

COMMENTARY

Mitochondrial dynamics in neuronal injury, development and plasticity

Kyle H. Flippo and Stefan Strack*

ABSTRACT

Mitochondria fulfill numerous cellular functions including ATP production, Ca^{2+} buffering, neurotransmitter synthesis and degradation, ROS production and sequestration, apoptosis and intermediate metabolism. Mitochondrial dynamics, a collective term for the processes of mitochondrial fission, fusion and transport, governs mitochondrial function and localization within the cell. Correct balance of mitochondrial dynamics is especially important in neurons as mutations in fission and fusion enzymes cause peripheral neuropathies and impaired development of the nervous system in humans. Regulation of mitochondrial dynamics is partly accomplished through post-translational modification of mitochondrial fission and fusion enzymes, in turn influencing mitochondrial bioenergetics and transport. The importance of post-translational regulation is highlighted by numerous neurodegenerative disorders associated with post-translational modification of the mitochondrial fission enzyme Drp1. Not surprisingly, mitochondrial dynamics also play an important physiological role in the development of the nervous system and synaptic plasticity. Here, we highlight recent findings underlying the mechanisms and regulation of mitochondrial dynamics in relation to neurological disease, as well as the development and plasticity of the nervous system.

KEY WORDS: Bioenergetics, Dynamin-related protein 1, Mitochondrial fission, Mitochondrial fusion, Neurodegenerative disease, Synaptic plasticity

INTRODUCTION

Mitochondria are commonly referred to as the ‘power house’ of the cell due to the dominant role they play in ATP production in eukaryotic cells. However, this is an oversimplification of mitochondrial physiology. In addition to carrying out ATP synthesis through oxidative phosphorylation, mitochondria are also important for Ca^{2+} signaling (Brini et al., 2014; Nicholls, 2005; Duchen, 2000; Clapham, 2007), cell death (Tait and Green, 2013; Duchen, 2000), steroid synthesis (Miller, 2011), reactive oxygen species (ROS) production and sequestration (Zorov et al., 2014; Hamanaka and Chandel, 2010; Shadel and Horvath, 2015; Accardi et al., 2014), and neurotransmitter synthesis and inactivation (Rowley et al., 2012; Bak et al., 2005; Waagepetersen et al., 2000). Given the importance of processes, such as ATP production, Ca^{2+} transients, neurotransmitter metabolism and ROS signaling, in synaptic transmission it is not surprising that recent work has illustrated that perturbations in mitochondrial physiology exert

profound effects on neuronal development and function. Mitochondrial ATP production, Ca^{2+} buffering, neurotransmitter metabolism and ROS signaling themselves are spatially and temporally regulated in neurons through mitochondrial localization (Brodin et al., 1999; Jayashankar and Rafelski, 2014; Li et al., 2004; Niescier et al., 2013; Rueda et al., 2014; Sheng, 2014; Vos et al., 2010), mitochondrial bioenergetics (Rueda et al., 2014; Dickey and Strack, 2011), and mitochondrial biogenesis (Cheng et al., 2012), all of which are strongly influenced by mitochondrial dynamics, which entails mitochondrial fission, fusion and transport.

Perturbations in mitochondrial dynamics, and altered expression and activity of fission and fusion enzymes has been observed in almost every major neurodegenerative disorder (Table 1), yet it remains unclear precisely how alterations in mitochondrial dynamics contribute to the pathology of these diseases (DuBoff et al., 2013; Hroudová et al., 2014; Reddy et al., 2011; Yu-Wai-Man et al., 2011; Cho et al., 2013; Büeler, 2009). Furthermore, the importance of mitochondrial dynamics has been documented in neuronal development (Li et al., 2004; Dickey and Strack, 2011; Chan, 2006; Ishihara et al., 2009) and survival (Slupe et al., 2013; Merrill et al., 2013; Dagda et al., 2008; Merrill et al., 2011; Cho et al., 2010; Nakamura et al., 2010). However, also there, the specific mechanisms often await elucidation. In this Commentary, we highlight recent findings that illustrate the importance of mitochondrial dynamics in neuronal development, synaptic transmission and disease. We also propose potential mechanisms on how mitochondrial dynamics might influence mitochondrial function in these instances by focusing on altered mitochondrial bioenergetics and localization within neurons.

The mitochondrial fission and fusion machinery

Mitochondria are morphologically diverse, ranging from near spherical objects to interconnected networks. Mitochondrial shape changes are brought about by opposing fission (or division) and fusion processes. Mitochondrial fission and fusion are catalyzed by a family of large GTPase enzymes that utilize GTP hydrolysis in order to remodel the two mitochondrial membranes. The enzyme responsible for fission of the outer mitochondrial membrane (OMM) is dynamin-related protein-1 (Drp1), whereas mitochondrial fusion requires coordination of three enzymes. Mitofusin 1 and 2 (Mfn1 and 2) promote fusion of the OMM, whereas optic atrophy 1 (Opa1) promotes fusion of the inner mitochondrial membrane (IMM) (Otera et al., 2013; Kasahara and Scorrano, 2014) (Fig. 1). It should be noted that, in addition to its role in promoting mitochondrial fusion, recent evidence suggests that Mfn2 plays an important role in regulating the association between the endoplasmic reticulum (ER) and mitochondria, and can localize to both the ER and to mitochondria. Importantly, ER-mitochondrial contact is necessary for both ER-mitochondrial Ca^{2+} signaling and mitochondrial fission. ER-mitochondrial contacts

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Table 1. Mitochondrial fission and fusion proteins in neurological diseases

