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Mitochondrial accumulation of APP and Aβ: significance for Alzheimer disease pathogenesis

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Abstract

Accumulating evidence suggest that alterations in energy metabolism are among the earliest events that occur in the Alzheimer disease (AD) affected brain. Energy consumption is drastically decreased in the AD-affected regions of cerebral cortex and hippocampus pointing towards compromised mitochondrial function of neurons within specific brain regions. This is accompanied by an elevated production of reactive oxygen species contributing to increased rates of neuronal loss in the AD-affected brain regions. In this review, we will discuss the role of mitochondrial function and dysfunction in AD. We will focus on the consequences of amyloid precursor protein and amyloid-β peptide accumulation in mitochondria and their involvement in AD pathogenesis.

Keywords: Alzheimer disease • mitochondria • oxidative stress • amyloid precursor protein • amyloid-β peptide

Introduction

Alzheimer disease (AD) is a devastating neurodegenerative disease characterized by a progressive decline in memory and cognitive functions such as language and perception [1]. It affects mostly elderly people and appears both sporadically (accounting for about 95% of all cases) and in familiar autosomal dominant (FAD) form [2, 3]. AD is characterized by two brain lesions, intraneuronal fibrillary tangles and extracellular amyloid plaques [4, 5]. Neurofibrillary tangles are composed of aggregated hyperphosphorylated τ protein. The role of aggregated τ in neurodegeneration is still controversial, as evidence points to either a toxic or protective role in the disease [6–8]. The major constituent of the amyloid plaques is a hydrophobic 39–43 amino acid peptide named amyloid-β peptide (Aβ), a cleavage product of the much larger transmembrane protein, amyloid precursor protein (APP) encoded by a single gene on chromosome 21 [9]. Aβ is generated from the C-terminal end of APP by the sequential action of β- and γ-secretases [10]. Amyloid formation is generally associated with the clinical manifestations of AD and the Aβ cascade hypothesis is the main pathogenic model of AD [11]. Almost all gene mutations linked to the early-onset FAD cases are associated with deregulated metabolism of APP resulting in enhancement of production of Aβ or increase of Aβ42/40 ratio [12]. Although the aetiology of sporadic AD is largely unknown, accumulating data suggest that mitochondrial dysfunction and oxidative stress occur in brain as well as in peripheral tissues of AD patients [13]. Mitochondria produce most of the cell energy, they are key regulators of cell survival and death, have a central role in ageing and have recently been found to harbour specific proteins implicated in AD. A better understanding of how mitochondrial functions change in aging and neurodegenerative diseases could reveal novel mitochondria-directed approaches for therapy of AD. Here we review and discuss the role of mitochondrial changes during aging and AD progression as well as recent findings connecting the amyloid cascade hypothesis and mitochondria.

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Age and AD related mitochondrial changes in brain and peripheral tissues

Today the free radical as well as the mitochondrial decline theories gains most in popularity among the different theories of aging. According to the free radical theory of aging, proposed by Harman [14], reactive oxygen species (ROS) produced in the body as a by-product of oxidative metabolism initiate changes associated with aging. Free radicals such as hydroxyl radical ($OH^{\cdot}$), superoxide anion radical ($O_2^{\cdot -}$), hydroperoxyl radical ($HO_2^{\cdot}$) or nitric oxide radical ($NO^{\cdot}$) can indiscriminately react with a wide variety of organic substrates causing peroxidation of lipids, cross-linking and modifications of proteins and mutations in DNA [15]. Cells have a variety of defence mechanisms to protect themselves against the harmful effects of ROS. Still, over time ROS-induced damage interfere with cell communication, disturb DNA, RNA and protein synthesis, decrease energy levels and generally impede vital chemical processes. Most of the cellular ATP is produced in mitochondria during respiration in a process called oxidative phosphorylation. This process encompasses electron transfer between protein complexes of respiratory chain in the inner mitochondrial membrane, vectorial transfer of protons into intermembrane space and ATP synthesis by ATP synthase upon re-entry of protons into the matrix [16]. During respiration a small portion of electrons passing through the electron transport chain, mostly at complex I and complex III, react with molecular oxygen and generate ROS through enzymatic and non-enzymatic reactions [17, 18]. The constant ROS production inside mitochondria subjects them to substantial free radical damage. Because the mitochondrion contains its own intron-less and histone-less ~16-kb circular DNA, a central role for mtDNA mutations caused by free radicals in aging has been postulated [19, 20]. In fact, mtDNA mutations have been shown to accumulate with aging in different tissues of various species [21–23]. As an extension and refinement of the free radical theory of aging the mitochondrial 'vicious cycle' theory was put forward [24]. This theory postulates that mtDNA mutations, which accumulate progressively during life, as a side effect of respiration, are directly responsible for a measurable deficiency in cellular oxidative phosphorylation activity leading to an enhanced ROS production. Trifunovic and colleagues have developed a 'mtDNA mutator' homozygote mouse expressing a proofreading-deficient form of the DNA polymerase (Polg) [25]. These mice have widespread tissue distribution of mutated mtDNA and a remarkable phenotype with several examples of premature aging. Interestingly, the increased levels of mtDNA mutations were not associated with increased ROS production [26] and this animal model has thus questioned the mitochondrial 'vicious cycle' theory. In a similar animal model developed by another group, the heterozygote $Polg^{+/D257A}$ mice were also shown to have strikingly high mtDNA mutation frequency albeit lower than $Polg^{D257A/D257A}$ mice [27] despite the fact that they lack the accelerating aging features of homozygous mice. It was found that there is a threshold of mtDNA mutations to reach the phenotypic expression, which differs in various tissues and ability of cells to repair large mtDNA deletions would determine tissue tolerance [28]. Despite the missing backward link between increased rate of mtDNA mutations and increased ROS production in the mtDNA mutator mice, another mouse model still points towards age-associated oxidative stress correlated with increased rate of mtDNA mutations [29]. In this model, mice expressing catalase targeted to mitochondria but not to peroxisomes or nuclei have extended lifespan by approximately 20%. Their results suggest that scavenging harmful ROS in mitochondria protect mtDNA and prolong lifespan [29].

