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Regulation and roles of mitophagy at synapses

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ABSTRACT

Maintenance of synaptic homeostasis is a challenging task, due to the intricate spatial organization and intense activity of synapses. Typically, synapses are located far away from the neuronal cell body, where they orchestrate neuronal signalling and communication, through neurotransmitter release. Stationary mitochondria provide energy required for synaptic vesicle cycling, and preserve ionic balance by buffering intercellular calcium at synapses. Thus, synaptic homeostasis is critically dependent on proper mitochondrial function. Indeed, defective mitochondrial metabolism is a common feature of several neurodegenerative and psychiatric disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), bipolar disorders and schizophrenia among others, which are also accompanied by excessive synaptic abnormalities. Specialized and compartmentalized quality control mechanisms have evolved to restore and maintain synaptic energy metabolism. Here, we survey recent advances towards the elucidation of the pivotal role of mitochondria in neurotransmission and implicating mitophagy in the maintenance of synaptic homeostasis during ageing.

1. Introduction

Neurons communicate with each other and/or different cell types, such as muscle cells, through specialized junctions, known as synapses. Synaptic information is transmitted by the release of synaptic vesicles filled with neurotransmitters (Rancz et al., 2007; Sudhof, 2013). Synaptic transmission influences neuronal development, connectivity, plasticity and subsequently cognitive function. Excitatory synapses depend heavily on energy metabolism, to facilitate neurotransmission and preserve ionic balance, by regulating membrane sodium and calcium pumps that rapidly restore ion gradients upon synapse stimulation (Alle et al., 2009; Harris et al., 2012; Rangaraju et al., 2014). Thus, synaptic homeostasis depends on constant availability of functional proteins required for energy metabolism.

Compared to most other cell types, neurons maintain a large number of mitochondria, give the high energy demands needed for preserving neuronal circuitry homeostasis and function (Garcia et al., 2019; Smith et al., 2016). Notably, neurons have a unique cellular architecture, characterized by small cell body volume, long axons and multiple dendritic branches. Therefore, cells have developed specialized molecular mechanisms for efficient distribution of mitochondria to distal neuronal parts, such as growth cones, Ravier nodes, pre- and post-synaptic endings, where high energy is required to maintain neuronal activity. In addition to energy generation, mitochondria also regulate intracellular ion homeostasis through cytoplasmic calcium buffering. Hence, proper mitochondrial activity is essential for neuronal function and maintenance of brain metabolism (Camandola and Mattson, 2017). Indeed, mitochondrial defects and energy supply insufficiency could promote synaptic impairment and axonal degeneration leading subsequently to compromised neuronal function and cognitive deficits (Harris et al., 2012).

In this review, we focus on the interplay between energy metabolism and synaptic homeostasis. We first describe the molecular mechanisms of mitochondrial distribution in neuronal cells. Furthermore, we discuss the essential role of mitochondria and mitophagy in synaptic formation and activity. Better understanding of the compartmentalized nature of mitochondrial quality control in neurons is a key requirement to elucidate the pathophysiological mechanisms, as well as to develop novel therapeutic interventions against several neurodegenerative and mental diseases.

2. Mitochondrial trafficking in neuronal processes

Despite their unique architecture and cellular polarization, neuronal metabolic requirements dictate a constant mitochondrial transport to areas with high-energy demands. Surprisingly, the majority of neuronal mitochondria is persistently immobile, known as stationary mitochondria, whereas there is a smaller portion of organelles moving along

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neuronal processes (Misgeld and Schwarz, 2017). Stationary mitochondria are mainly located in specific neuronal compartments, such as pre- and post-synaptic boutons, indicating an additional layer of regulation associated with neuronal homeostasis (Chang and Reynolds, 2006; Lees et al., 2020; Obashi and Okabe, 2013). Indeed, a very recent study demonstrated that mitochondria accrue locally at pre-synaptic terminals leading to increased synapse stability (Lees et al., 2020).

In parallel, specialized molecular mechanisms have developed to transfer mitochondria from soma to distal neuronal compartments and ensure mitochondria docking in particular areas supporting multiple neuronal activities (Boecker et al., 2020; Misgeld et al., 2007). Despite the fact that some organelles are moving bidirectionally (both anterograde and retrograde) along axons, it is demonstrated that neuronal mitochondria could change their direction, pause and/or switch from mobile to docking behaviour (Boecker et al., 2020; Misgeld et al., 2007; Misgeld and Schwarz, 2017; Obashi and Okabe, 2013; Sheng and Cai, 2012; Wehnekamp et al., 2019). Furthermore, the competing processes of fusion and fission that mediate fluctuations in organelle axonal translocation influence mitochondrial mobility (Lewis et al., 2018). The intricate mobility pattern of mitochondria is regulated by anterograde and retrograde motor proteins, docking and anchoring multiprotein complexes (Sheng and Cai, 2012).

