Mitochondrial dynamics in astrocytes

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Abstract
Astrocytes exhibit cellular excitability through variations in their intracellular calcium (Ca^{2+}) levels in response to synaptic activity. Astrocyte Ca^{2+} elevations can trigger the release of neuroactive substances that can modulate synaptic transmission and plasticity, hence promoting bidirectional communication with neurons. Intracellular Ca^{2+} dynamics can be regulated by several proteins located in the plasma membrane, within the cytosol and by intracellular organelles such as mitochondria. Spatial dynamics and strategic positioning of mitochondria are important for matching local energy provision and Ca^{2+} buffering requirements to the demands of neuronal signalling. Although relatively unresolved in astrocytes, further understanding the role of mitochondria in astrocytes may reveal more about the complex bidirectional relationship between astrocytes and neurons in health and disease. In the present review, we discuss some recent insights regarding mitochondrial function, transport and turnover in astrocytes and highlight some important questions that remain to be answered.

Introduction

Astrocytes are a subset of glial cells that provide trophic and structural support for neurons. Accumulating evidence over the last few years has revealed that astrocytes are active regulators of information processing in the central nervous system (CNS) [1–5]. At the synaptic level, where astrocyte processes can ensheath the pre- and post-synaptic elements (termed the tripartite synapse), they play many important regulatory roles that include clearance of synaptic transmitters, such as glutamate and ions such as potassium (K^+). Astrocytes integrate neuronal activity by increasing their intracellular calcium (Ca^{2+}) [6,7] levels and this can subsequently lead to the release of neuroactive substances (for example, glutamate, ATP and D-serine) that regulate synaptic communication [3,8–11]. The synaptically evoked astrocyte Ca^{2+} rise occurs in spatially restricted microdomains within astrocyte processes [12] and represents a crucial element of rapid bidirectional signalling between neurons and astrocytes [13,14].

Mitochondria are dynamic organelles highly efficient at producing cellular energy in the form of ATP and buffering intracellular Ca^{2+}. In neurons, they are transported on a rapid timescale and localize at sites of high energy consumption such as the axon initial segment, nodes of Ranvier and the pre- and post-synaptic domains [15–18]. In terms of astrocytes, their positioning could influence intracellular Ca^{2+} levels, which could be important for astrocyte Ca^{2+} signalling, i.e. Ca^{2+} wave propagation and Ca^{2+}-dependent gliotransmission that can have an impact on the signalling of nearby neurons. Mitochondria may also play a role in providing ATP for important astrocytic functions such as the glutamate–glutamine shuttle and ion homoeostasis at the tripartite synapse [19]. Their ability to be rapidly transported makes them ideal candidates for localized ATP provision and Ca^{2+} buffering within astrocytes. However, far less is known about the spatial regulation of mitochondria in astrocytic processes.

In the present paper, we highlight recent key findings regarding Ca^{2+} buffering by mitochondria in astrocytes and draw upon emerging evidence highlighting the importance of mitochondrial trafficking dynamics and positioning within astrocyte processes. We also briefly discuss what is known about mitochondrial turnover in astrocytes.

Mitochondrial calcium buffering in astrocytes

Ca^{2+} signalling is central for the regulation of astrocyte functions and for astrocyte–neuron communication. Mitochondria actively uptake Ca^{2+} via the mitochondrial Ca^{2+} uniporter (MCU), powered by a negative membrane potential and release free Ca^{2+} to the cytosol via the Na^+/Ca^{2+} exchanger (NCX) [12,20–24]. Release of Ca^{2+} from the endoplasmic reticulum (ER) internal stores, via activation of both inositol 1,4,5-trisphosphate receptors (IP_3Rs) and ryanodine/caffeine receptors [25], contributes to an increase in cytosolic Ca^{2+} ($[Ca^{2+}]_c$) in astrocytes. Using fluorescence imaging of cultured astrocytes, Boitier et al. [26] showed that the ER-induced $[Ca^{2+}]_c$ rise was followed by an increase in mitochondrial Ca^{2+} levels. Application of the protonophore carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), which leads to

Key words: calcium, fission, fusion, gliotransmission, mitophagy, trafficking.