A large number of reports support the fact that decreased mitochondrial functions are associated with aging. In general mitochondria isolated from old animals show lower respiration rates due to decreased activity of electron transfer chain enzymes [30, 31]. Respiratory chain complexes I and IV show significantly decreased enzymatic activities in mitochondria isolated from different tissues derived from old animals, whereas complexes II and III are less affected [32–36]. One plausible explanation for this phenomena is the large proportion of mtDNA encoded subunits of complex I (seven subunits) and complex IV (three subunits) in comparison to only one subunit of complex III and no nuclear encoded subunits in complex II. Time course analysis of mitochondrial DNA gene expression in C57BL6 mouse brain reveal increased expression of mitochondrial encoded genes of the respiratory chain at 12 and 18 months of age as compared to 2-month-old controls [37]. These data suggest age dependent compensatory mechanism for mitochondrial respiratory chain dysfunction.

Other mitochondrial enzymes such as adenine nucleotide translocase [38], nitric oxide synthase [39] and carnitine acyltransferase [40] show 30–60% decreased activities in senescent brain. The observed decrease of electron transfer in aged brain mitochondria is paralleled with the development of mitochondria with increased size [41] and higher proportion of depolarized non-functional mitochondria [42]. In summary, physiological aging is associated with general decline in mitochondrial functions.

Numerous reports show abnormal mitochondrial dysfunction associated with AD pathology. For example, a decline in cerebral glucose metabolism occurs before pathology and symptoms manifest, continues as symptoms progress and is more severe than in normal aging [43]. Studies of neural cells devoid in mtDNA and fused with platelets that lack intact nucleus (cybrid cells) provide important evidence for mitochondrial dysfunction in AD. In such cells that differ only in their mitochondrial content cybrids obtained from AD patients show decreased activities of mitochondrial enzymes and increased oxidative stress and, remarkably, elevated extra and intracellular levels of Aβ [41–45]. The cybrid model implicates mtDNA as important factor in AD pathogenesis and the mtDNA mutation rate is increased in AD as compared to age-matched controls. However, no consistent abnormalities in mtDNA associated with AD were reported [44], suggesting random mtDNA damage due to elevated ROS production.

Mitochondrial dysfunction of APP transgenic mice was demonstrated by an increased expression of genes related to mitochondrial energy metabolism as well as apoptosis [45]. These data

implicate mitochondrial stress as common denominator for AD and aging [37].

Among the most consistent findings in mitochondrial AD associated defects are decreased activities of α-ketoglutarate dehydrogenase [46, 47], pyruvate dehydrogenase [48] and cytochrome *c* oxidase (complex IV) of electron transfer chain [49, 50]. Interestingly, the activities of all of these enzymes are inhibited by Aβ [51] providing a possible link between amyloid cascade and mitochondrial dysfunction in AD. Crouch *et al.* showed that low molecular weight $A\beta_{1-42}$ oligomers inhibits cytochrome c oxidase in a copper-dependent manner [52]. Maximal cytochrome c oxidase inhibition was reached with Aβ solutions aged for up to 6 hrs. These $A\beta_{1-42}$ preparations consisted of around 10% toxic oligomeric species (~17 μM). The remaining 90% are monomeric Aβ, high molecular weight oligomers, protofibrils and fibrils and regarded as relatively non-toxic. It remains to be determined whether concentrations of low molecular weight $A\beta_{1-42}$ oligomers required to inhibit complex IV are present in AD brain mitochondria.