Mitochondrial distribution along neuronal processes is governed by cytoskeleton components. Long-range mitochondrial translocation is mainly dependent on microtubule-based motor proteins, which bind mitochondria directly or indirectly via adaptor molecules, and facilitate their transport along microtubule tracks (Barnhart, 2016; Sheng and Cai, 2012). Hence, microtubule polarity and organization drive the long-range mitochondrial transfer from cell body to distal neuronal compartments. Outer mitochondrial membrane Rho GTPase (Miro) and Milton/TRAK adaptor molecule interact with kinesin and dynein motor proteins to facilitate polarized mitochondrial transport (Devine et al., 2016; Glater et al., 2006; Stowers et al., 2002; van Spronsen et al., 2013). Indeed, Miro and Milton deficiency abolished mitochondrial transport to synapses resulting in synaptic deficits in flies (Guo et al., 2005; Stowers et al., 2002).

Short-range distribution and docking of mitochondria in growth cones and nerve terminal is primarily mediated by myosin proteins and actin cytoskeleton Interestingly, myosin 19 is characterized as a novel mitochondria-associated myosin that facilitates actin-based mitochondrial distribution in various cell types, including neurons (Fig. 1A) (Quintero et al., 2009). Furthermore, genetic studies in *Drosophila melanogaster* demonstrated that myosin 5 and 6 interfere with mitochondrial translocation in axons. Notably, knocking down of myosin 5 enhanced mitochondrial velocity promoting both retrograde and anterograde movements, whereas depletion of myosin 6 selectively augmented retrograde transport (Pathak et al., 2010). These results suggest that myosin motor proteins either compete with microtubule-based transport or promote mitochondrial docking along actin microfilament network by diminishing the association between mitochondria and microtubule tracks. Thus, coordinated function of microtubule- and actin-based motor proteins together with docking and anchoring adaptor molecules may contribute to the intricate saltatory motility pattern of mitochondria in neurons.

Recent studies have shown that mitochondrial motility progressively declines during neuronal development and maturation (Lees et al., 2020; Misgeld and Schwarz, 2017; Wehnekamp et al., 2019). Interestingly, mitochondrial stabilization is not influenced by neuronal activity and plasticity underlining that long-term immobilization of mitochondria in axons, dendrites and pre-synaptic boutons, is a hallmark of neuronal circuit maturation (Faits et al., 2016; Lee et al., 2018a; Lewis et al., 2016; Smit-Rigter et al., 2016). The stationary mitochondrial pool is required to sustain energy generation, cytoplasmic calcium levels and synaptic ion gradient enhancing neurotransmission. Several molecular mechanisms have described to regulate long-term mitochondrial arrest in axonal regions and synaptic terminals. These mechanisms are primarily dependent on the dissociation between mitochondria and kinesin motor proteins, followed by their subsequent interaction with cytoskeleton components (Devine et al., 2016). Elevation of cytoplasmic calcium concentration is the initial signal for mitochondrial arrest. In turn, EF-hand domain of Miro acts as a calcium biosensor inhibiting its assembly with kinesins and thereby promoting mitochondrial release from microtubule tracks (Macaskill et al., 2009). Furthermore, kinesins displacement from microtubule network is suggested to mediate direct binding of their motor domain with Miro leading eventually to mitochondrial capture (Wang and Schwarz, 2009). Syntaphilin was identified as a “static anchor” protein that selectively target axonal mitochondria (Chen and Sheng, 2013; Kang et al., 2008). Syntaphilin deficient mice displayed increased number of motile mitochondria in neuronal processes, whereas its overexpression abolished axonal transport of mitochondria in dorsal root ganglion (DRG) neurons (Zhou et al., 2016). Calcium-dependent recruitment of syntaphilin to axonal mitochondria promotes their immobilization. Interestingly, kinesins dissociate from Miro and subsequently interact with syntaphilin, which inhibits the ATPase activity of kinesins and facilitates mitochondrial displacement and docking (Chen and Sheng, 2013). Hence, syntaphilin could be used as a potential target to modulate mitochondrial mobility and investigate the impact of synaptic activity in the establishment of the stationary mitochondrial pool.

3. Mitochondrial homeostasis and synaptic plasticity

Multiple lines of evidence underscore the pivotal role of mitochondrial metabolism in the regulation of synaptic plasticity and transmission (Cheng et al., 2010; Mattson, 2007; Todorova and Blokland, 2017). Neuronal plasticity is a dynamic process orchestrated by cellular and molecular mechanisms that require gene expression, local protein synthesis, remodelling of cytoskeleton compartments, membrane and organelle trafficking (Greer and Greenberg, 2008; Raefsky and Mattson, 2017).