Neurological disorder	Gene mutated	Fission/fusion protein affected	Impact on mitochondrial length	Species	References
Amyotrophic lateral sclerosis	various	↑ Drp1	↓ (↑ fission)	rat	(Song et al., 2013)
Alzheimer's disease	various	↑ Drp1	↓ (↑ fission)	mouse	(Cho et al., 2009)
Huntington's disease	<i>Htt</i>	↑ Drp1	↓ (↑ fission)	human, mouse, rat	(Haun et al., 2013)
Autosomal-recessive spastic ataxia of Charlevoix–Sanguenay (ARSACS)	<i>Sacs</i>	↓ Drp1	↑ (↓ fission)	human, mouse	(Bradshaw et al., 2016; Girard et al., 2012)
Autosomal dominant Charcot–Marie–Tooth disease, axonal (CMT2K)	<i>GDAP1</i>	↓ GDAP1	↑ (↓ fission)	human	(Sivera et al., 2010; Niemann et al., 2009)
Autosomal recessive Charcot–Marie–Tooth disease, axonal, type 2K (ARCMT2K)	<i>GDAP1</i>	↓ GDAP1	↑ (↓ fission)	human	(Niemann et al., 2009; Baxter et al., 2002)
Autosomal recessive Charcot–Marie–Tooth disease, type 4A, demyelinating (CMT4A)	<i>GDAP1</i>	↓ GDAP1	↑ (↓ fission)	human	(Cuesta et al., 2002; Niemann et al., 2009)
Autosomal dominant Charcot–Marie–Tooth disease, axonal, type 2A2 (CMT2A2)	<i>Mfn2</i>	↓ Mfn2	↓ (↓ fusion)	human, rat	(Misko et al., 2012; Züchner et al., 2004)
Autosomal dominant optic atrophy (ADOA)	<i>Opa1</i>	↓ Opa1	↓ (↓ fusion)	human, rat	(Misko et al., 2012; Zanna et al., 2008)

Abbreviations: Drp1, dynamin-related protein 1; HTT, huntingtin; SACS, saccin; GDAP1, ganglioside-induced differentiation-associated protein 1; MF2, mitofusin 2; Opa1, optic atrophy 1.

have been proposed to mark fission sites on the OMM through pre-constriction of the OMM by promoting assembly of the mitochondrial fission machinery (Friedman et al., 2011; Hatch et al., 2014; Lee and Yoon, 2014). However, there is currently a debate as to whether Mfn2 promotes or inhibits the apposition between ER and mitochondrial membranes. Work by the Scorrano group has suggested that Mfn2 is necessary for ER-mitochondrial association because deletion of Mfn2 decreases this interaction (de Brito and Scorrano, 2008; Naon et al., 2016). However, other groups have challenged this model, suggesting instead that deletion of Mfn2 increases ER-mitochondrial association and, in turn, are questioning whether Mfn2, indeed, localizes to the ER (Filadi et al., 2015; Cosson et al., 2012). Regardless of the role Mfn2 has in

ER-mitochondrial contacts, the importance of mitochondrial fusion proteins – particularly in neuronal cells – is highlighted by the fact that hypomorphic mutations in *Mfn2* are most often responsible for autosomal dominant Charcot–Marie–Tooth (CMT) disease, axonal, type 2A2 (CMT2A2) (Chapman et al., 2013; Misko et al., 2012; Niemann et al., 2014; Yu-Wai-Man et al., 2011), a common peripheral neuropathy (Züchner et al., 2004), whereas mutations in *Opa1* are the most common cause of hereditary blindness, i.e. autosomal dominant optic atrophy (ADOA) (Yu-Wai-Man et al., 2011; Alexander et al., 2000; Zanna et al., 2008). CMT2A is the most common form of CMT comprising 20% of all diagnoses. In addition to the diagnostic peripheral neuropathy associated with CMT2A, some patients also experience sensorineural hearing loss, impaired vision and encephalopathy (Stuppia et al., 2015). Similarly, although ADOA is clinically characterized by degeneration of the optic nerve, ~20% of patients present with extraocular phenotypes, such as peripheral neuropathy and sensorineural hearing loss (Lenaers et al., 2012). Although very rare, *de novo* hypomorphic mutations in *DNM1L*, the gene encoding Drp1, are equally devastating and can cause severely impaired development of the nervous system, which leads to epileptic encephalopathy, development delay, pain insensitivity and even – depending on the mutation – early postnatal death (Fahrner et al., 2016; Sheffer et al., 2016; Waterham et al., 2007).

In contrast to Opa1 and mitofusin, which are anchored in the IMM and OMM, respectively, localization of Drp1 to mitochondria is transient and relies on its interaction with distinct mitochondrial adaptor proteins. Multiple adaptor proteins for Drp1 have been identified, suggesting a complex regulatory network that controls Drp1 localization at mitochondria. Mitochondrial fission factor (Mff) is likely to play a central role in the recruitment of Drp1 to mitochondria; but other proteins have also been identified, including Mid49 and Mid51 (also known as Mief2 and Mief1, respectively), and Fis1 (Otera et al., 2013). It has been proposed that these adaptor proteins function coordinately in order to segregate active and inactive forms of Drp1 at mitochondria (Prudent and McBride, 2016; Wilson et al., 2013). This also suggests that mitochondrial localization of Drp1 is insufficient to induce fission, with recent work illustrating a role for actin and actin-associated proteins in assembly of the fission machinery. However, assembly of the fission machinery itself does not always progress to fission of

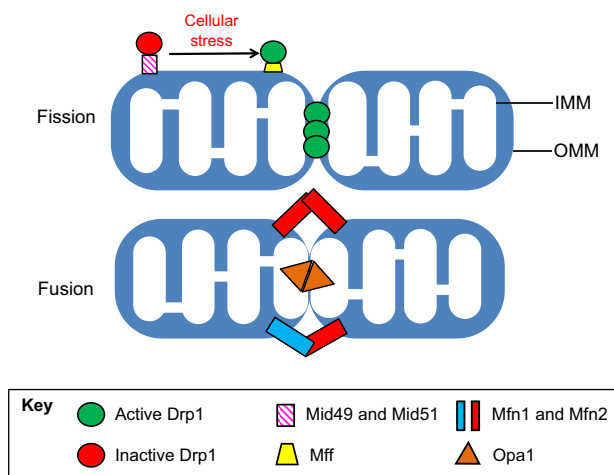


Fig. 1. GTPases catalyze mitochondrial fission and fusion. Mitochondrial fission occurs through oligomerization of active Drp1 and constriction of the outer mitochondrial membrane (OMM). The mitochondrial adaptor protein Mff appears to be important for coordinating the oligomerization of active Drp1, whereas Mid49 and Mid51 are thought to localize inactive Drp1 to the OMM in preparation for future fission events. Mitochondrial fusion requires the coordination of mitofusin1 and 2 (Mfn1 and Mfn2) at the OMM, and Opa1 at the inner mitochondrial membrane (IMM). Homo- or heterodimerization of Mfn1 and Mfn2 on opposing OMM surfaces promote fusion of the OMM. Similarly, homodimerization of Opa1 at the IMM promotes fusion of mitochondrial matrix compartments.

the OMM (Hatch et al., 2014, 2016; Ji et al., 2015). In support of this latter model, recent work by Adachi and colleagues suggests that the fission activity of Drp1 is also dependent upon phospholipid composition of the OMM (Adachi et al., 2016) and requires coordination with dynamin 2 in order to accomplish fission of the OMM (Lee et al., 2016).

