed mitophagy), thus maintaining overall mitochondrial quality [89,137]. Mitophagy is also required for the regulation of the mitochondrial number to match the metabolic or developmental demands [138,139]. The Parkinson's disease-related protein kinase (PINK1; PTEN-induced putative Ser/Thr kinase) and a RING domain-containing E3 ubiquitin ligase Parkin are involved in mitophagy. PINK1 is steadily targeted and degraded on healthy mitochondria. Upon membrane depolarization, it is stabilized on the MOM, activated by phosphorylation on the serine residues, and recruits cytosolic Parkin to ubiquitinate damaged mitochondria prior to autophagosome formation [140 142]. In addition to the above-described mitophagy pathway, Parkin also mediates proteasome-dependent degradation of MOM proteins, leaving proteins of the inner compartments undegraded [143,144]. This reaction promotes the rupture of the MOM [144,145]. Thus, Parkin regulates the degradation of the mitochondrial outer and inner membranes by temporally differentiated mechanisms through proteasome- and autophagy-dependent pathways.

The process of mitophagy involves several mitochondrial ssion and fusion proteins. As described already, hFis1 induces mitochondrial fragmentation and enhances mitophagy [87]. Furthermore, reduced hFis1 expression in β-cells by RNAi decreases mitophagy and results in the accumulation of oxidized mitochondrial proteins, reduced respiration, and impaired insulin secretion [89]. In adult cardiac myocytes, Drp1 is required for mitophagy induced by BNIP3. Overexpression of the dominant negative form of Drp1 results in decreased mitochondrial ssion and decreased mitophagy [146]. Parkin-dependent ubiquitination of Mfn1 and Mfn2 and its proteasome and p97-dependent degradation result in increased mitochondrial ssion to promote mitophagy [147 149]. Reichert and collaborators recently demonstrated that mild oxidative stress (moderate levels of ROS) speci cally triggers mitophagy in a Drp1-dependent manner without inducing non-selective autophagy [150]. Hypoxia induces marked accumulation of GFP-LC3 puncta and extensive mitochondrial fragmentation. Recently, a MOM 155-amino acid protein FUNDC1 was reported to mediate hypoxia-induced mitophagy [151]. It is anchored to MOM through the C-terminal three transmembrane domains, extruding the N-terminal segment with the LC3-intracting region to the cytoplasm. FUNDC1 is steadily phosphorylated at Tyr18, presumably by Src-kinase, and de-phosphorylation under hypoxic conditions enhances its interaction with LC3 for selective mitophagy. The FUNDC1-dependent pathway is distinct from BNIP3-dependent and hypoxia-induced mitophagy [152].

6. Regulation of mitochondrial ssion by post-translational modi cations of Drp1

Various stressors outside or inside cells induce mitochondrial ssion to remodel mitochondria and alter cellular function [153]. During apoptosis, cytoplasmic Drp1 is translocated to the mitochondria and induces mitochondrial fragmentation prior to caspase activation by the release of cytochrome *c* [154]. Such increased ssion events are also important for the autophagic clearance of depolarized (or dysfunctional) mitochondria [88,89]. Overexpression of wild-type Drp1 does not lead to mitochondrial fragmentation, suggesting that a simple alteration of Drp1 levels does not change mitochondrial s-sion, whereas the regulation of Drp1 properties, such as mitochondrial translocation, higher order assembly, or GTPase activity is rather critical. Thus, regulation of Drp1 and/or its interacting proteins by post-translational modi cations is important for Drp1 cycling between the cytosol and mitochondria (Fig. 3).