Abbreviations: AD, Alzheimer's disease; CNS, central nervous system; Drp1, dynamin-related protein 1; ER, endoplasmic reticulum; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; IP_3R, inositol 1,4,5-trisphosphate receptor; MAM, mitochondria-associated ER membrane; MCU, mitochondrial Ca^{2+} uniporter; mGluR, metabotropic glutamate receptor; NCX, Na^+/Ca^{2+} exchanger; OMM, outer mitochondrial membrane; ONH, optic nerve head; PD, Parkinson's disease; PINK1, phosphatase and tensin homologue deleted on chromosome 10 (PTEN)-induced putative kinase 1; ROS, reactive oxygen species; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase; SOC, store-operated Ca^{2+} channel; TRAK, trafficking protein, kinesin-binding.

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a collapse of the mitochondrial membrane potential and a diminished Ca^{2+} buffering capacity of mitochondria, along with oligomycin (ATP synthase inhibitor), decreased the rate of $[Ca^{2+}]_c$ decay. This suggests that mitochondria play a crucial role in clearance of physiological Ca^{2+} loads, generated by the ER [26]. Moreover, altering mitochondrial Ca^{2+} buffering with FCCP or CGP37157, a mitochondrial NCX blocker, which induces Ca^{2+} accumulation in the mitochondria, resulted in increased or reduced glutamate release by astrocytes respectively [27]. These data suggest that $[Ca^{2+}]_c$ levels are directly correlated with glutamate release and altering the Ca^{2+} buffering capacity of mitochondria can affect this phenomenon [27]. Taken together, these results reveal that the ability of mitochondria to buffer Ca^{2+} in astrocytes is important in terms of both astrocyte functionality and the downstream implications for neural signalling.

Depletion of the internal ER stores in astrocytes results in Ca^{2+} entry from the extracellular space via store-operated Ca^{2+} channels (SOCs) (for example, transient receptor potential channels) [28–31], which triggered significant mitochondrial Ca^{2+} transients. This indicates functional cross-talk between the plasma membrane and mitochondrial domains. In addition, compromised mitochondrial Ca^{2+} buffering, due to NCX silencing, caused reductions in SOC entry, amplitude and rate. These results suggest that mitochondrial Ca^{2+} handling, and in particular NCX activity, is of major importance for the regulation of Ca^{2+} influx into the cell through SOCs [21]. The depleted ER internal stores are subsequently replenished via sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPases (SERCAs) [32]. Simpson and Russell [33] showed that mitochondria are found in close association with high density SERCA-expressing ER sites in cultured astrocytes. One possibility for this close association is that mitochondrial Ca^{2+} uptake via the MCU may regulate Ca^{2+} levels around nearby IP_3Rs. Indeed, Csordas et al. [34] showed using drug-inducible fluorescent inter-organelle linkers that increasing the distance between the ER and the outer mitochondrial membrane (OMM) to $\sim$15 nm enhanced the efficiency of Ca^{2+} transfer from the ER to the mitochondria, in a H9c2 cell line. In contrast, reduction in the mitochondria–ER gap to 5 nm blocks the accommodation of IP_3Rs (shown to protrude $\sim$10 nm from the ER membrane) and thus prevents efficient Ca^{2+} transfer [34,35]. Moreover, several structural and functional studies have revealed zones of close contact between ER and mitochondria called mitochondria-associated ER membranes (MAMs) [36]. MAMs enable highly efficient transmission of Ca^{2+} and lipid exchange from ER to mitochondria [37,38]. However, discerning the components of ER–mitochondria contact sites and regulation of Ca^{2+} and lipid exchange at the MAM contact sites within astrocytes remains to be fully explored.

Mitochondrial trafficking in astrocytic processes

The ability of mitochondria to spatially match energy provision and Ca^{2+} buffering to neuronal demand is an important aspect of neuronal signalling [15,16,18,39,40]. In neurons, mitochondria are mobilized in order to replenish the usable pool of mitochondria in axons and dendrites and to transport damaged mitochondria back to the soma for degradation [17,41]. Although significant progress has been made to elucidate the mechanisms that regulate mitochondrial trafficking dynamics in neurons, far less is known about the mechanisms regulating mitochondrial dynamics in astrocytes.