Post-translational regulation of mitochondrial fission and fusion

A range of post-translational modifications (PTMs) of mitochondrial fission and fusion enzymes and their associated proteins provide an additional layer of regulation that allows for dynamic control over connectivity of the mitochondrial network. Drp1 undergoes several PTMs with phosphorylation of specific serine residues being the best characterized (Fig. 2). In neurons, protein kinase A (PKA)-mediated Drp1 phosphorylation of a highly conserved serine residue (S637 in human Drp1 isoform 1), inhibits Drp1 function and mitochondrial fission, whereas dephosphorylation of this residue has the opposite effect (Cribbs and Strack, 2007; Chang and Blackstone, 2007; Cereghetti et al., 2008). By contrast, phosphorylation of Drp1 at S616 by cyclin dependent kinases, protein kinase C (PKC) or extracellular signal regulated kinases (ERKs) (Lee and Yoon, 2014), has been shown to promote Drp1 activity and mitochondrial fragmentation in mitotic cells, as well as in neurons (Cho et al., 2014; Tang et al., 2016). In addition to phosphorylation of Drp1, nitrosylation of its cysteine residue 644 triggered by nitric oxide (NO), has been suggested to facilitate Drp1-mediated mitochondrial fission and neuronal death in Alzheimer’s disease (AD) (Nakamura et al., 2010; Cho et al., 2009) and Huntington’s disease (HD) (Haun et al., 2013). However, other groups did not observe increased Drp1 S-nitrosylation but, instead, proposed increased phosphorylation of S616 and the subsequent recruitment of Drp1 to mitochondria as a mechanism to trigger the mitochondrial fragmentation found in neurodegenerative diseases (Bossy et al., 2010; Zhang et al., 2016). In addition, Drp1 is SUMOylated at multiple lysine residues, which has been suggested to stabilize Drp1 oligomers at the OMM in order to promote mitochondrial fission and initiate apoptosis (Wasiak et al., 2007; Harder et al., 2004; Guo et al., 2013a; Figueroa-Romero et al.,

2009). Drp1 is also subject to alternative splicing and can include up to three alternative exons that give rise to eight different protein isoforms in mammals (Fig. 1B). Interestingly, inclusion of specific alternative exons determines the subcellular localization and function of Drp1 (Strack et al., 2013). PTMs of the mitochondrial adaptor protein Mff also promote recruitment of Drp1 to the OMM. In mouse embryonic fibroblasts, cellular stress induced by inhibitors of the mitochondrial electron transport chain triggers AMP-kinase-mediated phosphorylation of Mff at S155 and S172, culminating in recruitment of Drp1 and mitochondrial fission (Toyama et al., 2016).

Also acutely controlled by PTMs is mitochondrial fusion. Phosphorylation of Mfn2 by the mitochondrion-targeted PTEN-induced putative kinase 1 (PINK1) at threonine (T111) and S442 allows Mfn2 to serve as a docking station for parkin (PARK2) at the OMM, which is necessary for mitophagy of cardiac mitochondria (Chen and Dorn, 2013). Furthermore, phosphorylation of Mfn2 at S27 can activate ubiquitin-mediated degradation of Mfn2 during cellular stress, leading to mitochondrial fission and apoptosis in cells of the U-2 OS cell line (Leboucher et al., 2012). Phosphorylation of Mfn1 by ERK2 (also known as MAPK1) inhibits Mfn1 oligomerization, thereby promoting mitochondrial fission and apoptosis (Pyakurel et al., 2015). Besides phosphorylation, acetylation of Mfn1 triggers its degradation during cellular stress through ubiquitylation mediated by the E3 ligase MARCH5 (Park et al., 2014). Finally, crosslinking of mitofusins by the formation of disulfide-bonds – a process that is dependent on oxidation of glutathione and hydrolysis of GTP – has been suggested to contribute to mitochondrial hyperfusion, a protective mechanism in response to oxidative stress (Shutt et al., 2012).

In terms of the post-translational regulation of inner membrane fusion, the proteolytic cleavage of Opa1 has been identified as an important modulator of mitochondrial dynamics. Proteolytic cleavage of Opa1 influences Opa1 function and localization because proteolytic cleavage at certain sites produces soluble isoforms that appear to promote mitochondrial fission, whereas other cleavage products remain anchored at the IMM and, so, capable of membrane fusion (Ishihara et al., 2006; MacVicar and Lane, 2014; Mishra et al., 2014). The inactivating cleavage of Opa1

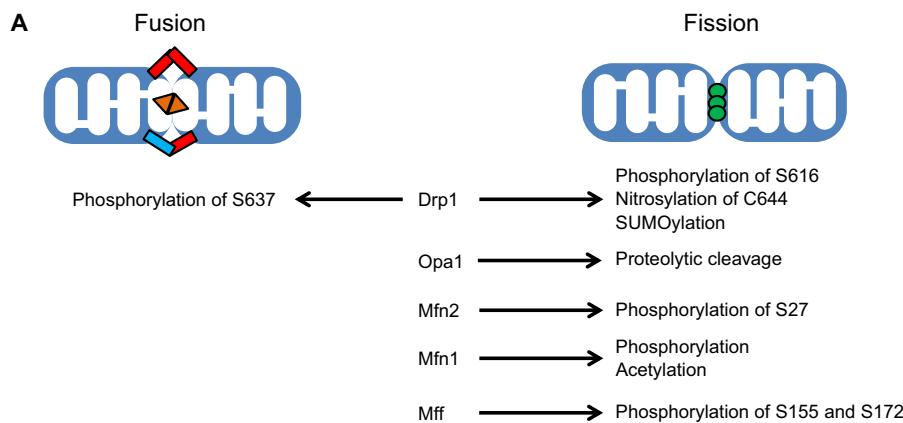


Fig. 2. Post-translation modification (PTM) of mitochondrial fission and fusion proteins.

(A) Drp1 can undergo a variety of PTMs, leading to either fission or fusion of mitochondria. PTMs of most other mitochondrial fission and fusion proteins appear to result exclusively in fission; however, the characterization of their PTMs is far from comprehensive. (B) Location of Drp1 S616 and S637 (human isoform 1) in different Drp1 splice variants and the inclusion of alternative exons based on the PhosphoSitePlus database (<http://www.phosphosite.org/homeAction.action>) (Hornbeck et al., 2015).

