

Physiological and Pathological Significance of Dynamin-Related Protein 1 (Drp1)-Dependent Mitochondrial Fission in the Nervous System

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Mitochondria are essential for proper neuronal morphogenesis and functions, as they are the major source of energy for neural development. The dynamic morphology of mitochondria determines the key functions of mitochondria. Several regulatory proteins such as dynamin-related protein 1 (Drp1) are required to maintain mitochondrial morphology via a balance between continuous fusion and fission. Activity of Drp1, a key regulator in mitochondrial fission, is modulated by multiple post-translation modifications and receptor interactions. In addition, numerous researches have revealed that the regulation of Drp1 activity and mitochondrial dynamics is closely associated with several neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. In this article, we concisely review the recent findings about the biological importance of Drp1-mediated mitochondrial fission in neurons under physiological and pathological conditions.

Key words: Drp1, neurodegeneration, mitochondria, fission, neuron

INTRODUCTION

Mitochondria support multiple aspects of neuronal development and functions by providing cellular energy, maintaining the intracellular calcium levels, generating reactive oxygen species and regulating the release of apoptogenic proteins such as cytochrome c and apoptosis-inducing factor [1]. Neurons are highly polarized cells with distinct axons and dendrites, and they have a high demand for energy to maintain neural circuits. Therefore, the quantity, quality and localization of mitochondria are all important for appropriate function of neurons [2]. For example, neuronal

mitochondria are enriched in the synaptic structures and nodes of Ranvier where immediate supply of cellular energy and active buffering of calcium are required [3].

Recently, it has been demonstrated that morphological dynamics of mitochondria is also involved in the maintaining their function and distribution in neurons [1]. Mitochondrial morphology is determined by a balance between continuous fusion and fission [4], which is regulated by large GTPase dynamin-related proteins including Mitofusin 1/2 (Mfn1/2), Optic atrophy 1 (Opa1) and Dynamin-related protein 1 (Drp1) [5]. Mfn1/2 is located on the outer membrane of mitochondria (OMM), and binding and homo- or hetero-dimerization of mitofusins promote fusion of two neighboring OMM. Sequentially, Opa1, resided on the mitochondrial inner-membrane (IMM), fuses IMM by GTP hydrolysis. On the other hand, Drp1 is primarily localized at cytosol. For mitochondrial fission, Drp1 translocates from cytosol to OMM, and divides a mitochondrion into two pieces by GTP hydrolysis.

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Mitochondrial fission is predominantly controlled by the activity of Drp1, and the frequency of mitochondrial fission determines the quality as well as morphology of mitochondria. Because mitochondria are essential for the neuronal functions, increasing amounts of evidence support the idea that Drp1 plays critical roles in the physiological function and pathological progression of the nervous system.

MOLECULAR REGULATORY MECHANISMS FOR THE DRP1 FUNCTION

Drp1 protein has four domains: GTPase domain, middle do-

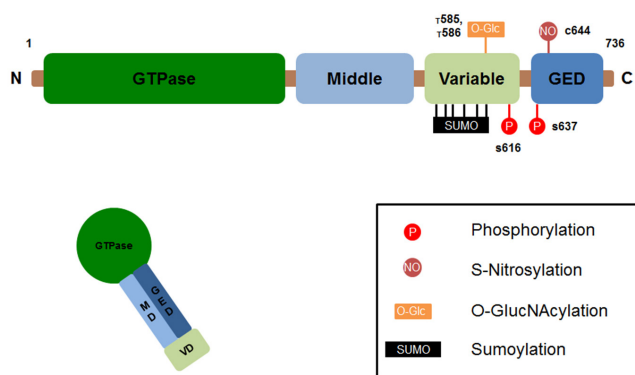


Fig. 1. Schematic illustration of Drp1 structure. (A) Domain structure and post-translational modifications of Drp1. Multiple post-translational modifications regulate GTPase activity of Drp1. The number of amino acids is based on Drp1 splicing variant 1 in human. (B) Folded structure of Drp1. VD, variable domain; GED, GTPase effector domain; MD, middle domain; P, phosphorylation; NO, S-nitrosylation; O-Glc, O-GlcNAcylation; SUMO, sumoylation.

main, variable domain, and GED domain (Fig. 1) [6]. Unlike other dynamin family proteins, however, Drp1 does not have a lipid-interacting pleckstrin homology domain [7], and thus the anchorage of Drp1 to the mitochondrial membrane is mediated by its receptor binding. Crystal structural study revealed that the variable domain of Drp1 acts as a hinge, forming a T-shaped dimer or tetramer [8, 9]. Therefore, fission-promoting activity of Drp1 is controlled by many post-translational modifications around the variable domain, including phosphorylation, sumoylation, ubiquitination and S-nitrosylation (Fig. 1, Table 1). Post-translational modifications and the alterations in its receptor functions also contribute to the activity of Drp1. S-nitrosylation is irreversible protein modification process, which is associated with neurodegenerative changes of proteins [10]. On the other hand, other post-translational modifications can be reversible, suggesting that these changes occur for the physiological control of mitochondrial dynamics.