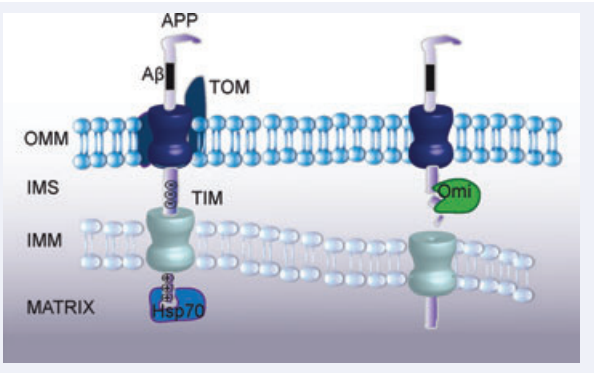

Fig. 1 APP is localized to mitochondria. APP N-terminal contains a mitochondrial targeting sequence (positively charged aa 40, 44 and 51) and is incompletely imported into mitochondria due to an acidic stop translocation sequence (aa 220–290). Subsequently APP may either be degraded by proteases such as Omi in the intermembrane space or form supercomplexes with the TOM and TIM complexes whereby blocking the import machinery.

Effect of APP accumulation on mitochondrial functions

Recently it was shown that mitochondria isolated from affected AD brain regions contain substantial amounts of APP as compared to age-matched controls [53]. APP forms stable translocation intermediate complexes with translocase of the outer membrane (TOM) in the AD mitochondria (Fig. 1). APP also links TOM and translocase of the inner membrane (TIM) together. Mitochondrial accumulation of APP correlates with decreased ability of mitochondria to import nuclear encoded proteins and with decreased activity of cytochrome c oxidase in AD-affected brain regions [53]. It was suggested that APP has a typical endoplasmic reticulum (ER) signal peptide followed by a cryptic mitochondrial targeting signal, whereas a more C-terminally located domain enriched in acidic amino acids acts as a mitochondrial translocation arrest sequence [54]. Expression of full length APP, in contrast to APP lacking the acidic domain, impedes mitochondrial functions suggesting that APP exert its toxicity mainly *via* formation of protein translocation intermediates [54]. In yeast *in vivo* mitochondrial targeting of a chimeric mitochondrial precursor resulted in irreversible accumulation of precursor protein in mitochondrial translocation contact sites, progressive zippering of the outer and the inner membranes, cell growth arrest and cell death [55, 56]. Interestingly, transgenic mice overexpressing APP show accumulation of APP in brain mitochondria in contrast to the non-transgenic age-matched animals [54]. In 2008, Hauptmann and colleagues reported early mitochondrial dysfunction associated with increased ROS production, decreased mitochondrial membrane potential, ATP level and complex IV activity in double Swedish and London mutant APP transgenic mice [57]. In this animal model a several fold increased expression of APP in the brain was detected. At present it is not clear whether the observed mitochondrial toxicity is due to APP or Aβ accumulation.

A combination of several factors may cause abnormal accumulation of APP in the mitochondrial translocation channels during AD progression. APP is normally targeted to ER on its way to the plasma membrane and the cryptic mitochondrial targeting signal is not exposed. Recently, an inducible cytosolic endoprotease that specifically activates mitochondrial cryptic signals by removal of the preceding ER signal peptide was characterized [58]. Upon activation of this protease several proteins including CYP1A1, glucocorticoid receptor, retinoid X receptor and p53 underwent cleavage-dependent mitochondrial import. Although *in vitro* mitochondrial import of APP does not require removal of ER signal peptide [54] it cannot be excluded that such processing occurs *in vivo*.

Post-translational modifications represent another mechanism that can control protein targeting to mitochondria. For example, post-translational N-terminal myristoylation of pro-apoptotic protein BID is required for targeting its proteolytic fragment to mitochondria during apoptosis [59]. Phosphorylation also can regulate the movement of proteins from cytosol to mitochondria [60] or promote mitochondrial localization of ER-targeted proteins [61]. Enhanced mitochondrial targeting was shown to be mediated through increased binding of client phosphoproteins to cytosolic chaperones [60, 62, 63]. Selective mitochondrial relocalization of proteins can also be achieved without their post-translational modification. Conformational change in the pro-apoptotio protein BAX structure as the result of cellular stress promotes its rapid translocation into mitochondria [64, 65]. Moreover, in response to decreased mitochondrial membrane potential parkin is re-localized from cytosol to mitochondria, which results in elimination of mitochondria by autophagosomes [66]. All together the changes in intracellular homeostasis during the initial course of AD can potentially increase mitochondrial APP targeting, resulting in mitochondrial

dysfunction and acceleration of disease progression. Complete impairment of protein import into mitochondria will eventually result in collapse of mitochondrial function and ultimately to neuronal cell death. Therefore it can be assumed that during the AD progression a gradual accumulation of APP in the mitochondrial import channels is a dynamic and regulated process.