6.1. Phosphorylation

During mitosis, human Drp1 is activated by the Cdk1/cyclin B-mediated phosphorylation of Ser616 (Ser585 in rat Drp1) in the variable domain. This mitotic phosphorylation promotes Drp1-dependent mitochondrial ssion and facilitates the proper distribution and segregation of mitochondria into daughter cells [155] (Fig. 3). This Drp1 phosphorylation and subsequent mitochondrial targeting are regulated by a small Ras-like GTPase RALA, its effector RALBP1, and mitotic kinase Aurora A [156]. During metaphase, Aurora A phosphorylates Ser194 of RALA and alters the subcellular location of the RALA/RALBP1 complex from the plasma membrane to the mitochondria. RALA/RALBP1 stimulates Cdk1/cyclin B kinase activity at the mitochondrial membrane, and mediates the phosphorylation of Drp1, followed by Drp1 oligomerization and subsequent mitochondrial ssion [156] (Fig. 3). Furthermore, Mff RNAi, but not hFis1 RNAi, decreases the recruitment of both RALA/RALBP1 and Drp1 to mitochondria at mitosis [156]. Under oxidative stress conditions, protein kinase Cδ mediates phosphorylation of Ser579 in human Drp1 isoform 3 (corresponding to Ser616 in the human Drp1 isoform 1), leading to mitochondrial fragmentation and impaired mitochondrial function, which contributes to hypertension-induced brain injury [157] (Fig. 3). As mentioned already, in pulmonary arterial hypertension, a lethal syndrome characterized by pulmonary vascular obstruction with pulmonary artery smooth muscle hyperproliferation, mitochondrial mitotic ssion by Cdk1/cyclin B-mediated Drp1-Ser616 phosphorylation causes hyperproliferation of vascular smooth muscle cells leading to pulmonary arterial remodeling [135]. Mdivi-1, a chemical inhibitor speci c to Drp1, reduces this process in a dose-dependent manner.

Unlike Cdk1/cyclin B, cAMP-dependent protein kinase A (PKA) phosphorylates Ser637 in the GED domain of human Drp1 (also referred in the literature as Ser600, 617, and 656, depending on species and splice variants). This modi cation inhibits mitochondrial ssion through the inhibition of the intra-molecular interaction between GTPase and GED domains, GTPase activity, and eventually mitochondrial recruitment of Drp1 [158,159] (Fig. 3). Under nutrient starvation conditions, for example, mitochondrial ssion is repressed by PKA-dependent phosphorylation of Drp1-Ser637 due to increased cAMP levels [160,161] and coincident dephosphorylation of Drp1-Ser616(P) [161], resulting in mitochondrial elongation as well as a higher density of cristae and a capacity for ef cient ATP production. This response protects mitochondria from autophagosomal degradation and sustains cell viability (Fig. 3) [160,161]. Conversely, mitochondrial targeting of a PKA inhibitor promotes mitochondrial fragmentation [162]. Calcineurin dephosphorylates Drp1-Ser637(P) and stimulates Drp1 translocation to the mitochondria [163] (Fig. 3). In neurons and nonneuronal cells, the A kinase anchoring protein 1 (AKAP1, also identi ed as the small GTPase Rab32) [164] localized on the MOM is involved in the mitochondrial recruitment of Drp1 [162]. The PKA/AKAP1 complex regulates the phosphorylation of Drp1-Ser637 to support mitochondrial network integrity and neuronal survival (Fig. 3). Previous experiments suggested that the phosphorylation of Ser637 compromises mitochondrial recruitment of Drp1 and stress-induced dephosphorylation in the cytoplasm facilitates the mitochondrial translocation of Drp1. Strack and collaborators, however, demonstrated that the phosphorylation of Drp1-Ser637 by PKA/AKAP1 traps Drp1 in large and slowly recycling complexes on the mitochondria to induce mitochondrial network extension [162].

As mentioned above, in a cellular model of HD expressing huntingtins with a longer polyglutamine repeat (mutant Htts), the increased dephosphorylation of Drp1 by hyperactivation of calcineurin induces mitochondrial ssion and cristae disruption, which leads to an increased response to apoptotic stimuli [158,159,165]. Dephosphorylation of Drp1-Ser637 is also implicated in programmed necrosis [166]. Programmed necrosis induced by tumor necrosis factor-α requires the activation of receptor-interacting Ser/Thr kinases RIP1 and RIP3, the mixed lineage kinase domain-like protein MLKL, and the mitochondrial protein phosphatase PGAM5 present as two splice variants, PGAM5L and PGAM5S [166]. Upon necrosis induction, the PGAM5S/PGAM5L complex on the mitochondrial membrane recruits Drp1 and activates its GTPase activity by the dephosphorylation of