Recently, Jackson et al. [42] revealed that mitochondria are non-uniformly distributed along the fine processes of astrocytes (<600 nm in diameter) in organotypic slices, confirming previous results from astrocyte cultures [43,44] and *in vivo* [45,46]. Their trafficking was bidirectional with 44 % of mitochondria moving in the retrograde direction (towards the cell body) and 56 % moving in the anterograde direction (away from the cell body). This differed from neurons where 61 % of the mitochondria were moving in the retrograde direction. Interestingly, mitochondria moved significantly more slowly and covered shorter distances in astrocytic processes compared with dendrites. These results suggest that astrocyte mitochondrial trafficking might be governed by different motor proteins [42]. In neurons, long-range anterograde and retrograde mitochondrial trafficking involves kinesin superfamily 5 (KIF5) and dynein motors respectively, which bind to the microtubule cytoskeleton [18,47]. Short-range trafficking in neurons is instead regulated by actin filaments [48–50]. Jackson et al. [42] report that mitochondrial trafficking within astrocytic processes seems to be regulated by both microtubule and actin cytoskeletons, where pharmacological disruption of microtubule or actin assembly significantly decreased the fraction of moving mitochondria in organotypic slices. Kremneva et al. [51], however, report that microtubules are the main regulators of mitochondrial trafficking in cultured astrocytes. Interestingly, it has been shown that astrocytes express kinesin motor proteins (KIF11 and KIF22) [52]; however, their involvement in regulating astrocyte mitochondrial trafficking has yet to be determined.

In neurons, another key regulator of mitochondrial trafficking is Miro1, an OMM Rho-GTPase protein containing two GTP-binding domains separated by a linker region containing two Ca^{2+}-binding EF-hand motifs [53] (Figure 1A). Miro1 is crucial for mitochondrial transport in both axons and dendrites [54–56] by coupling mitochondria to kinesin or dynein motors via the adaptor protein trafficking protein, kinesin-binding (TRAK) (of which there are two isoforms, TRAK1 and TRAK2) [40,57–59,59a]. Currently, three models have been proposed to describe the role of Miro1 as a Ca^{2+} sensor in regulating mitochondrial mobility in neurons [60] (Figure 1B). First, mitochondrial transport is mediated by linking Miro1 to KIF5 and the KIF5-binding protein TRAK2. Ca^{2+}-binding to Miro1's EF-hand motifs dissociates Miro1, along with TRAK2, from KIF5, which remains bound to the microtubules [40]. In the second model, the KIF5 tail is linked to Miro1 via TRAK2 in a Ca^{2+}-independent manner, freeing the motor domain to engage with microtubules. Ca^{2+} binding to the EF-hand

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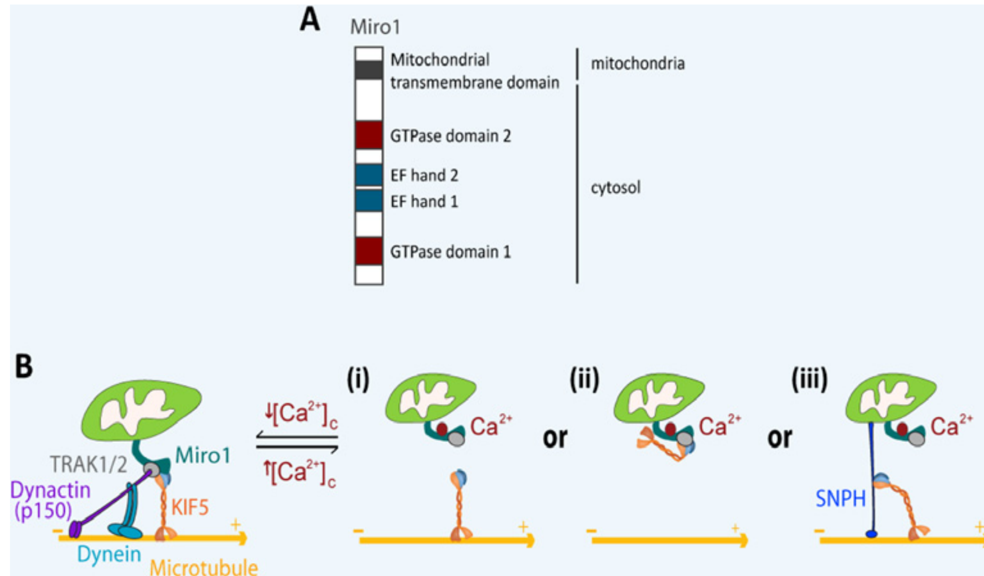
Figure 1 | Miro1 and the machinery of mitochondrial transport in neurons