B

Splice form	Mouse			Human				Rat
	111	000	100	011	010	001	000	111
No. of alternative exons	3	0	1	2	1	1	0	3
S616 (human isoform 1)	S622	S579	S592	S616	S605	S590	S579	S635
S637 (human isoform 1)	S643	S600	S613	S637	S626	S611	S600	S656

is catalyzed by the peptidases YME1L and OMA1, which are activated during cellular stress (MacVicar and Langer, 2016). Mitochondrial depolarization, an indicator of mitochondrial dysfunction, activates both peptidases, which results in Opal cleavage, mitochondrial fission and, finally, mitophagy of damaged mitochondria (MacVicar and Langer, 2016; Song et al., 2007; Korwitz et al., 2016; MacVicar and Lane, 2014). Ultimately, the complex coordination of PTMs of mitochondrial fission and fusion proteins dictates the mitochondrial architecture, which, in turn, impacts on mitochondrial function.

Mitochondrial transport and bioenergetics

Mitochondria utilize microtubules and their associated motor proteins dynamin and kinesin to disperse within the cell. The size of mitochondria as determined by the equilibrium between fission and fusion influences mitochondrial transport to a significant degree, with either extreme mitochondrial fission or fusion capable of stalling mitochondrial transport in neurons (Zanna et al., 2008; Misko et al., 2012; Chapman et al., 2013; Baloh, 2008; Barbosa et al., 2014; DuBoff et al., 2013; Sheng, 2014). Neurons are especially sensitive to perturbations in mitochondrial transport given the length and complexity of their axons and dendrites. As such, aberrant mitochondrial transport results in deficits in ATP production, which is important for neurotransmitter synthesis, vesicular recycling and maintenance of the membrane potential (Birsá et al., 2013; Brodin et al., 1999; Saxton and Hollenbeck, 2012). Mitochondrial transport along microtubules is a Ca^{2+} -sensitive process; here, the mitochondrial adaptor proteins of the Miro family serve as Ca^{2+} sensors that regulate the interaction of mitochondria with members of the trafficking kinesin protein (TRAK)/Milton family of motor adaptors and the motor protein kinesin-1 (Tang, 2015; Lee and Lu, 2014; Lin and Sheng, 2015; Sheng, 2014). The binding of Ca^{2+} to the calmodulin-like EF-hands in Miro proteins inhibits kinesin-mediated transport of mitochondria, which has been suggested to localize mitochondria to active synapses (Sheng, 2014; Lee and Lu, 2014; Liu and Hajnóczky, 2009).

Neurons are excitable cells and, therefore, require the ability to maintain large ionic gradients across the plasma membrane. This is mostly accomplished through the activity of the plasmalemma Na^+/K^+ -ATPase and requires the sustained production of high levels of ATP, which – in neurons – is provided almost exclusively through oxidative phosphorylation at the IMM. Mitochondrial fission and fusion shape ATP supply in neurons in multiple ways. Mitochondrial fusion increases the mitochondrial membrane potential in neurons (Dickey and Strack, 2011) and the ability to maintain ATP levels in response to hypoxia (Khacho et al., 2014; Mishra and Chan, 2016; Schrepfer and Scorrano, 2016); presumably, this is a result of improved efficiency of oxidative phosphorylation (Westermann, 2012). Conversely, enhancing mitochondrial fission in neurons depolarizes mitochondria (Dickey and Strack, 2011) and has been associated with decreased levels of cellular ATP (Ju et al., 2007). Mitochondrial Ca^{2+} uptake is an electrophoretic process and, thus, requires a negative mitochondrial membrane potential while also being regulated by the mitochondrial Ca^{2+} uniporter (MCU) (Niescier et al., 2013). Interestingly, mitochondrial Ca^{2+} uptake can enhance ATP production by stimulating mitochondrial dehydrogenases and transporters that fuel the Krebs cycle (Rueda et al., 2016; Glancy and Balaban, 2012). Furthermore, Ca^{2+} -mediated regulation of mitochondrial transport and bioenergetics has been suggested to position mitochondria in an activity-dependent manner, matching ATP production with demand. In this scenario, mitochondria detach

from microtubules when they encounter a region of elevated Ca^{2+} , such as an active synaptic bouton. Here, the now stationary mitochondria help return Ca^{2+} to baseline levels by directly taking up Ca^{2+} and by providing ATP for the pumps that either extrude Ca^{2+} or shunt Ca^{2+} into the endoplasmic reticulum (Zündorf and Reiser, 2011). Mitochondrial Ca^{2+} uptake further boosts ATP synthesis, which is necessary for high-frequency neurotransmitter release (Verstreken et al., 2005; Sheng, 2014; Rangaraju et al., 2014; Stephen et al., 2015).

In the process of supplying ATP, mitochondria – through the electron transport chain – generate reactive oxygen species (ROS), which mediate oxidative stress. Precipitous mitochondrial fission or fragmentation has been observed to accompany ROS production and oxidative damage in response to a variety of neuronal insults (Yu et al., 2006; Cho et al., 2012; Ebenezer et al., 2010; Gan et al., 2014; Grohm et al., 2010). However, mitochondria-derived ROS have also been shown to act as a homeostatic signaling molecule in various physiological processes, including synaptic transmission (Shadel and Horvath, 2015; Hamanaka and Chandel, 2010). During synaptic transmission, the mitochondrial ATP generation produces ROS, which can regulate the strength of synaptic transmission. Specifically, it was shown that mitochondria-derived ROS selectively recruit $\alpha 3$ subunit-containing GABA_A receptors to inhibitory synapses in order to increase the frequency and amplitude of inhibitory postsynaptic currents (IPSCs) in cerebellar stellate neurons (Accardi et al., 2014). Future studies are required to determine how mitochondrial dynamics impact on ROS-mediated regulation of synaptic transmission. Although mitochondrial dynamics serve important physiological roles in providing ATP and regulating Ca^{2+} and ROS signaling in neurons, there is also a strong body of evidence suggesting that aberrant mitochondrial dynamics contribute to neurological disease through these processes as well.