Given the fact that neuronal plasticity relies on several ATP-dependent cellular processes, including actin cytoskeleton remodelling, generation of membrane potential, synaptic vesicle biogenesis and recruitment, as well as exocytosis-endocytosis cycles among others, mitochondria possess a central regulatory role in multiple aspects of synaptic homeostasis. In addition to their central bioenergetic activities, mitochondria are also involved in the regulation of calcium and redox homeostasis. Therefore, it is not surprising that mitochondrial function is associated with the modulation of synaptic plasticity and neurotransmission. Pharmacological inhibition of mitochondrial metabolism results in impairment of synaptic activity, whereas enhancement of mitochondrial function boosts synaptic density highlighting the crucial role of mitochondria both in synaptic structural alterations and signal transmission (Billups and Forsythe, 2002; Li et al., 2004; Medler and Gleason, 2002; Todorova and Blokland, 2017). Furthermore, inhibition of electron transport chain results in diminished synaptic vesicle release and impaired neuronal plasticity (Ivannikov et al., 2013). Congruently, a very recent study revealed that local mitochondrial depletion led to defective protein synthesis and eventually to synaptic plasticity and spine morphology defects underlining the essential role of stationary mitochondria in synapse homeostasis (Rangaraju et al., 2019).

Genetic studies in *D. melanogaster* uncovered that dynamin-related protein 1 (DRP1) deficiency abolished mitochondrial fission and disturbed organelles distribution in neurons leading to subsequent depletion of mitochondrial population from pre-synaptic endings. DRP1 mutant flies failed to sustain normal levels of neurotransmission due to their inability to preserve and re-fill the pool of synaptic vesicles (Verstreken et al., 2005). Interestingly, exogenous supplementation of ATP partially rescued synaptic defects indicating that reserve pool of vesicles relies on mitochondrial ATP production (Verstreken et al., 2005). Thus, impairment of energy metabolism has detrimental effects

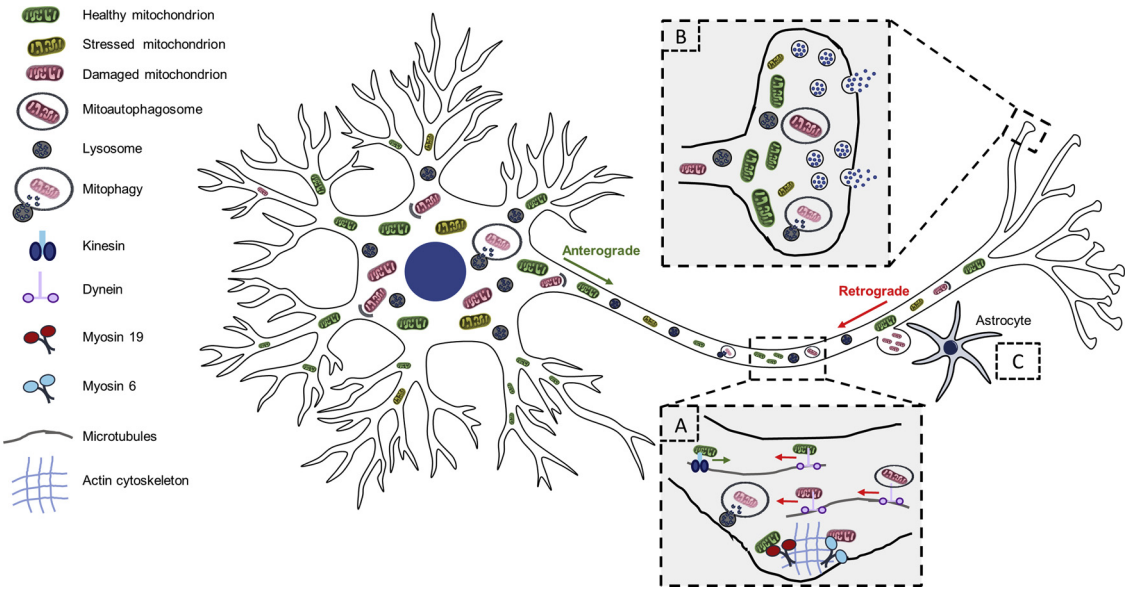

Fig. 1. Compartmentalization of mitophagy in neuronal cells. Neurons contain increased mitochondrial number to cover their energy demands. Mitochondria are transported on microtubule network via kinesin and dynein motor proteins to distal neuronal compartments, such as synapses. Moreover, myosin 19 mediates actin-based mitochondrial distribution. Stressed and damaged organelles and/or mitoautophagosomes perform retrograde movement to be degraded in soma through mitophagy, where mature lysosomes are mainly located. Furthermore, mitophagy might be also taking place distally both in axons and synapses to maintain stationary mitochondria activity (A, B). Myosin 6 traps and isolates damaged organelles in "actin cages" promoting their mitophagic degradation (A). In addition to axonal and synaptic mitophagy, a pool of defective mitochondria can be transcellularly degraded in neighbouring astrocytes (C).

not only in neurotransmission *per se*, but also in the mobilization of synaptic vesicles reserve pool regulating synaptic strength (Fig. 1B).