(**A**) Schematic diagram of Miro1's functional domains, which consist of two GTPase domains flanking two Ca^{2+}-binding EF-hand domains. Miro1 also has an outer-mitochondrial transmembrane domain. (**B**) Under basal conditions, Miro1 forms a complex with TRAK1/TRAK2 and KIF5. This complex binds the microtubule network and allows anterograde transport. As well as binding kinesins, Miro1 can bind dynein motors via TRAK2 and this instead allows retrograde transport. During activity there is a concomitant influx of Ca^{2+}, which binds to Miro1 causing a conformational change in its structure resulting in its uncoupling from the transport network. Upon uncoupling, mitochondria are rendered immobile and in this way they are retained at sites of high Ca^{2+}. Three possible models have been proposed for the uncoupling of Miro1 from the microtubule network. (i) KIF5 remains bound to the microtubule network [40], (ii) KIF5 remains bound to the Miro1/TRAK2 complex upon uncoupling [55], or (iii) detachment from KIF5 occurs with subsequent interaction of the mitochondrial tether syntaphilin (SNPH) [61].



motifs triggers the direct interaction of the motor domain with Miro1, thus preventing the motor from engaging with microtubules [56]. In the third model, the detachment of KIF5 from Ca^{2+}-bound Miro1 triggers its interaction with syntaphilin, and the KIF5–syntaphilin anchors mitochondria on to the microtubules [61]. In addition, Miro1 has been suggested to play a role in the health and organization of mitochondria by regulating fission and fusion, mitochondrial degradation (mitophagy) and mitochondria–ER interactions [39,62,63]. However, nothing is currently known about Miro or its binding partners in astrocytes, raising the critical question of whether, if present, Miro plays an equally important role in regulating astrocyte mitochondrial dynamics.

Many potential routes exist for elevating astrocyte $[Ca^{2+}]_c$ and factors that determine which route is involved include age or stage of development and brain region [64]. The most established route is via synaptic glutamate activation of metabotropic glutamate receptors (mGluRs), in particular mGluR5, which trigger G-protein-coupled receptor cascades that induce a Ca^{2+} rise from internal stores [8,64–66]. Glutamate released during synaptic activity can be taken up by astrocyte glutamate transporters (GLT-1 and GLAST) along with sodium (Na^+) ions. The subsequent conversion of glutamate into non-toxic glutamine along with restoring homoeostatic levels of Na^+ within the cell is energetically demanding [67,68]. Here, the role of mitochondrial ATP provision could be of particular importance (Figure 2Ai). Interestingly, the astrocyte glutamate transporters have been shown to form complexes with glycolytic enzymes and mitochondria [69,70]. The co-transported Na^+ favours the reversal of the plasma membrane NCX, which extrudes Na^+ and thus induces a rapid local Ca^{2+} rise [14,71,72]. Here, mitochondria could be important for local Ca^{2+} buffering. Tan et al. [73] have shown that with pharmacological elevations of intracellular Ca^{2+}, released from internal stores, using 4Br-A23187 in primary cortical astrocytes, mitochondria became shorter with a reduction in motility. Furthermore, Jackson et al. [42] revealed that pharmacological inhibition of the NCX increased the percentage of mobile mitochondria in organotypic slice astrocytes. To a similar degree, tetrodotoxin, a Na^+ channel blocker, also increased the fraction of moving mitochondria with a corresponding increase in the distance between mitochondria and GLT-1 puncta in organotypic slice astrocytes [42]. These results infer that glutamate activates astrocytic glutamate transporters, possibly inducing NCX-dependent elevations in intracellular astrocytic Ca^{2+}, which could trigger the