Mitochondrial fission in cerebral ischemia

Mitochondrial fragmentation is observed in mice following middle cerebral artery occlusion (MCAO) and reperfusion *in vivo* (Barsoum et al., 2006). *In vitro* models of cerebral ischemia confirm these findings, as glutamate excitotoxicity (Kumari et al., 2012; Niizuma et al., 2010) and hypoxia (Sanderson et al., 2015) on their own can induce mitochondrial fragmentation. The relationship between mitochondrial fission and ischemic injury appears more than correlative, as inhibition of Drp1 with the small-molecule inhibitor mdivi-1 was shown by several groups to decrease infarct volume following MCAO (Grohm et al., 2012; Li et al., 2015; Zhang et al., 2013). However, a number of surprising observations question the interpretation that inhibition of Drp1 protects neurons from ischemic death by preventing mitochondrial fission. First, mdivi-1 has several off-target effects, including blocking of a delayed-rectifying K^+ channel at the plasma membrane and inhibition of mitochondrial outer membrane permeabilization in a Drp1-independent manner (Rosdah et al., 2016; Kushnareva et al., 2012; So et al., 2012). Second, glutamate excitotoxicity causes mitochondrial fission through a Drp1-independent mechanism in primary hippocampal cultures (Young et al., 2010). Furthermore, inhibition of calcineurin-mediated activation of Drp1 and mitochondrial fission prior to deprivation of oxygen and glucose improves neuronal survival but does not prevent mitochondrial fission during and following injury (Slupe et al., 2013). If inhibition of Drp1 does not prevent ischemia-induced mitochondrial fission, how does it mediate neuroprotection? Mitochondrial fission is thought to sensitize neurons to insults, such as oxidative stress and excitotoxicity, because the subsequent decrease in the ability of the

fragmented mitochondria to produce ATP ultimately impairs their capability to detoxify excess ROS and sequester or extrude intracellular Ca^{2+} (Reddy et al., 2011). In support of this, knockdown of MCU prevents NMDA-mediated excitotoxicity induced mitochondrial depolarization and improves neuron survival (Qiu et al., 2013). Similarly, promotion of mitochondrial elongation prior to injury through inhibition of Drp1 could improve neuronal survival as it increases mitochondrial membrane potential and, in turn, bioenergetic capacity, helping neurons weather an ischemic energy crisis (Fig. 3).

Mitochondrial dynamics in neurodegenerative diseases

Among neurological disorders, mitochondrial fragmentation is not unique to cerebral ischemia and excessive mitochondrial fission has been described in most major neurodegenerative disorders. Indeed, fragmentation of the mitochondrial network has been observed in AD and HD patients; in both cases, fragmentation is thought to depend on increased mitochondrial localization and fission activity of Drp1 (Cho et al., 2010; Haun et al., 2013). Amyloid plaques composed of $\text{A}\beta$ -peptides and neurofibrillary tangles containing hyper-phosphorylated Tau are central to the pathology of AD, and both $\text{A}\beta$ and Tau have been linked to mitochondrial fission. Degradation of $\text{A}\beta$ in a cell line overexpressing amyloid precursor protein (APP) prevents mitochondrial fragmentation (Wang et al., 2008). Conversely, introduction of $\text{A}\beta$ into a neuroblastoma cell line causes mitochondrial fission (Manczak et al., 2010). Additionally, a direct interaction between Drp1 and $\text{A}\beta$, as well as evidence for increased mitochondrial fission, was observed in samples of human AD patients; here, increased association of Drp1 with $\text{A}\beta$ was indicative of disease progression (Manczak et al., 2011). Drp1 has also been shown to interact with hyper-phosphorylated Tau, which promotes the GTPase activity of Drp1 and mitochondrial fission in

samples of human AD patient (Manczak and Reddy, 2012). However, another group reported that expression of human Tau in mouse neurons impairs mitochondrial fission through an actin-dependent mechanism that disrupts Drp1 localization to mitochondria and contributes to neurodegeneration (DuBoff et al., 2012). Despite these conflicting findings, the available evidence clearly points to an imbalance between mitochondrial fission and fusion in AD. In contrast to AD, the general consensus in the case of HD is that increased mitochondrial fission is associated with – and perhaps contributes to the pathogenesis of – the disease. According to two independent reports for which post-mortem brain samples from HD patients were analyzed, Drp1 interacts with mutant huntingtin (Song et al., 2011; Shirendeb et al., 2012). Moreover, Drp1 GTPase activity is increased and mitochondria are fragmented in brain samples of HD patients, suggesting that mutant huntingtin also promotes activation of Drp1 (Song et al., 2011; Shirendeb et al., 2012). In support of a pathological role for mitochondrial fission in HD, inhibition of Drp1 with a cell-permeable peptide inhibitor (p110-TAT) was shown to improve mitochondrial function and neuronal survival in induced pluripotent stem cell (iPSC)-derived neurons from HD patients (Guo et al., 2013b). In addition to AD and HD, increased Drp1 activity and mitochondrial fission have also been observed in Parkinson's disease (PD). Dominant-negative inhibition of Drp1 in a *PINK1*^{-/-} mouse-knockout model of PD, as well as in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinsonism in mice, prevented neuronal death and rescued the impaired dopamine release that is associated with PD (Rappold et al., 2014). More recent work on the MPTP mouse model of PD supports these findings, as p110-TAT-peptide-mediated inhibition of Drp1 localization to mitochondria attenuated the death of dopaminergic neurons (Filichia et al., 2016).

While excessive mitochondrial fission is a common feature among neurodegenerative disorders, excessive mitochondrial fusion can be just as detrimental. Impaired mitochondrial fission has been observed in autosomal recessive spastic ataxia of Charlevoix–Saguenay (ARSACS), a neurodegenerative disease characterized by early-onset cerebellar ataxia and spasticity (Bouhhal et al., 2011). ARSACS results from loss-of-function mutations in the gene encoding saccin, a huge protein thought to function as a Hsp70 co-chaperone (Parfitt et al., 2009). Saccin localizes to mitochondria where it interacts with Drp1 and might be involved in the assembly of higher-order complexes containing the fission enzyme. Consistent with this notion, fibroblasts from ARSACS patients exhibit severe hyperfusion of the mitochondrial network, which is recapitulated by knockdown of saccin in neurons (Girard et al., 2012). Additionally, fibroblasts from ARSACS patients and fibroblasts in which saccin had been knocked down both show a decrease in the formation of Drp1 foci at mitochondria, suggesting that saccin stabilizes active fission complexes at mitochondria (Bradshaw et al., 2016). In ARSACS patients, impaired mitochondrial fission may interfere with mitochondrial transport to distal neurites. Alternatively or additionally, impaired fission may disrupt mitophagy, a process necessary for the removal of damaged mitochondria; this ultimately leads to increased oxidative stress and neuronal degeneration. As described above, the latter mechanism has also been proposed to be involved in familial forms of PD that are caused by mutations in PINK1 and Parkin (Youle and van der Bliek, 2012).