Serine 637 (S637) of human Drp1 can be phosphorylated by protein kinase A (PKA), and it suppresses mitochondrial translocation and GTPase activity [11, 12]. On the other hand, Drp1^{S637} is dephosphorylated by calcineurin (PP2B), which promotes mitochondrial fragmentation [13]. In neurons, Drp1^{S637} can also be phosphorylated by CaMK1 α following neuronal activation [14]. Interestingly, phosphorylation of Drp1 by CaMK1 α conversely enhances the mitochondrial fragmentation, since phosphorylated Drp1 by CaMK1 α shows higher affinity with mitochondrial effector molecule, Fis1. In addition, Rho-associated coiled-coil containing protein kinase 1 (ROCK1) also phosphorylates Drp1^{S637}, resulting in mitochondrial fragmentation under hyper-

Table 1. Post-translational modifications of Drp1

Modifications	Sites	Upstream regulators	Effects	References
Phosphorylation	S616	CDK1	Activation	Taguchi et al., 2007
		Erk1/2	Activation	Yu et al., 2011
		PKC δ	Activation	Qi et al., 2011
	S637	PKA	Inactivation	Chang and Blackstone, 2007; Cribbs and Strack, 2007b
		CaMK1 α	Activation	Han et al., 2008a
Dephosphorylation	S637	ROCK1	Activation	Wang et al., 2012c
		Calcineurin (PP2B)	Activation	Cereghetti et al., 2008
S-nitrosylation	C644	Nitric oxide	Activation	Cho et al., 2009
SUMOylation	Multi-sites in variable domain	MAPL	Activation	Braschi et al., 2009
DeSUMOylation		SEN5	Inactivation	Zunino et al., 2007b
Ubiquitination		MARCH5	Inactivation	Nakamura et al., 2006a; Yonashiro et al., 2006b
		Parkin	Inactivation	Wang et al., 2011b
O-GlcNAcylation	T585, T586	O-GlcNAc-transferase	Activation	Gawlowski et al., 2012b

glycemic condition [15]. Considering that phosphorylation at the same site by different kinases showed entirely opposite effects on Drp1 activity, it appears that not only the status of Drp1 phosphorylation but also the type of upstream kinases determines the functional consequences. The precise mechanisms underlying these interesting phenomena remain to be explored.

Another phosphorylation site, serine 616 (S616), can also be phosphorylated by many kinases including CDK1 [16], ERK1/2 [17] and PKC δ [18]. Phosphorylation at this site enhances the activity of Drp1 under certain circumstances such as mitosis, high glucose or oxidative stress conditions, which results in mitochondrial fragmentation. However, when its phosphomimetic or phosphodeficient mutant, Drp1(S616D) or Drp1(S616A) respectively, was simply overexpressed in HeLa cells, mutant Drp1 failed to alter mitochondrial morphology [13]. It implies that the effect of Drp1^{S616} phosphorylation may depend on intracellular context and/or upstream kinases rather than phosphorylation status. More recently, it has been identified that CDK5 can also phosphorylate Drp1^{S616}, which induces mitochondrial fragmentation by the mobilization of Drp1 to mitochondria from microtubule [19]. However, CDK-dependent phosphorylation of Drp1^{S616} inhibits the oligomerization of Drp1 bound on microtubule or mitochondria. These results imply that CDK-dependent phosphorylation of Drp1^{S616} may have reciprocal effect on mitochondrial translocation and oligomerization of Drp1. Considering that neurons have high activity of CDK5, it is interesting to explore whether phosphorylation of Drp1^{S616} can serve as an activating or inactivating mechanism for Drp1 in the nervous system.