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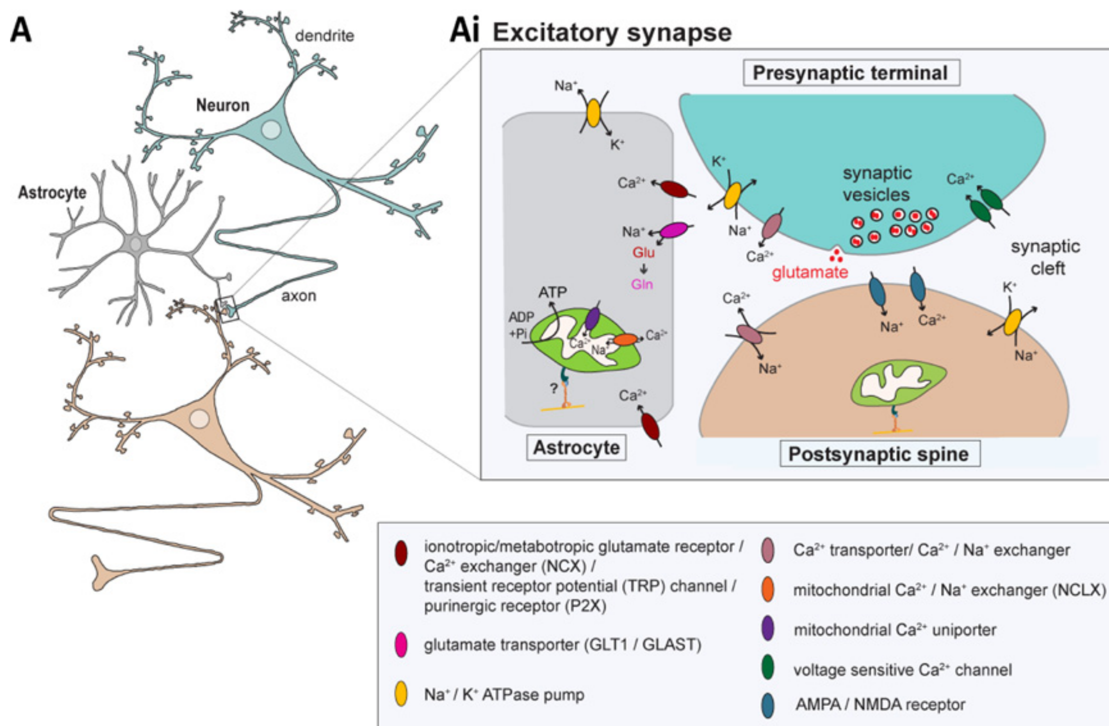
Figure 2 | **Local excitatory synaptic signalling and the contribution of astroglial perisynaptic processes**

(**A**) Schematic diagram of synaptic interactions occurring between neuronal dendrites and separate axonal boutons, from neighbouring neurons, forming the excitatory synapse. Here, astrocytes can project their processes into the synaptic cleft where they can influence synaptic signalling. (**Ai**) Synaptic release of glutamate activates receptors (of which many are expected to be involved, depending on the brain region) and glutamate transporters on the astrocyte membrane. Glutamate (Glu) entering via glutamate transporters can either be actively converted into glutamine (Gln), lactate or glucose (to be stored as glycogen) or be used to generate ATP production via mitochondrial oxidation. Na^+ influx occurs alongside glutamate entry and this rise can induce the reverse mode of the NCX, resulting in Ca^{2+} influx. This is another possible route for astrocyte Ca^{2+} entry (the other being activation of receptors), which can consequently be taken up by the mitochondrial uniporter and re-released in exchange for Na^+. Ca^{2+} release by mitochondria can then induce the exocytotic release of glutamate. The Na^+/K^+-ATPase is important for ionic homoeostasis during astrocyte depolarization by actively extruding Na^+ that accumulates with glutamate uptake. These actions reveal the intricate and crucial regulatory role provided by astrocytes at the excitatory synapse and the importance of mitochondrial ATP provision and Ca^{2+} buffering within their processes.



localization of mitochondria near glutamate transporters. This therefore highlights the significance of mitochondrial trafficking, which may allow astrocytes to localize energy production and Ca^{2+} buffering to specific areas requiring functional mitochondria.