Mitochondrial dynamics and nervous system development

In developing neurons, mitochondria have been found concentrated near growth cones, where they can satisfy the high metabolic

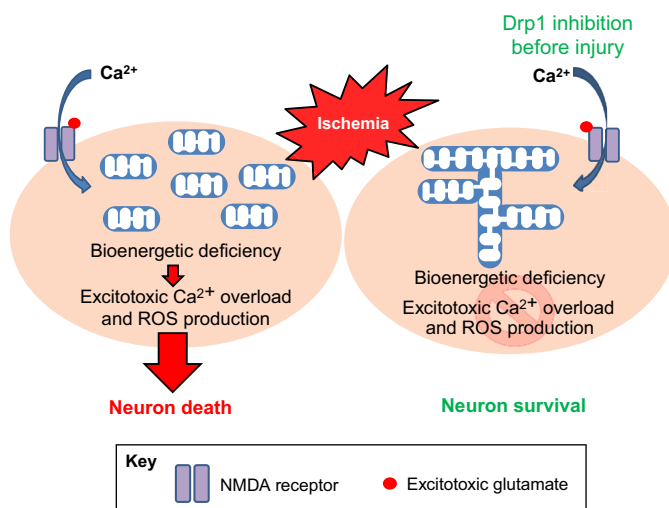


Fig. 3. Neuroprotective effects of inhibiting mitochondrial fission through inhibition of Drp1. During cerebral ischemia, mitochondrial fission occurs and has been proposed to contribute to ischemic injury as inhibition of Drp1; thus, mitochondrial fission attenuates neuron death. However, inhibition of Drp1 does not prevent hypoxia or glutamate-mediated mitochondrial fission, suggesting the protective effect of Drp1 inhibition is due to increased connectivity of the mitochondrial network prior to ischemic insult. Essentially, the promotion of mitochondrial fusion by inhibiting Drp1 improves the bioenergetic capacity of mitochondria, which in turn prevents bioenergetic deficiency and collapse of ionic homeostasis that is normally observed during glutamate excitotoxicity and hypoxia. Here, the ability to maintain ionic homeostasis until reperfusion is presumed to prevent neuron death.

requirements of these motile structures (Morris and Hollenbeck, 1993). In addition to providing ATP, mitochondria also regulate intracellular Ca^{2+} dynamics, which, in turn, strongly influences growth cone extension and collapse (Bolsover, 2005; Kaczmarek et al., 2012). Interestingly, mitochondrial fission/fusion appears to influence the decision making of growth cones with regard to their directionality. Driving mitochondrial elongation by pharmacological inhibition of Drp1, or Mfn2 overexpression *in vitro* enabled the crossing of growth cones in rat retinal ganglion cells (RGCs) into stripes of inhibitory growth factors at the expense of crossing into stripes of permissive factors (Steketee et al., 2012). These results highlight the important role of mitochondrial dynamics in local signaling responses to growth factors that, in turn, influence growth cone development and synapse formation.

In addition to exerting important roles in presynaptic development, mitochondrial dynamics also contribute to the development of postsynaptic dendritic spines. Activity-dependent transport of mitochondria to dendrites is required for the maintenance of dendritic spines in cultured hippocampal neurons (Li et al., 2004). Furthermore, increasing mitochondrial fragmentation by overexpression of Drp1 enhances synapse formation, whereas dominant-negative inhibition of Drp1 has the opposite effect in cultured hippocampal neurons, indicating a prominent role for mitochondrial dynamics in synaptogenesis (Dickey and Strack, 2011; Li et al., 2004) (Fig. 4). In support of an importance of Drp1 for synapse development, brain-specific Drp1-KO mice die shortly after birth due to impaired forebrain development and synapse formation that likely is a result of mitochondrial aggregation, their impaired transport and defective mitophagy in neurons (Ishihara et al., 2009; Wakabayashi et al., 2009). Two recent studies that use mice with postnatal Drp1 deletion in forebrain neurons support these findings; the mice exhibited impaired mitochondrial ATP production, hippocampal atrophy, defects in synaptic transmission, as well as deficits in learning and memory (Shields et al., 2015; Ottinghaus et al., 2016).

Typically, dendritic arborization and dendritic spine formation are positively correlated. However, in contrast to the increase in dendritic spine formation associated with mitochondrial fragmentation in cultured hippocampal neurons, overexpression of Drp1 and mitochondrial fragmentation actually stunt dendritic arborization, with the opposite being the case when Drp1 is inhibited (Dickey and Strack, 2011). This uncoupling of dendritic outgrowth and dendritic spine formation may be explained by the effects of mitochondrial morphology on mitochondrial bioenergetics. Hyperpolarization of mitochondria by using L-carnitine phenocopies the augmented dendritic arborization and decrease in dendritic spine formation that is observed when inhibiting Drp1; however, L-carnitine does so without altering mitochondrial morphology. Interestingly, inhibition of Drp1 and mitochondrial elongation increases the mitochondrial membrane potential, whereas mitochondrial fission decreases (depolarizes) mitochondrial membrane potential in cultured hippocampal neurons (Dickey and Strack, 2011). Perhaps an increase of mitochondrial fusion and mitochondrial membrane potential would decrease cytosolic Ca^{2+} levels – through enhanced mitochondrial Ca^{2+} uptake – ultimately impairing dendritic spine formation (Fig. 4B). However, at the same time, given the increase in membrane potential and Ca^{2+} buffering, ATP production would likely be improved which would allow for greater dendritic arborization.

In addition to the morphology of existing mitochondria, mitochondrial biogenesis also contributes to the development of the nervous system. Mitochondrial biogenesis serves to increase mitochondrial mass, which is a necessary checkpoint for initiating neuronal differentiation and development (Agostini et al., 2016). Moreover, the stimulation of dendritic spine development by brain-derived neurotrophic factor (BDNF) is, at least partly, dependent upon peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PPARGC1A, hereafter referred to as PGC1- α), a master transcriptional regulator of mitochondrial biogenesis (Cheng et al., 2012). Accordingly, knockdown of PGC1- α inhibits, whereas