SUMOylation has been known to increase the stability of Drp1 on OMM that is required for efficient fission of mitochondria [20, 21]. For example, ectopic expression of SUMO1 stabilizes mitochondrial-targeted Drp1 in a Bax/Bak-dependent manner leading to mitochondrial fragmentation during apoptotic cell death [21]. Currently, the SUMOylation of Drp1 is regulated by SUMO E3 ligase MAPL [22] and SUMO protease SENP5 [23].

Drp1 activity can be regulated by ubiquitination, which is mediated by mitochondria-associated RING-finger E3 ubiquitin ligase (MARCH5/MITOL). Both overexpression of ligase activity-defective MARCH5 and gene knockdown induce hyperaccumulation of Drp1 on OMM, but leads to opposite effect which is mitochondrial elongation [24] or fragmentation [25]. These contradictory outputs result from diverse effects of MARCH5 on dynamin-related proteins; MARCH5 promotes Mfn2-mediated mitochondrial fusion, while it also ubiquitinates Drp1 [26]. In addition, Drp1 can also be ubiquitinated by Parkin

implicated in Parkinson's disease [27]. This modification promotes proteasome-dependent degradation of Drp1, and pathogenic mutation or knockdown of Parkin induces increased level of Drp1 and aberrant mitochondrial fragmentation.

In rat neonatal cardiac myocytes, Drp1 can be O-GlcNAcylated by O-GlcNAc-transferase at Threonine 585 and 586 in the variable domain [28]. This modification decreases the level of phosphorylated Drp1^{S637}, which is consistent with a reciprocal relationship between O-GlcNAcylation and phosphorylation in several cases [29]. In fact, O-GlcNAcylation of Drp1 increases the levels of GTP-bound active Drp1 and leads to fragmentation of mitochondria.

MITOCHONDRIAL RECEPTORS FOR DRP1

Upon exposure to stimuli for mitochondrial fission, Drp1 is recruited from cytosol to putative fission sites on OMM. Recruitment of Drp1 is accompanied by several receptors on the OMM, and the interaction promotes Drp1-mediated mitochondrial fission. Fis1 was first identified as a Drp1 receptor in yeast [30]. In yeast, Fis1 is indirectly associated with Drp1 by two adaptor proteins, Mdv1 [31] and Caf4 [32]. However, mammalian homologues of Mdv1 and Caf4 have not been identified. In mammal, Drp1 interacts with Fis1 for mitochondrial fission [33], but Drp1 can also be recruited to mitochondria in a Fis1-independent manner [34, 35]. This implies alternative pathways of Drp1-mediated mitochondrial fission. Recent reports have demonstrated that mitochondrial fission factor (Mff) directly interacts with Drp1. In both *in vivo* and *in vitro* conditions, Mff binds Drp1 to promote mitochondrial fragmentation [36]. Consistently, knock-down of Mff releases Drp1 from fission foci on OMM and causes mitochondrial elongation. However, molecular mechanism underlying the foci formation by Drp1 and Mff is still unclear. MiD49 and MiD51 (MIEF1) also binds to Drp1, but these bindings inhibit mitochondrial fission [37, 38].

In addition, cytoskeleton is also involved in mitochondrial translocation of Drp1. Disruption of F-actin inhibits mitochondrial translocation of Drp1 under mitochondrial depolarization [39], and impaired microtubule sequesters Drp1 away from mitochondria [19]. Interestingly, Drp1 also interacts with Mfn2 to inhibit mitochondrial fusion [40], suggesting that mitochondrial recruitment of Drp1 is involved in the mitochondria fusion as well. After mitochondrial recruitment, Drp1 forms ring- or spiral-like oligomers and weakly constricts at a putative fission site [41]. Finally, GTP hydrolysis promotes a complete fission of mitochondria [42].