Mitochondrial morphology in astrocytes

Mitochondrial morphology is diverse and dynamic, varying between different cell types, and within individual cells [74]. Mitochondrial fission and fusion events regulate mitochondrial morphology, promote the exchange of genetic material and proteins between mitochondria, an essential process required for proper mitochondrial function [74]. These opposing processes are complex and involve a host of proteins with fission being mediated by dynamin-related protein 1 (Drp1) and fission 1 protein (Fis1) and fusion being mediated by mitofusins (Mfn1 and Mfn2) and optic atrophy 1 (OPA1) [75–77].

Motori et al. [79] showed that under physiological conditions, individual astrocytic mitochondria undergo fission and fusion events in acute cortical slices with an even split of events, i.e. 50 % of events were fission and 50 % were fusion. Importantly, the intracellular environment can have an impact on mitochondrial morphology. Indeed, Tan et al. [73] showed that pharmacological Ca^{2+} elevations in cultured astrocytes resulted in remodelling or rounding of mitochondria. Similarly, in neurons, influx of Ca^{2+} resulting from glutamate treatment also altered mitochondrial morphology [78]. Although the direct impact of mitochondrial morphology on function is not immediately clear, morphology may influence energy production and Ca^{2+} buffering functions;

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for example, longer mitochondria may allow Ca^{2+} buffering and ATP dispersal over a larger cytoplasmic area [74].

Interestingly, the balance between mitochondrial fusion and fission events is altered in astrocytes challenged with cellular injury. During cortical injury or treatment with pro-inflammatory stimuli in acute slices, an increase in mitochondrial fission was observed along with an increase in the phosphorylated/activated form of Drp1 [79]. The inflammatory insult was transduced into nitric oxide (NO) production, which mediated the activation of Drp1 and subsequently induced mitochondrial fission in astrocytes. The fragmented astrocytic mitochondria exhibited compromised ATP production and increased reactive oxygen species (ROS) generation, affecting cell survival [79]. NO itself can regulate gene transcription, impair mitochondrial respiration or directly induce cell death by apoptosis. Interestingly, inducible nitric oxide synthase expression was enhanced in astrocytes (significantly more than in neurons) in human Alzheimer's disease (AD) tissue [80] and mouse models of AD [81]. These results reveal the ability of astrocytes to rapidly react to pro-inflammatory stimuli. This highlights the importance of clarifying the mechanisms regulating mitochondrial morphology in astrocytes as well as how it is dysregulated during pathology.

Mitochondrial quality control mechanisms and their implications in astrocytes

Increasing evidence has revealed that aberrant mitochondrial dynamics, function and turnover are associated with pathological changes in several neurodegenerative diseases [82,83]. Indeed, there are several mitochondrial quality control mechanisms that are utilized to ensure mitochondrial integrity, including repair of dysfunctional mitochondria by fusion with healthy mitochondria [83,84] and selective removal of irreversibly damaged mitochondria by mitophagy, a cargo-specific subset of autophagy [85].

The main pathway for selective mitochondrial degradation involves activation of the phosphatase and tensin homologue deleted on chromosome 10 (PTEN)-induced putative kinase 1 (PINK1) and the E3 ubiquitin ligase parkin. Upon mitochondrial damage, PINK1 accumulates in its full-length form on the OMM, recruits parkin from the cytosol and activates it by phosphorylation. Activated parkin then ubiquitinates various substrates on the OMM [VDAC1 (voltage-dependent anion channel 1), Drp1, Mfns, TOM (translocase of OMM)-20 and -40] [86–93]. Ubiquitinated mitochondria are subsequently recognized by ubiquitin-binding autophagic components, e.g. LC3 (microtubule-associated protein light chain 3), HDAC6 (histone deacetylase 6) and p62 sequestosome, that promote the formation of autophagosomes and induce mitochondrial clearance by fusion with lysosomes. Loss-of-function mutations in PINK1/parkin have been associated with forms of early-onset Parkinson's disease (PD) [94,95]. More recently, Miro1 was shown to interact with PINK1 and parkin [90,96,97], and was found to be ubiquitinated upon mitochondrial damage [63,98,99], suggesting a possible link between Miro-mediated transport pathways and PD-associated disruption of mitochondrial turnover.