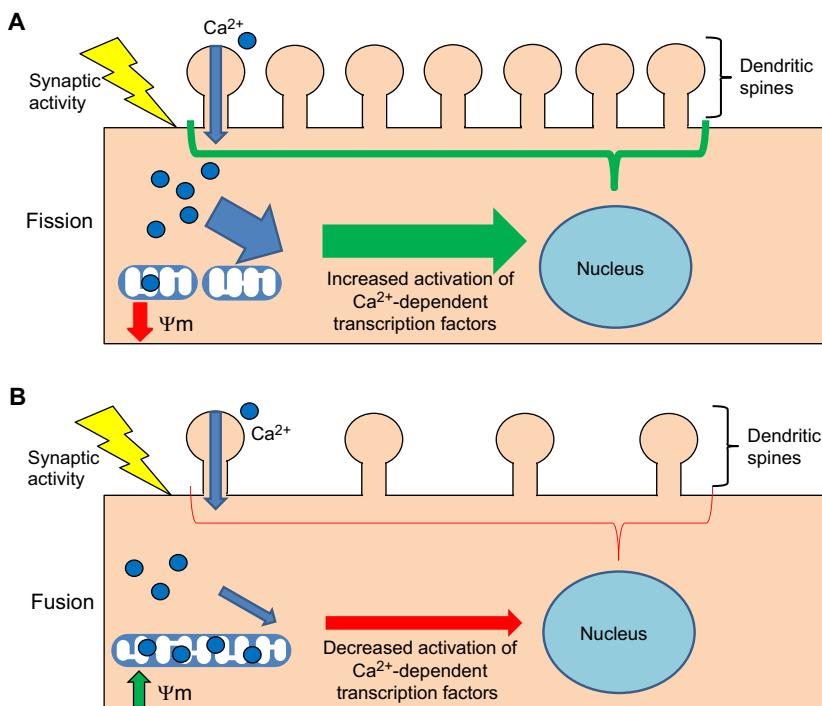


Fig. 4. Mitochondrial fission and fusion impact on dendritic spine development. Promotion of mitochondrial fusion or fission, respectively, increases or decreases the mitochondrial membrane potential (Ψ_m), indicated by small green or red arrow, respectively. In turn, an increase or decrease of Ψ_m presumably increases or decreases mitochondrial Ca^{2+} buffering, respectively. (A) Reduced mitochondrial Ca^{2+} buffering as a result of mitochondrial fission likely increases cytosolic Ca^{2+} levels (thick blue arrow), thereby augmenting dendritic spine development (bold green bracket) through activation of Ca^{2+} -sensitive transcriptional reprogramming. (B) In contrast, increased mitochondrial Ca^{2+} buffering as a result of fusion likely decreases cytosolic Ca^{2+} concentration (thin blue arrow), thereby impairing synaptic activity-dependent dendritic spine development (thin red bracket) through decreased activation of Ca^{2+} -dependent transcriptional programs (thin red arrow).

overexpression of PGC1- α increases mitochondrial biogenesis and dendritic spine formation (Cheng et al., 2012).

Mitochondrial dynamics in synaptic transmission and plasticity

Besides regulating the development of the nervous system, mitochondrial dynamics retain an active role in synaptic function and plasticity within mature neurons. In lamprey, frequently firing tonic dorsal column synapses have approximately twice the number of mitochondria as infrequently firing phasic reticulospinal synapses (Brodin et al., 1999). Additionally, mitochondria isolated from lamprey tonic synapses convert glutamine to glutamate more efficiently than mitochondria from phasic synapses (Shupliakov et al., 1995). At the *Drosophila* neuromuscular junction (NMJ), synaptic potentiation induced by tetanic stimulation, i.e. post-tetanic potentiation (PTP) of motor nerves increases transport of mitochondria to synaptic terminals (Tong, 2007). Accordingly, inhibition of mitochondrial ATP production with the complex I inhibitor rotenone interfered with synaptic accumulation of mitochondria and PTP. By contrast, boosting mitochondrial ATP production in motor axons by genetic methods increased PTP (Tong, 2007). Further illustrating the importance of synaptic localization of mitochondria in short-term synaptic plasticity, Drp1 loss-of-function mutations in *Drosophila* depleted mitochondria from motor nerve terminals and inhibited mobilization of the reserve pool of synaptic vesicles (Verstreken et al., 2005) (Fig. 5). The resulting transmission failure at high-stimulation frequencies could be rescued by application of exogenous ATP, suggesting that, in the absence of mitochondria, ATP production is insufficient to power the processes required for high-frequency synaptic transmission (Verstreken et al., 2005).

In addition to mitochondrial ATP production, mitochondrial Ca^{2+} buffering is also important in PTP. For instance, PTP at the crayfish NMJ can be blocked by agents that inhibit mitochondrial Ca^{2+} uptake and, therefore, appears to depend on a slow and sustained release of Ca^{2+} from mitochondria (Fig. 5) (Tang and Zucker,

1997). Furthermore, at the calyx of Held, the largest synapse in the mammalian nervous system, Ca^{2+} sequestration by presynaptic mitochondria accelerates recovery from presynaptic depression following bursts of moderate stimulation (Billups and Forsythe, 2002). In both studies, inhibitors of Ca^{2+} uptake and release from the ER had no effect on short-term synaptic plasticity, suggesting mitochondria are the predominantly Ca^{2+} buffering reservoirs at presynaptic sites.

Postsynaptic mitochondria are thought to contribute to synaptic plasticity in a manner similar to that in presynaptic mitochondria. Mitochondria are found in the dendritic shaft, but are notably absent from most dendritic spines, the sites of excitatory input in mammalian neurons. Mirroring activity-dependent presynaptic capture of mitochondria, electrical stimulation of cultured hippocampal cultures triggers the transport of mitochondria to active dendritic areas (Li et al., 2004). Also, Drp1-dependent mitochondrial fission is necessary for dendritic localization of mitochondria (Fig. 5), as well as spine formation and synaptogenesis in hippocampal cultures (Li et al., 2004; Dickey and Strack, 2011). Ca^{2+} uptake by postsynaptic mitochondria has been shown to be necessary for long-term potentiation (LTP) of nociceptive inputs to the spinal cord (Fig. 5), which is thought to underlie the development of chronic pain. In mice, depolarizing mitochondria or blocking the MCU interfered with N-methyl-D-aspartate (NMDA)-induced Ca^{2+} rises in mitochondria but not in the cytosol, therefore attenuating spinal LTP and mechanical hyperalgesia following injury (Kim et al., 2011).