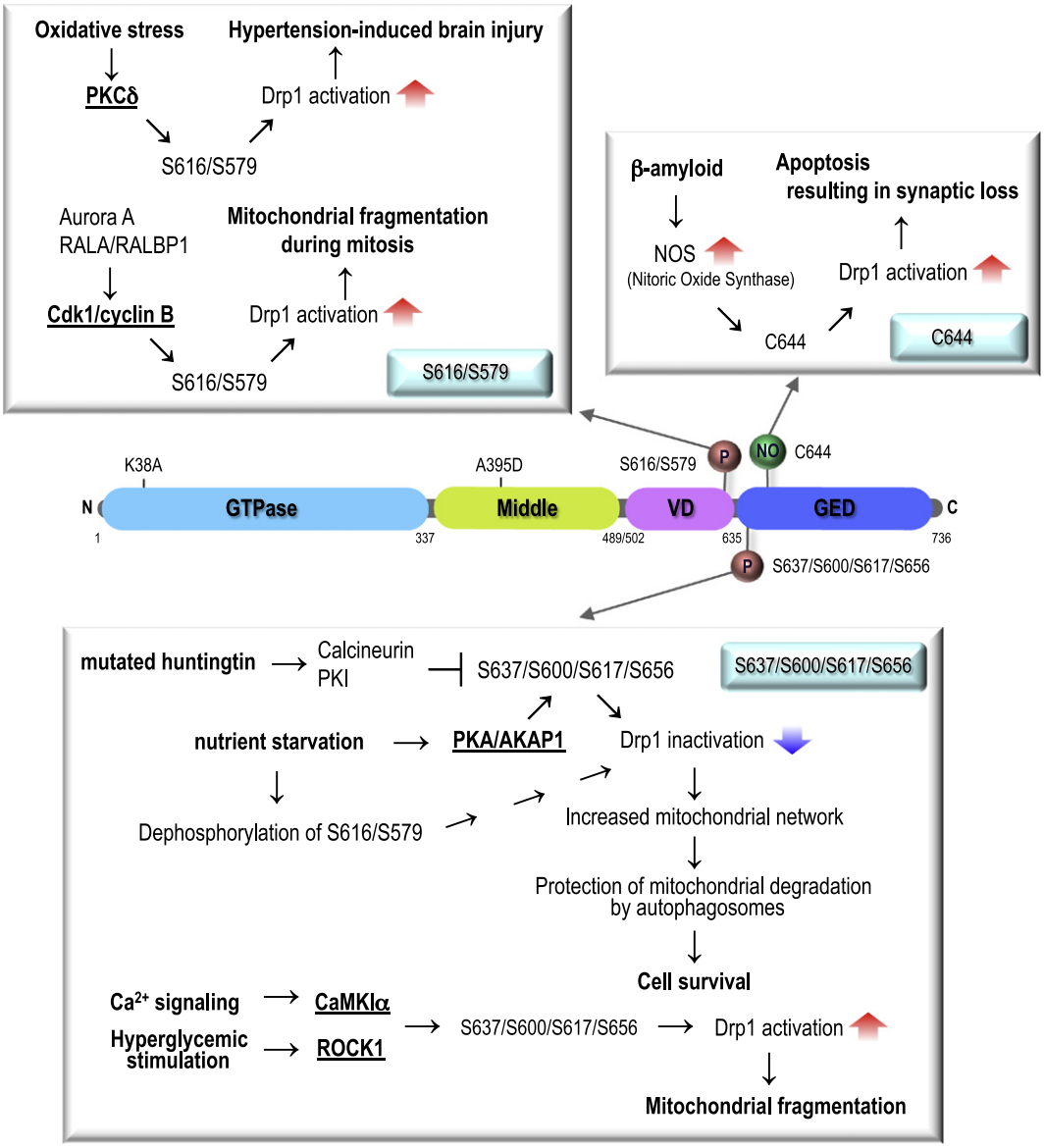

Fig. 3. Domain structure of Drp1 and summary of the regulation in Drp1 by post-translational modi cations. Drp1 activity is regulated by various post-translational modi cations and changes in these modi cations are related to several disorders. All amino acid numbering is based on all Drp1 splice variants in human and rat. VD, variable domain (also called insert-B); GED, GTPase effector domain; P, phosphate; NO, nitric oxide; NOS, NO synthase.

Ser637(P). Increased Drp1 activity causes mitochondrial fragmentation, the disruption of mitochondrial functions, and entry into programmed necrosis [166].