Interestingly, parkin dysfunction has been shown to impair astrocyte mitochondrial function and contribute to the pathogenesis of PD [100]. Glial cells cultured from parkin-null mice exhibited increased expression of pro-apoptotic proteins and reduction in heat-shock protein 70 (cytoprotective), together contributing to decreased neuroprotection [101,102]. Expression of proteins containing other PD-related gene mutations (for example, DJ-1 mutations) in cultured astrocytes have been shown to similarly impair mitochondrial dynamics, compromise neuroprotective ability and thus render neurons more sensitive to toxic insults [103–105]. Moreover, Ledesma et al. [100] have shown that astrocytes express lower levels of parkin, which has been suggested to exhibit different subcellular localizations compared with neurons. These data warrant further investigation into the importance of cell-specific differences in parkin expression and distribution, differential cellular response to lack of parkin, and the subsequent effect on PD pathogenesis.

Regulation of the autophagy machinery is crucial for mitochondrial clearance and affects mitochondria function in astrocytes. For example, knockout of the gene encoding Atg7, essential for autophagosome production and lipidation of LC3B, in astrocytes resulted in highly hyperfused mitochondria, ROS production and ultimately cell death [79]. Importantly, Motori et al. [79] highlight that a failure to maintain the functional architecture of mitochondria affects astrocyte survival.

A recent discovery by Davis et al. [106] revealed that retinal ganglion cell axons shed mitochondria at the optic nerve head (ONH), which are internalized and degraded by adjacent astrocytes. Axonal mitochondria accumulate in large protrusions that are subsequently pinched off to form membrane-enclosed evulsions. Axonal mitochondria were confirmed within adjacent astrocytes, exhibiting high phagocytic activity (high expression of phagocytic marker Mac2) [106]. This process, termed transcellular mitophagy, is predicted to be a widespread phenomenon, occurring elsewhere in the CNS [106]. These data suggest a potential mechanism of mitophagy employed within astrocytes as well as astrocyte influence on neuronal mitophagy. However, there are still major mechanistic questions that remain regarding the components involved in the mitophagy pathway in astrocytes, for example, the timescale in comparison with neurons and dysregulation of mitophagy during pathology.

Concluding remarks

Currently, there is great interest regarding the complex relationship between astrocytes and neurons. Although studies have revealed the importance of mitochondrial function in astrocytes [107,108], shedding light on the specific mechanisms that regulate mitochondrial dynamics in astrocytes would significantly enhance our understanding

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of information processing in the CNS during health and disease. It is expected, but still only inferred, that neuronal activity regulates mitochondrial trafficking within astrocyte processes [42]. Thus it remains to be determined whether mitochondrial trafficking is regulated directly by glutamate transport or modulated by a subsequent process such as the activation of the Na^+/K^+-ATPase. However, it is known that mitochondrial NCX-mediated Ca^{2+} elevations modulate glutamate release and, in this way, astrocytic mitochondria may play a role in preventing excitotoxic cell death in the CNS [14].

Specialized astrocyte processes are found to be highly mobile and spontaneously advance or retract from active synapses in brain slices [109–111]. It remains to be clarified whether mitochondria are present in these extremely fine/perisynaptic astrocyte processes. Together with the recent study revealing differences in astrocytic mitochondrial trafficking dynamics in comparison with dendritic mitochondria [42], it will be interesting to determine whether Miro-mediated mitochondrial trafficking dynamics are conserved in astrocyte processes or whether alternative mechanisms are employed. This will, perhaps, shed light on how astrocyte mitochondria are selectively retained at sites of high Ca^{2+} or glutamate release in response to neuronal activity, which could have important consequences for astrocyte function and consequently neuronal activity regulation by astrocytes.

Understanding the consequences of alterations in mitochondrial morphology on their functionality (ATP production and Ca^{2+} buffering) will determine the impact not only on overall cellular health but also on the mechanisms contributing to disease progression. The identification of transcellular mitophagy has also raised several interesting questions, for example, whether this is indeed a widespread phenomenon occurring in the CNS and exactly how astrocyte mitophagy may influence neuronal function. More importantly, mitophagy regulation in astrocytes remains to be defined. Overall, discerning the more specific mechanisms of mitochondrial dynamics and function in astrocytes will be pivotal for advancing our understanding of the astrocyte–neuron bidirectional relationship and how this has an impact on synaptic function.

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