In addition to energy production, mitochondria affect neurotransmission by modulating cytoplasmic calcium homeostasis (Engelman and MacDermott, 2004). Administration of cyclosporine A, a chemical agent that blocks the mitochondrial permeability transition pore, impaired synaptic plasticity and augmented basal neurotransmission due to alteration of resting calcium levels at pre-synaptic terminals (Cook et al., 2009; Levy et al., 2003). Axonal transport deficits of Miro mutant flies reduced synaptic mitochondrial population and promoted pre-synaptic calcium accrual leading eventually to impaired synaptic transmission (Guo et al., 2005). Congruently, mammalian studies in hippocampal neurons have shown that modulation of mitochondrial mobility and/or activity impairs synaptic signal transmission by regulating calcium signalling (Gazit et al., 2016; Kang et al., 2008). Recently, it was shown that mitochondrial presence at pre-synaptic boutons is associated with decreased calcium concentration that depends on mitochondrial calcium uniporter (MCU) activity (Kwon et al., 2016). Interestingly, LKB1 kinase regulates the expression levels of MCU modulating mitochondrial calcium uptake and subsequently neurotransmitter release. Indeed, overexpression of MCU restored mitochondrial calcium clearance and synaptic defects of LKB1-deficient cells (Kwon et al., 2016). Altogether, these findings suggest the essential role of mitochondrial homeostasis in synaptic plasticity and neurotransmission.

4. Mitophagy in neuronal cells

Several molecular mechanisms have evolved to orchestrate energy metabolism and maintain mitochondrial network integrity and function. Mitochondrial proteases, proteasome system, mitochondrial derived vesicles (MDVs), fusion/fission machinery and the mitochondrial unfolded protein response pathway (mtUPR) are well-studied quality control mechanisms that restore and preserve the most salvageable organelles (Evans and Holzbaur, 2019; Held and Houtkooper, 2015; Palikaras and Tavernarakis, 2014). However, excessive mitochondrial damage promotes mitochondrial network fragmentation through unopposed fission events leading to generation of smaller and isolated organelles. In turn, these damaged mitochondria are eliminated via mitochondrial selective autophagy, known as mitophagy (Palikaras et al., 2018).

Mitophagy is a ubiquitous endomembrane sorting and degradation mechanism that removes dysfunctional and/or superfluous mitochondria and modulates mitochondrial number preserving energy metabolism and cellular viability (Palikaras et al., 2018). The rate of mitochondrial turnover differs between cell types and depends on energy requirements of the biological systems. Recent studies underscore the pivotal contribution of mitophagy in neuronal function and implicate mitophagy deregulation in synaptic homeostasis, as well as in the development and progression of several psychiatric and neurodegenerative disorders (Evans and Holzbaur, 2019; Hou et al., 2019; Lou et al., 2019; Murphy and Hartley, 2018; Palikaras et al., 2018).

The PINK1/Parkin pathway is the most well-studied molecular mechanism that regulates mitochondrial elimination during challenged conditions (Harper et al., 2018; Montava-Garriga and Ganley, 2019; Sekine and Youle, 2018). Furthermore, several studies have demonstrated the existence of PINK1- and Parkin-independent signalling cascades revealing multiple mitochondrial proteins (e.g. FUNDC1, BNIP3, NIX, PHB2) or lipids (e.g. cardiolipin) that serve as mitophagy receptors in response to environmental and/or developmental stimuli (Evans and Holzbaur, 2019; Montava-Garriga and Ganley, 2019; Palikaras et al., 2018). However, the molecular mechanisms of mitophagy were mostly investigated *in vitro* using cell lines that overexpress key mitophagy components following artificial stimulation by general chemical agents. Therefore, multiple aspects regarding the *in vivo* regulation of steady-state mitophagy levels, known as basal mitophagy, remain relatively elusive. The generation of transgenic animals expressing mitophagy reporters, such as mitoKeima and mito-QC, to assess mitochondrial removal improved our understanding about mitophagy levels under physiological and non-stress conditions (McWilliams et al., 2016; Sun et al., 2015). Interestingly, basal mitophagy induction varies not only across tissues, but also between cell types in the same tissue (McWilliams et al., 2016, 2019; Sun et al., 2015). Moreover, genetic studies in flies and rodents uncovered that

endogenous PINK1 is dispensable for basal mitophagy in many high metabolic organs and cell types, including neuronal cells (Lee et al., 2018b; McWilliams et al., 2018). These findings provoked a debate on multiple features of mitophagy execution under normal conditions highlighting also the complex regulatory network of basal mitophagy *in vivo*.