In contrast to the above-mentioned mode, Ca^{2+}/calmodulin-dependent protein kinase Iα mediates phosphorylation of Ser600 in human Drp1 isoform 3 (corresponding to Ser637 in human Drp1 isoform 1), which induces mitochondrial fragmentation during voltage-dependent Ca^{2+} channel-associated Ca^{2+} signaling in cultured hippocampal neurons [167] (Fig. 3). Furthermore, hyperglycemia triggers the activation of Rho-associated coiled coil-containing protein kinase 1 (ROCK1; Ser/Thr kinase; Fig. 3). It directly phosphorylates Ser600 in the GED domain of mouse Drp1 isoform b (corresponding to Ser 637 in human Drp1 isoform 1), which stimulates Drp1 recruitment to the mitochondria and mitochondrial ssion, eventually leading to diabetic nephropathy [168]. As described above, LRRK2 regulates mitochondrial ssion by increasing the mitochondrial recruitment of Drp1 by direct interaction with Drp1 [111,112]. LRRK2 kinase activity plays a critical role in this process, whereas the phosphorylation sites of Drp1 or its regulating proteins have not yet been identi ed.

To summarize, phosphorylation of Drp1 at the same GED domain residues is likely to have opposite effects on the mitochondrial ssion activity in different cells or tissues, or under different culture conditions. Why phosphorylation at the same site has opposite effects on mitochondrial morphology remains to be clari ed.

6.2. S-Nitrosylation

Nitric oxide (NO) is implicated in neuronal cell survival and death [169]. *S*-Nitrosylation is a ubiquitous protein modi cation in redox-based signaling. β-amyloid protein, a key mediator of Alzheimer's disease, stimulates NO production to cause *S*-nitrosylation of Drp1 at Cys644 within the GED domain, which enhances GTPase activity and Drp1 oligomer formation in association with excessive mitochondrial ssion in neurons, leading to synaptic loss and neuronal damage in the brains of Alzheimer's disease patients, and a mutation of Cys644 prevents mitochondrial fragmentation and blocks the neurotoxicity induced by NO or β-amyloid protein [170] (Fig. 3), although this model has been challenged [171]. During myogenic differentiation, the short mitochondria in myoblast cells change into the extensively elongated

network observed in myotubes. Palma et al. demonstrated that the inhibition of Drp1-mediated mitochondrial ssion by NO generated by neuronal NO synthase in a cGMP-dependent manner is critical for myogenic differentiation [172]. The mechanistic details by which the mitochondrial network extension induced by the NO inhibition of Drp1 primes myogenic differentiation are not known.

6.3. SUMOylation

The SUMO protein also affects Drp1 activity. Overexpression of SUMO1 stabilizes Drp1 on the mitochondrial membrane in a Bax/Bak-dependent manner and induces mitochondrial ssion, suggesting that SUMOylation is a step in the regulation of Drp1 during early apoptosis progression [127]. Mitochondrial SUMO E3 ligase (MAPL for mitochondrial-anchored protein ligase) has been identi ed as SUMO E3 ligase for Drp1 and its overexpression stimulates mitochondrial ssion [173]. Conversely, overexpression of the SUMO protease SENP5 decreases Drp1 SUMOylation (deSUMOylation) and rescues SUMO1-induced mitochondrial ssion [174]. Interestingly, SENP5 resides primarily within the nucleoli in addition to a pool in the cytosol in interphase. In G2/M phase, nucleoli SENP5 translocates to the mitochondrial membrane and deSUMOylates Drp1, leading to mitochondrial fragmentation. Conversely, knockdown of SENP5 arrests the cell cycle precisely at the time when the protease is translocated to the mitochondria; thus, SENP5 is a key player in cell-cycle progression and might coordinate mitochondrial division and the cell cycle through the deSUMOylation of Drp1 [175,176]. It is not known why SENP5-dependent deSUMOylation of Drp1 has reverse effects on mitochondrial morphology, but it might depend on external parameters such as cellular status. Recent reports indicated that MAPL is incorporated within unique, Drp1-independent, mitochondria-derived small vesicles that are transported to peroxisomes and the process is regulated by Vps35 involved in vesicle transport from the endosome to the Golgi apparatus [177,178]. Communication with peroxisomal membranes might thus in uence mitochondrial morphology or lipid biosynthesis.