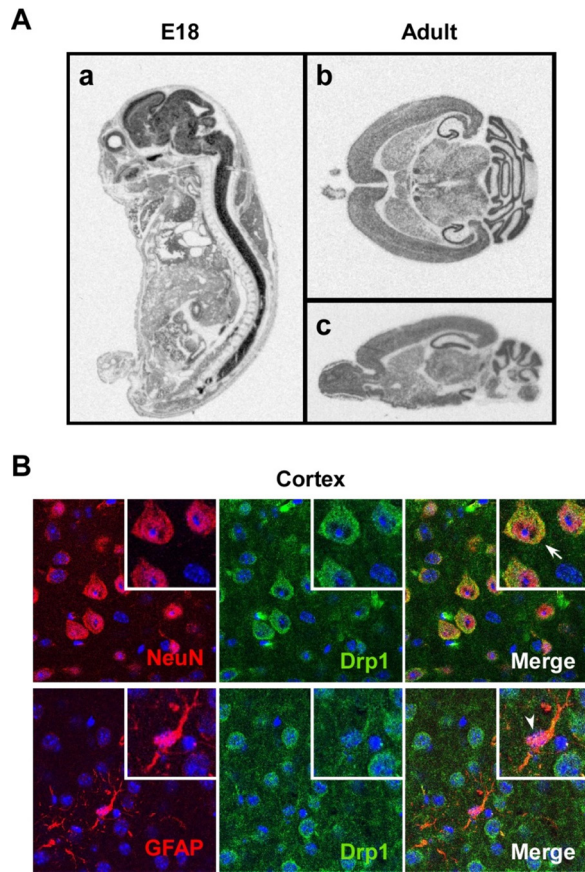


Fig. 2. High level of Drp1 expression in the nervous system. (A) *In situ* hybridization of Drp1 mRNA in rat embryo (a) and adult brain (b, c). (B) Immunohistochemistry of Drp1 protein in rat cerebral cortex. NeuN and GFAP are used as markers for post-mitotic neurons and astrocytes, respectively.

MITOCHONDRIAL DYNAMICS AND NEURONAL FUNCTION UNDER PHYSIOLOGICAL CONDITIONS

Drp1 is a key player for the regulation of mitochondrial dynamics in neurons. Drp1 is highly expressed in post-mitotic neurons. RNA *in situ* hybridization revealed that Drp1 mRNA is highly enriched in the central nervous system including spinal cord and brain, compared to non-neural tissues of rat embryo (Fig. 2Aa). In adult brain, regions containing neuronal cell bodies exhibit increased levels of Drp1 mRNA (Fig. 2Ab, c). In addition, immunohistochemistry shows that Drp1 is predominantly expressed in neurons labeled by NeuN, a neuronal marker, rather than glial cells marked by GFAP (Fig. 2B). These data suggest that Drp1 may be a neuron-specific regulator of mitochondrial dynamics. Drp1 plays a role in mitochondrial transport for synaptic plasticity [14, 43], and neuronal growth [44, 45]. The balance between mitochondrial fission and fusion is important for cellular homeostasis in neuron. Neuronal activation induces

mitochondrial fragmentation, which results in redistribution of mitochondria in dendritic spines for local ATP supply and Ca^{2+} buffering [46]. Consistently, inhibition of mitochondrial fission leads to defects on synaptic morphogenesis and plasticity [14, 43, 46, 47] and axonal growth [44, 45].

Drp1 is essential for neuronal development, function and survival. Neuron-specific Drp1^{-/-} mice died shortly after birth, resulting from developmental defects in nervous system [44]. In addition, a patient with dominant-negative mutation in Drp1 showed similar defects in brain development [48, 49]. These findings imply that Drp1-mediated mitochondrial fission is required for neuronal development. During the development of nervous system, the length of mitochondria changes dynamically. Recently, we found that mitochondria in developing chick motoneurons progressively shortened *in vivo*, accompanied by a marked induction of Drp1 expression [45]. The blockade of Drp1 activity markedly increased the mitochondrial length, suggesting that increased Drp1 expression contributed to the developmental adjustment of mitochondrial length. On the other hand, it is known that mitochondria progressively elongate in cultured mouse cortical neurons [50-52]. Exact cause for this difference is yet unclear, but we found that Drp1 expression level is also progressively increased during the maturation of neurons *in vitro*, suggesting that transcriptional program for the Drp1 expression is conserved *in vitro*. Maturation of cultured neurons *in vitro* is temporally associated with marked growth of the neuronal processes, thereby demanding massive biogenesis of mitochondria. Mitochondrial length is influenced by multiple factors including fusion/fission ratio, biogenesis and mitophagic degradation. Therefore, it appears that increased Drp1 and fission rate may not be sufficient to explain the mismatch between development-related shortening and biogenesis-related elongation of mitochondria in neurons.