Although several studies demonstrated that mitochondrial damage triggers mitophagy in primary neuronal cells, the spatial regulation of neuronal mitophagy is not well-understood. Parkin-mediated mitophagy is induced upon mitochondrial depolarization in mouse cortical neurons (Cai et al., 2012). Neuronal mitophagy takes place in the somatodentritic compartment of neurons, where mature lysosomes mainly localize, and is accompanied with elevated retrograde transport indicating that dysfunctional mitochondria travel back to the soma for degradation (Fig. 1) (Cai et al., 2012). Congruently, basal mitophagy is also restricted in the cell bodies of Purkinje cells in mito-QC transgenic animals underlining the *in vivo* compartmentalization of neuronal mitophagy (McWilliams et al., 2016). Surprisingly, another study using optogenetic tools to selectively induce mitochondrial damage showed that local mitophagy is triggered in the axons of hippocampal neurons (Ashrafi et al., 2014). Indeed, autophagosomal proteins and axonal lysosomes are recruited on depolarized organelles to facilitate their distal axonal degradation in a PINK1/Parkin dependent manner (Fig. 1A) (Ashrafi et al., 2014). Recently, it was also reported in flies that Parkin deficiency did not promote accumulation of defective organelles in motor axons or neuromuscular junctions; instead, axons contained fewer mitochondria, which were characterized by normal shape, motility pattern and metabolism. However, defects in mitochondrial morphology where restricted to neuronal cell bodies (Sung et al., 2016). Interestingly, syntaphilin is released from axonal mitochondria promoting mitochondrial motility and vesicle generation under mild stress conditions (Lin et al., 2017). These vesicles are different from MDVs, since their formation is independent of Parkin, DRP1 and the autophagic machinery (Evans and Holzbaur, 2020; Lin et al., 2017). Together, these findings support a model where syntaphilin-vesicles are generated in axons under mild stress conditions, whereas PINK1/Parkin-dependent mitophagy is triggered under more severe conditions to eliminate dysfunctional organelles. An alternative model, which could explain the restriction of damaged mitochondria in neuronal cell bodies, could be the existence of a molecular filter that selectively allows only healthy mitochondria to enter neuronal processes and, thereby, limiting the impact of mitochondrial damage. Thus, reported differences in mitophagy compartmentalization might depend on specific neuronal cell type morphology and activity.

Apart from mitochondria and oxidative phosphorylation (OXPHOS) for ATP production, neuronal cells also rely on glycolysis to meet their energy requirements. Glycolysis generates less ATP per cycle but operating at a higher rate. Notably, glycolytic enzymes have an essential role in brain energy metabolism (Yellen, 2018). Genetic studies in *C. elegans* revealed that glycolytic proteins are dynamically localized at pre-synaptic boutons fuelling the synaptic vesicle cycle (Jang et al., 2016). Neurons switch to glycolysis preserving their synaptic function during conditions of increased neuronal stimulation or hypoxic stress (Jang et al., 2016). Moreover, during retinal ganglion cell differentiation, NIX-dependent mitophagy is activated promoting mitochondrial removal and metabolic rewiring towards glycolysis (Esteban-Martinez et al., 2017). These findings indicate mitophagy as central metabolic regulator particularly at intensely active compartments, such as synaptic terminals. Thus, the coordination between the selective removal of damaged mitochondria from synapses, together with the dynamic distribution of glycolytic enzymes might be pivotal to maintain synaptic homeostasis in health and disease.

Neurons utilize multiple surveillance mechanisms to sustain their energy homeostasis. In addition to somatodendritic and axonal mitophagy, a cell non-autonomous cytoprotective mechanism, termed transcellular mitophagy, was found to mediate the removal of defective mitochondria (Fig. 1C) (Davis et al., 2014). Retinal ganglion cells belong to a subset of neurons characterized by very long processes. Interestingly, a pool of axonal mitochondria was transported and degraded in adjacent astrocytes that are located at the optic nerve head (Davis et al., 2014). Transcellular mitophagy suggests that similar mitochondrial degradation events might occur in other neurites with similar morphological and anatomical features, such as motorneurons and nerve cells of superordinate centers that regulate somatomotor, visceromotor and limbic system. The fact that these neuronal cells are more vulnerable to degeneration in several pathological conditions, including PD, amyotrophic lateral sclerosis (ALS) and Charcot-Marie-Tooth 2a, underlines the urgent need for redundant mechanisms to regulate the removal of defective mitochondria (Braak et al., 2004; Sasaki et al., 2005; Sau et al., 2011).

5. Molecular insights into mitophagy regulation at synapses

Given the fact that a substantial portion of synaptic area is occupied by mitochondria and the intensive metabolic activity of synapses, there is an urgent need for a local specific mitochondrial maintenance system to sustain stationary mitochondrial pool homeostasis. Thus, synaptic mitophagy might be a critical denominator of neuronal plasticity and circuits preserving energy homeostasis and subsequently synaptic function (Fig. 1B). Although we are just scratching the surface, emerging findings indicate that mitophagy deregulation might cause synaptic failure and neuronal cell death (Evans and Holzbaur, 2019; Lou et al., 2019; Palikaras et al., 2018).

A recent study demonstrated that myosin 6 is a novel regulator of mitophagy. Under normal conditions, cytosolic myosin 6 is localized to the vicinity of intracellular vesicles and at the plasma membrane, whereas stress-induced mitochondrial damage triggers its recruitment to defective organelles. Interestingly, myosin 6 interacts directly with Parkin-generated poly-ubiquitin chains supporting the degradation of dysfunctional organelles. Notably, myosin 6 mediates the formation of actin-based cages around damage mitochondria preventing their reintegration to the healthy mitochondrial network and promoting their isolation (Fig. 1A) (Kruppa et al., 2018). Congruently, the crystal structure of C-terminal cargo-binding domain of myosin 6 revealed its ability to generate complexes with autophagy receptors further supporting its vital role in mitophagy regulation (Hu et al., 2019). Indeed, myosin 6 deficient cells are characterized by increased mitochondrial population, compromised mitochondrial respiration rates, and defective metabolic responses (Kruppa et al., 2018). Considering the well-established function of myosin 6 in synapse formation, retrograde trafficking and neurotransmission, these findings suggest a possible association between mitophagy, synaptic integrity and function (Kneussel and Wagner, 2013). However, the impact of myosin 6 on mitophagy need to be validated *in vivo* and further investigated in neuronal cells using the newly developed mitophagy reporter transgenic animals.