Mitochondrial Ca^{2+} uptake has also been implicated in LTP within the hippocampus (Fig. 5). Indeed, inducing hippocampal LTP following high-frequency stimulation of the performant path increases mitochondrial Ca^{2+} uptake in the dentate gyrus as detected by $^{45}\text{Ca}^{2+}$ (Stanton and Schanne, 1986). Mitochondrial ATP production also clearly plays a role in LTP in the CA1 region of the hippocampus, as low doses of rotenone inhibit LTP induced by high frequency stimulation of the Schaffer collaterals – axonal collaterals projecting from the CA3 region to the CA1 region –

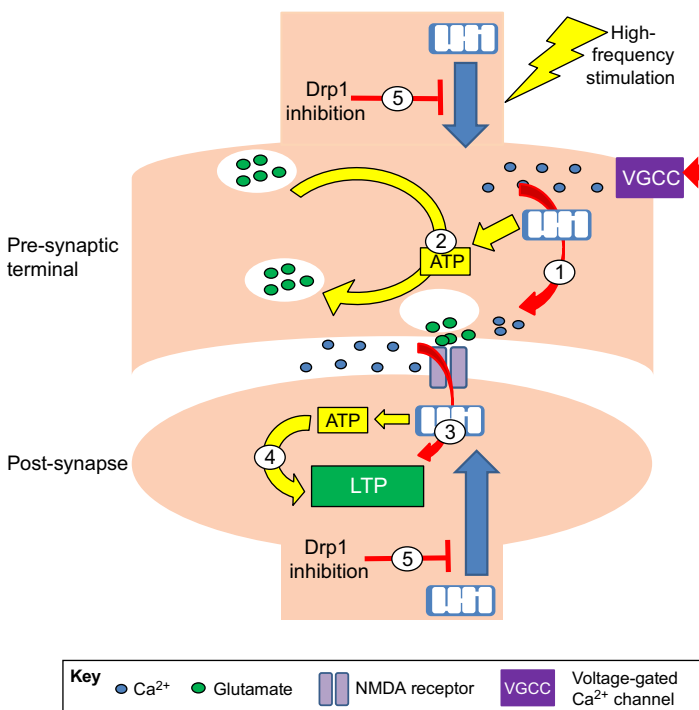


Fig. 5. Mitochondrial dynamics in synaptic transmission.

Multiple mitochondrial functions are necessary for correct synaptic function. (1) Presynaptic mitochondrial Ca^{2+} buffering during high frequency stimulation allows for controlled and sustained release of Ca^{2+} , thereby improving the release efficiency of neurotransmitters (e.g. glutamate). (2) Presynaptic mitochondrial ATP production supports synaptic vesicle recycling and mobilization of the reserve vesicle pool, both of which allow for sustained neurotransmitter release during high frequency stimulation. (3) Postsynaptic mitochondrial Ca^{2+} buffering is necessary to induce LTP in the hippocampus and the spinal cord. (4) Postsynaptic mitochondrial ATP production is also important to induce LTP, given that mitochondrial ATP production is inhibited in response to rotenone, thereby impairing hippocampal LTP during high frequency stimulation. (5) Inhibition of Drp1 and mitochondrial fission impairs activity-dependent transport of mitochondria to synapses both pre- and postsynaptically. However, it is important to note that multiple studies suggest that terminals and spines that lack mitochondria are still capable of maintaining basal synaptic transmission.

without affecting basal transmission (Kimura et al., 2012). A more recent study extends these findings to LTP and long-term depression (LTD) at the cortico-striatal synapse and, furthermore, shows that rotenone-induced deficits of synaptic plasticity can be rescued by antioxidants (Martella et al., 2016).

Mitochondrial fission and fusion likely impact on pre- and postsynaptic activity by influencing mitochondrial ATP production and Ca²⁺ cycling, as well as transport of mitochondria to these sites. However, whether localization of mitochondria to synaptic boutons and to the vicinity of dendritic spines is necessary to fulfill the local requirements for ATP synthesis and Ca²⁺ handling remains a topic of debate. Arguing against an obligate role for the presence of mitochondria near the active zone, serial section transmission electron microscopy (ssTEM) revealed that ~50% of the Schaffer collateral terminals in the CA1 region of the rat hippocampus do not have any mitochondria (Shepherd and Harris, 1998). Moreover, *Drosophila* expressing hypomorphic Drp1 mutants, which prevent axonal transport of mitochondria to the NMJ, exhibit only impaired neurotransmission during intense stimulation (Verstreken et al., 2005). More recently, hippocampal neurons cultured from Drp1 KO mice were shown to have ATP deficits specifically at nerve terminals, resulting in impaired synaptic vesicle cycling. Whereas Drp1 KO neurons had fewer axonal mitochondria, the deficits in ATP were similar among the synaptic boutons with or without mitochondria, indicating that an intrinsic bioenergetic deficit, rather than mislocalization of mitochondria, accounts for impaired neurotransmission in the Drp1 KO mice (Shields et al., 2015). Although rapid diffusion of ATP and spatiotemporal energy buffers, such as the phosphocreatine shuttle (Andres et al., 2008; Linton et al., 2010), can obviate the need for local ATP production by mitochondria, Ca²⁺ cycling and the production of ROS, and other short-lived metabolites might, nevertheless, depend to a greater extent on the precise localization of the organelle.

Concluding remarks

Mitochondrial dynamics is precisely regulated and likely influences every aspect of mitochondrial function. Because neurons have huge metabolic demands and cannot easily be replaced, these cells are especially sensitive to perturbations of the mitochondrial fission/fusion equilibrium and, indeed, mutations in the widely expressed mitochondria-shaping enzymes, such as Drp1, the mitofusins or Opa1, largely cause neurologic symptoms. Excessive mitochondrial fission or fusion can promote neuronal death and synaptic dysfunction, highlighting the delicate balance between these processes that must be maintained for optimal function. However, the complex regulation of mitochondrial dynamics provides many therapeutic targets for the treatment of cerebral ischemia, traumatic brain injury and other neurological disorders. Drp1 has been put forward as a drug target for preventing mitochondrial fragmentation under diverse pathological conditions (Rosdah et al., 2016). Indeed, currently available Drp1 inhibitors have shown neuroprotective properties in animal models of HD (Guo et al., 2013b), PD (Hatch et al., 2014; Filichia et al., 2016) and ischemic stroke (Grohm et al., 2012; Li et al., 2015; Zhang et al., 2013). However, given the widespread expression of the fission enzyme and the detrimental consequences on the development and function of the nervous system upon deletion of Drp1, targeting neuron-specific regulators of Drp1 or other components of the mitochondrial fission/fusion machinery are potentially safer therapeutic strategies. Development of such therapies will require a sustained effort from the research community that is aimed to better understand the cellular signaling mechanisms that impact on mitochondrial form and function.

Competing interests

The authors declare no competing or financial interests.

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