MITOCHONDRIAL DYNAMICS AND NEURONAL CELL DEATH AND PATHOLOGIES

The molecular links between Drp1 and cell death are first suggested by the fact that Drp1-containing fission foci also contains apoptosis-promoting Bcl-2 family molecules such as Bak, Bax and truncated Bid [21, 53-55]. In fact, mitochondrial fission is necessary for the execution of apoptosis. For example, suppression of Drp1 activity reduces mitochondrial fission and inhibits mitochondrial translocation of Bax which is essential for the execution of apoptosis [53, 56]. Conversely, the priming of mitochondrial fragmentation by Drp1 overexpression augments the extent of apoptosis [55], but Drp1 overexpression itself does

not induce cell death in many cell types. Recently, we provided *in vivo* evidence that mitochondrial fission is associated with the neuronal death. During the chick motoneuron development, mitochondrial length is dramatically reduced by the time of initiation of naturally occurring programmed cell death (PCD) [45], suggesting that mitochondrial shortening can reduce the threshold of neuronal apoptosis as a PCD onset mechanism. Supporting this, overexpression of Drp1 triggered premature onset of PCD in motoneurons (our unpublished data). However, suppression of Drp1 activity induced neuronal death in the chick [45] and mouse [49] development models. These results indicate that the mitochondrial dynamics and Drp1 activity is set for the maximal survival of neurons during the development, but the impaired balance of Drp1 activity results in neuronal cell death.

Changes in the mitochondrial dynamics are found in many neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). It has been postulated that the imbalance of mitochondrial fusion/fission is associated with disease-related mitochondrial dysfunctions [57]. In case of AD, mitochondrial fragmentation is progressively increased during the progression of disease in patients and transgenic mouse models [58, 59]. Upon induction of mitochondrial fragmentation, interactions between Drp1 and A β or phosphorylated Tau are also progressively increased [60, 61]. Although it is yet unclear how pathological changes enhance the interactions between Drp1 and AD-related proteins, it has been reported that post-translational modifications of Drp1 such as S-nitrosylation and phosphorylations are observed in AD patients [10, 62]. Therefore, it is plausible that AD-dependent modifications of Drp1 can enhance the fission activity as well as protein interactions.

In the pathology of PD, mitochondrial changes are well-documented. Especially, genetic mutations in the autophagic processes, such as Parkin and PINK, are frequently associated with PD. For example, Parkin and PINK play roles in the tagging of impaired mitochondria for autophagic degradation. Considering that mitochondrial fission is necessary for the efficient autophagic removal of damaged mitochondria, it has been proposed that mitochondrial fission machineries are affected by PD pathogenesis. As described in above, Parkin ubiquitinates Drp1 and promotes proteasome-dependent degradation of Drp1. Therefore, pathogenic mutation of Parkin results in the abnormal accumulation of Drp1, which promotes excessive mitochondrial fragmentation [27]. These mechanisms may be associated with PD-dependent mitochondrial dysfunctions. Drp1 also interacts with another PD-related molecule, leucine-rich repeat kinase 2 (LRRK2) [63]. This interaction enhances mitochondrial

translocation of Drp1 and leads to excessive mitochondrial fragmentation.

The expression of Drp1 is increased in the striatum and cortex of HD patients, which may influence mitochondrial dysfunction in HD [64, 65]. Mutant huntingtin (mHtt) abnormally recruits Drp1 on OMM and subsequently promotes GTPase activity of Drp1, resulting in excessive mitochondrial fragmentation [66, 67]. Recent report has revealed that hyper-activation of Drp1 is mediated by S-nitrosylation, as a result of nitric oxide produced by mHtt [68], as also found in AD.

While mitochondrial fragmentation is not yet reported in ALS patients, an animal model of ALS, mutant SOD1 G93A (mSOD1) mouse, exhibited reduced mitochondrial length in motoneuron at the pre-symptomatic period [69, 70]. Furthermore, prevention of Drp1 activity significantly decreased cell death of spinal motoneuron in rats [71]. During the pathological process of ALS, the axonal transport of mitochondria is also impaired in affected motoneuron [72, 73]. These impairments are rescued by suppressing Drp1 [71], supporting the idea that mitochondrial fission machineries are tightly linked with axonal transport mechanism [3, 71, 74].

CONCLUSION

Dysregulation of Drp1-dependent mitochondrial fission is commonly observed in neurodegenerative diseases, despite the fact that no genetic mutation of Drp1 has been identified. Drp1 is therefore considered as a candidate target for a disease-modifying drug to enhance neuronal survival and their functions. For instance, chemical inhibitor of Drp1 (mdivi-1) can effectively suppressed retinal neurodegeneration following acute ischemia [75]. Suppression of basal Drp1 activity, however, causes impairments in synaptic plasticity and axonal integrity. Therefore, it is necessary to study the disease-specific modification of Drp1 and its implications, as it may provide a better insight to understand and treat neurodegenerative diseases.

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