Disrupted in schizophrenia 1 (DISC1) is a critical factor that modulates multiple signalling pathways orchestrating neuronal development and synaptic plasticity. Mutations in DISC1 gene are associated with several mental illnesses, such as schizophrenia, bipolar disorder and depression (Sachs et al., 2005; Thomson et al., 2016). Recent studies revealed that the pleiotropic function of DISC1 depends on its ability to bind and generate multimeric complexes, with a variety of proteins, including GSK3 (glycogen synthase kinase 3), PDE4B (phosphodiesterase 4/phosphodiesterase 4B), APP (amyloid precursor protein), FEZ1 (fasciculation and elongation protein zeta 1), LIS1 (lissencephaly 1), KIF5A and Milton/TRAK, among others, thereby influencing neuronal outgrowth, as well as, dendritic and spine maturation (Tropea et al., 2018). Moreover, DISC1 associates directly with syntaphilin modulating mitochondrial docking in axons upon neuronal stimulation (Park et al., 2016). The spatial localization pattern of DISC1 uncovered its enrichment in the post-synaptic density suggesting its

contribution to synaptic transmission and plasticity (Carlisle et al., 2011; Hayashi-Takagi et al., 2010). Multiple lines of evidence associate the propensity of DISC1 to self-assemble and form multimeric ordered aggregates with the development of psychological conditions (Atkin et al., 2012; Leliveld et al., 2008, Leliveld et al., 2009). Indeed, increased levels of insoluble DISC1 aggregates were found in post-mortem brain samples from patients suffering from schizophrenia or mood disorders (Leliveld et al., 2008). In addition to DISC1-dependent mitochondrial transport regulation in neuronal processes, the involvement of DISC1 is also appreciated in mitochondrial activity (Millar et al., 2005; Norkett et al., 2017; Ogawa et al., 2014; Park et al., 2010; Pinero-Martos et al., 2016; Taya et al., 2007). Further supporting the role of DISC1 in energy metabolism, a very recent study found that DISC1 is a potent modulator of mitophagy (Wang et al., 2019). Notably, DISC1 overexpression promotes mitochondrial clearance through a direct interaction between its LIR (LC3-interacting region) motif with the LC3 autophagosomal protein. Although these findings underscore the pivotal role of DISC1 in the regulation of mitochondrial homeostasis, trafficking and synaptic function, it is unclear whether its mitophagic activity *per se* could be associated with the development and progression of mental disorders.

6. Mitophagy impairment and synaptic deterioration in age-related neurodegeneration

Mounting evidence highlights impairment of mitochondrial metabolism and defective mitophagy as hallmarks of ageing and age-associated neurodegeneration (Cummins et al., 2019; Fang et al., 2019b; Lautrup et al., 2019; Lopez-Otin et al., 2013; Lou et al., 2020). Accumulation of damaged organelles due to mitophagy deregulation results in energetic crisis, which subsequently mediate synaptic failure and neuronal degeneration culminating in brain damage and cognitive deficits (Camandola and Mattson, 2017; Evans and Holzbaur, 2020; Palikaras et al., 2018). Interestingly, axonal and synaptic mitochondria are differentially affected with age (Lores-Arnaiz et al., 2016; Stauch et al., 2014). Particularly, synaptic mitochondria display increased calcium buffering and decreased energy production capabilities, whereas axonal mitochondria sustain their bioenergetic capacities (Lores-Arnaiz et al., 2016). Therefore, the maintenance of synaptic mitochondrial homeostasis seems to be a top-class priority to modulate age-dependent synaptic deterioration and cognitive decline.

It is widely reported that synaptic failure is one of the major contributors of AD cognitive and mental deficits (Lamprecht and LeDoux, 2004; Morrison and Baxter, 2012; Scheff et al., 2006; Selkoe, 2002). Recent studies have shown that DISC1 attenuates the generation of amyloid-β (Aβ) plaques implicating its function in AD pathogenesis (Beecham et al., 2009; Deng et al., 2016; Shahani et al., 2015; Young-Pearse et al., 2010). Interestingly, DISC1 hippocampal overexpression triggered mitophagy leading to restoration of mitochondrial metabolism, synaptic plasticity impairment and cognitive defects in the APP/PS1 mouse model, highlighting the importance of DISC1 mitophagic role in AD development and progression (Wang et al., 2019).