6.4. Ubiquitination

In addition to SUMOylation, ubiquitination regulates Drp1 activity. March5 (also known as MITOL), a mitochondria-associated RING- nger E3 ubiquitin ligase, ubiquitinates Drp1 on the MOM, although the effect of March5-dependent ubiquitination of Drp1 on mitochondrial dynamics remains controversial. March5 knockdown or overexpression of the March5 mutant lacking ubiquitin ligase activity induces mitochondrial fragmentation [179,180]. Karbowski et al., however, later demonstrated that March5 knockdown, as well as overexpression of the RING-inactive March5 mutant, induces abnormal mitochondrial accumulation of Drp1 in association with abnormal mitochondrial elongation and their interconnections [181]. In addition, March5 might play a more general role in the quality control of mitochondria by ubiquitinating mutated, damaged, or misfolded proteins accumulated in the MOM, as was observed for a mutated version of SOD or expanded polyQ proteins [182,183]. In addition, recent work revealed that March5 protects neuronal cells from mitochondrial damage caused by the accumulation of *S*-nitrosylated microtubule-associated protein 1B-light chain 1 (MAP1B-LC1). March5 ubiquitinates *S*-nitrosylated LC1 and promotes its degradation via the ubiquitin-proteasome pathway [184]. Thus, the precise roles of March5 in mitochondria still remain controversial.

6.5. O-GlucNAcylation

Gawlowski et al. recently demonstrated O-GlcNAcylation at Thr-585 and Thr-586 in the insert B-domain (VD) of Drp1 in rat neonatal cardiac myocytes; the reaction was signi cantly augmented by the inhibition of N-acetyl-glucosaminidase, leading to elevated levels of GTP-bound active Drp1, its mitochondrial translocation, and the induction of mitochondrial fragmentation [185]. The reaction was also stimulated upon shifting cells from low to high glucose medium, concomitant with an increase in the Drp1 expression level. Interestingly, this reaction paralleled a decrease in Ser637-phosphorylated Drp1. The reaction was detected for cardiac Drp1 in type 2 diabetic model mice and, therefore, might be linked to the development of diabetes-induced mitochondrial dysfunction and cardiovascular complications. The epistatic relation between Ser637 phosphorylation and Thr585/586 O-GluNAcylation in the mitochondrial ssion pathway is not known.

To summarize, how these multi-site posttranslational modi cations on Drp1 are coordinated to regulate overall mitochondrial morphology and cellular function remains for important future investigation.

7. Perspectives

Although key players regulating mammalian mitochondrial ssion (Drp1, Mff, MiD49/51, and hFis1) and regulatory factors (such as GDAP1, Endo B1, and sacsin) were identi ed over the past decade, the exact molecular mechanisms, their coordination, and the physiologic functions in distinct tissues remain poorly understood compared with the fusion reaction, i.e., functional division of Mff and MiD/MIEF proteins in the mitochondrial recruitment of Drp1, regulation of assembly and disassembly of the Drp1 foci, and coordination of post-translational modi cations of Drp1 linked to cellular signaling pathways. Furthermore, the function of hFis1 in the regulation of mitochondrial dynamics and its physiologic relevance must be investigated, because its contribution to Drp1-dependent mitochondrial ssion seems to be small [72]. Although the IMM protein MTP18, identi ed as a transcriptionally regulated target of phosphatidylinositol 3-kinase signaling, regulates mitochondrial ssion coupled with Drp1 [186,187], no further information is currently available on how the Drp1-dependent ssion machinery of MOM cooperates with the MTP18-dependent MIM ssion system or with other unknown machinery.

Importantly, recent studies revealed that ER-mitochondria contacts (mitochondria associated membrane structures) are involved in regulating mitochondrial energy, lipid metabolism, calcium signaling, and mitochondrial ssion, as described above [58 60,188,189]. The identi cation of additional structural components involved in the reactions and regulation of their assembly will reveal novel aspects of cell physiology regulation through communication between the mitochondria and ER.

Number of reports indicate that physiologic signi cance of mitochondrial ssion differs depending on the cell-types or tissues. Studying how mitochondrial ssion in uences cell-speci c functions in various tissues is a challenging task. Furthermore, investigation of the coordination of cellular signaling pathways projecting to the mitochondrial ssion machinery and their physiologic function will provide exciting breakthroughs in the elds of cell biology and clinical medicine.

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