Indeed, mitochondrial turnover is diminished in post-mortem hippocampal samples and iPSC (induced pluripotent stem cells) derived from cortical neurons of AD patients, as well as in mouse and nematode AD models (Cummins et al., 2019; Fang et al., 2019b). Pharmacological stimulation of mitophagy ameliorates several AD pathological features, such as mitochondrial dysfunction, Tau and Aβ protein aggregation, neuroinflammation and cognitive decline, indicating that defective execution of mitochondrial degradation is an early event during AD development (Fang et al., 2019b). Supplementation of urolithin A (UA), a natural dietary, microflora-derived metabolite, was shown to induce mitophagy and promotes neuroprotection and lifespan extension in both nematodes and mice (Fang et al., 2019b; Ryu et al., 2016). Moreover, UA-induced mitophagy preserves mitochondrial homeostasis and restores muscle function in mice, nematodes and humans (Andreux et al., 2019; Ryu et al., 2016). In addition to UA, upregulation of NAD^+ intracellular levels were shown to promote mitophagy and neuroprotection in AD and Werner syndrome models (Fang et al., 2019a, b; Hou et al., 2018). Interestingly, treatment with nicotinamide riboside (NR), a precursor NAD^+ molecule, decreased apoptosis, neuroinflammation and restored hippocampal synaptic plasticity in AD mouse model (Hou et al., 2018). Surprisingly, UA and NR treatments upregulate the expression levels of several synaptic genes highlighting their potential synaptogenic abilities (Fang et al., 2019a; b). Although the beneficial effects of UA and NR on cellular and organismal physiology have been shown, the molecular mechanisms of their synaptic function need further investigation.

PD is the second most common neurodegenerative pathology and its primary manifestations are movement disabilities, such as tremor, gait and balance difficulties, and non-motor pathological symptoms, including hyposmia, depression, anxiety and daytime sleepiness among others (Chen et al., 2015; Mazzoni et al., 2012). Several genes associated with familial form of PD are central modulators of synaptic activity and formation, such as a-synuclein and LRRK2 (Dauer and Przedborski, 2003; Polymeropoulos et al., 1997; Soukup et al., 2018). Therefore, it is not surprising that synaptic failure is a pathogenic feature that probably precedes the degeneration of dopaminergic neurons. Many studies have implicated mitochondrial defects in the progressive loss of dopaminergic neuronal cells in PD (Park et al., 2018; Soukup et al., 2018). Although impaired mitochondrial function is characterized in neuronal cell bodies, little is known about mitochondrial function and homeostasis in axonal regions and synapses. Recently, post-mortem brain tissue examination revealed that PD neuronal axons contain more and healthier mitochondria compared to age-matched controls (Reeve et al., 2018). Another interesting observation was the decreased number of mitochondria-empty synapses in PD samples. These findings suggest that surviving dopaminergic neurons enhance mitochondrial trafficking and recycling in an attempt to sustain functional organelles at synapses. Despite the fact that this could be a compensatory mechanism against synaptic loss to enhance neurotransmission, it has also detrimental consequences for cellular viability since oxidative damage is increased due to impaired mitophagy (Soukup et al., 2018). The E3 ubiquitin ligase Parkin is conserved throughout taxa and its high expression levels were detected in mouse, rat and human brain samples (Scuderi et al., 2014). Interestingly, several studies have shown that Parkin is associated with multiple synaptic proteins, including endophilin A, CASK and PICK1 among others, influencing synaptic transmission and plasticity (Cortese et al., 2016; Fallon et al., 2002; Helton et al., 2008; Joch et al., 2007; Kitada et al., 2009; Maraschi et al., 2014; Rial et al., 2014; Trempe et al., 2009). Emerging findings suggest that mitochondrial calcium homeostasis is directly regulated by Parkin activity (Key et al., 2019; Matteucci et al., 2018). Interestingly, Parkin promotes the degradation of MCU1 and MCU2 proteins, which are the core components of the MCU machinery (Matteucci et al., 2018). Moreover, a very recent study identified ATP1A2 (sodium/potassium-transporting ATPases) and HPCA (Hippocalcin) calcium regulators as potential substrates of Parkin in neurons (Key et al., 2019). In addition to Parkin, PINK1 deficiency leads to elevation of cytoplasmic calcium levels through the modulation of MCU activity and thereby mediates neuronal cell death (Gandhi et al., 2009; Marongiu et al., 2009; Soman et al., 2017). Although, PINK1 and Parkin are appreciated as critical modulators of mitochondrial calcium homeostasis and thereby influencing synaptic function, it remains elusive whether these effects are either protein or mitophagy specific.

Mutations in *PINK1/PARK6* and *Parkin/PARK2* genes, which are the central mitophagy regulators, are associated with PD pathogenesis. However, PINK1 and Parkin deficient mice do not display any substantial PD-related phenotype (Goldberg et al., 2003; Kitada et al., 2007; Perez and Palmiter, 2005). Interestingly, an elegant study identified that exhaustive exercise and elevated mtDNA (mitochondrial DNA) damage led to dopaminergic neuronal loss and motor defects in

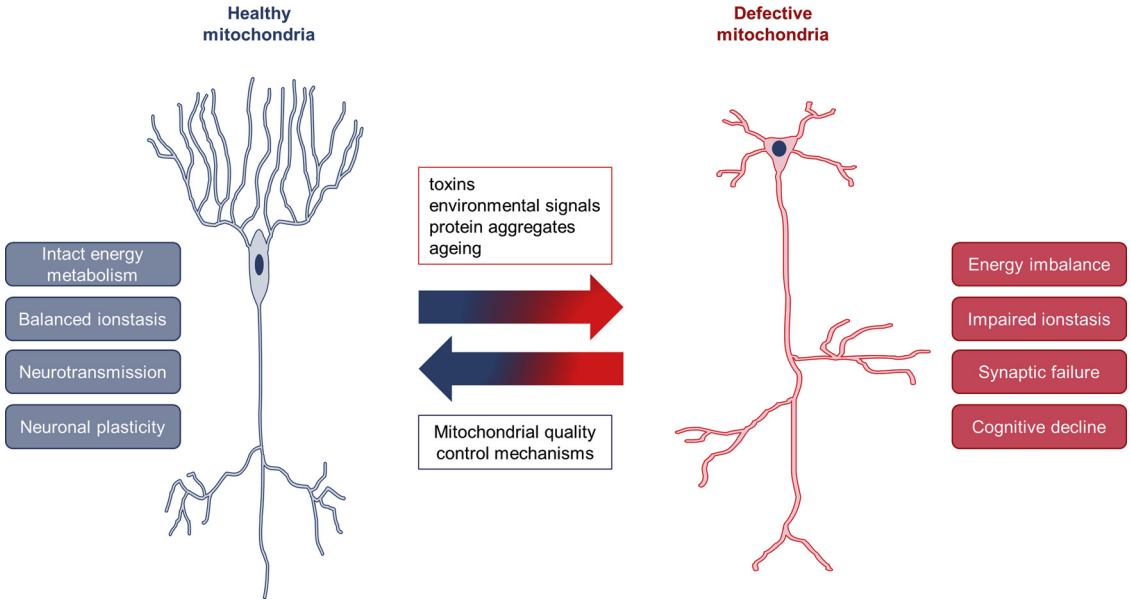

Fig. 2. Defective mitochondrial population influences neuronal homeostasis. A healthy mitochondrial population preserves intact energy metabolism, balanced ionic gradients, synaptic transmission and plasticity promoting neuronal circuit formation and function. Several stress-inducing factors, such as mitochondrial toxins, environmental stimuli, protein aggregates, and ageing, impair mitochondrial function resulting in energetic crisis and ionic imbalance, which subsequently mediate synaptic failure, neuronal degeneration culminating in brain damage and cognitive decline. Stimulation of mitochondrial quality control mechanisms, such as mitophagy, restores mitochondrial homeostasis protecting against deterioration of nervous systems.

PINK1 and Parkin deficient mice via the induction of STING-mediated neuroinflammation (Sliter et al., 2018). These results indicate that excessive organismal stress conditions required for triggering disease pathology in sensitive genetic backgrounds.

Despite the vital role of mitophagy in the maintenance of synaptic plasticity and cognition, very few studies have examined basal or stress-induced mitophagy *per se* on synaptic failure under physiological and pathological conditions. Therefore, future studies should utilize the already developed transgenic animals expressing mitophagy reporters in combination with novel high-resolution imaging techniques to shed lights on the molecular mechanisms of synaptic mitophagy in health and disease.

7. Conclusions

Convergent evidence supports a model whereby mitochondrial homeostasis is tightly associated with synaptic activity and plasticity. Indeed, synapses are vulnerable to oxidative and proteotoxic stress leading to accumulation of protein aggregates and dysfunctional organelles (Nikoletopoulou and Tavernarakis, 2018; Soukup et al., 2018). Hence, the elimination of defective mitochondria from pre- and post-synaptic endings and the maintenance of healthy stationary mitochondrial pool are perquisites for synaptic function (Fig. 2).

The physiological role of mitophagy in neuronal development and connectivity is steadily emerging. However, the role of mitophagy in synaptic homeostasis is relatively a new field in neuroscience. Thus, little is known about mitochondrial activity state, biogenesis and turnover at synapses. Future work should concentrate on *in vivo* mitophagy reporters investigating basal mitophagy levels at synapses in different brain regions or neuronal sub-populations and try to understand the impact of mitophagy in neurodegeneration. Furthermore, important questions remain to be addressed regarding mitochondrial proteostasis at synaptic boutons. Determination of protein synthesis rates and analysis of mitochondrial protein composition will further elucidate the unique features of synaptic mitochondria. The fact that synaptic proteins regulate multiple aspects of mitochondrial biology and their genes are found to be mutated in several neurodegenerative and mental disorders, including AD, PD and schizophrenia among others, underscores mitochondrial and synaptic deficits as early pre-symptomatic features (Lee et al., 2018a; Soukup et al., 2018). Since there is not any effective medication against psychiatric and neurodegenerative pathologies, there is an urgency to identify “early” biomarkers for diagnosis. Therefore, the development of novel therapeutic strategies to modulate synaptic mitophagy in spatiotemporal manner could confer neuroprotection and prevent synaptopathies before irreversible brain damage.

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