Several distinct mechanisms are known for mitochondrial protein turnover: autophagic degradation of entire organelles [67], the proteolysis of proteins within the matrix or intermembrane space [68], and proteasome-dependent outer mitochondrial membrane-associated degradation [69]. Careful balance between mitochondrial fission and fusion ensures the mitochondrial quality; however, mitochondria lacking electrochemical potential for extended periods will be selectively cleared through steady-state autophagy, or mitophagy [66, 70]. The destruction of whole organelle will eliminate non-functional mitochondria as well as prevent uncontrolled release of mitochondrial intermembrane space proapoptotic proteins into cytosol. Interestingly, abnormal mitochondrial morphology, dynamics and distribution have been observed both in fibroblasts from sporadic AD patients as well as in mice overexpressing APP [71, 72]. Furthermore, overexpression of APP in neuroblastoma cell lines displayed fragmentation and abnormal distribution of mitochondria caused by an imbalance of the mitochondrial fission and fusion system, leading to mitochondrial and neuronal dysfunction [72]. This indicates that the balance between mitochondrial fission and fusion can be affected by mitochondrial accumulation of APP or Aβ, or that a change in the fission and fusion balance towards increased fission during AD progression could result in mitochondrial APP and Aβ accumulation.

Mitochondrial protein turnover is a balance between protein synthesis inside the organelle, import of nuclear encoded proteins from cytosol and protein degradation by mitochondrial proteases. Several proteases have been identified and extensively studied in mitochondria. For example, the mitochondrial protease HtrA2/Omi has been studied in connection to APP turnover [73, 74]. HtrA2/Omi was able to cleave APP *in vitro*, inside mitochondria *in vivo* [73] as well as during ER-associated APP degradation [74]. HtrA2/Omi is a serine protease located in the intermembrane space of mitochondria. During cell death HtrA2/Omi translocates from intermembrane space to cytosol and enhances apoptosis *via* cleavage of inhibitory apoptosis proteins and subsequent activation of caspases [75, 76]. However, animal studies using mice with a targeted deletion of the HtrA2/Omi gene [77] as well as motor neuron degeneration 2 mice (*Mnd2*) [78] expressing homozygous Ser276Cys mutation in the HtrA2/Omi protease domain that greatly reduces its catalytic activity, did not exhibit decreased apoptosis. On the contrary HtrA2/Omi-deficient mice exhibit neurodegenerative phenotype, failed to gain weight and die prematurely clearly demonstrating the essential protective role of HtrA2/Omi *in vivo* [79]. Recent data indicate that HtrA2/Omi can play a role in the mitochondrial quality control response monitoring and controlling protein folding in the mitochondrial intermembrane space [80, 81]. HtrA2/Omi protease activity is regulated through serine phosphorylation having stimulating (Ser142) [82] or inhibitory (Ser212) [83] effect. HtrA2/Omi protease activity in mitochondria is also regulated by protein-protein interactions *via* the PDZ domain of HtrA2/Omi [79]. Such interactions can either promote or inhibit apoptosis probably depending on the function of HtrA2/Omi-interacting partner protein [82, 84–86]. Interestingly HtrA2/Omi interacts with presenilin-1, a catalytic subunit of the γ-secretase that cleaves APP to produce Aβ peptide [86].

It remains to be tested if the inhibition of the APP accumulation in mitochondrial import channels or increased proteolysis of APP would protect cells from mitochondria-mediated injury and potentially slow down AD progression.

Mitochondria as a target and mediator of Aβ toxicity

We and others have shown that Aβ is accumulating in mitochondria from post-mortem AD brain, living patients with cortical plaques and TgAPP mice [87–90]. In TgAPP mice mitochondrial Aβ accumulation occurs prior to plaque formation, indicating that this is an early event in the pathogenesis [87]. Even though both APP [54] and γ-secretase complexes [91] have been detected in mitochondria it is not likely that Aβ is produced locally in mitochondria. The reason is that the import of APP is arrested due to an acidic domain at amino acids 220–290 leaving the Aβ-region outside the membrane. Since γ-secretase cleaves its substrates by regulated intramembrane proteolysis such localization of APP excludes it as a γ-secretase substrate in mitochondria. Therefore, Aβ found in AD mitochondria must have been taken up. We decided to investigate these uptake mechanisms using isolated mitochondria treated with Aβ in the absence or presence of antibodies or inhibitors directed to various mitochondrial translocases and pores [90]. The uptake of Aβ was not affected by the presence of antibodies directed towards the voltage-dependent anion channel nor in the presence of cyclosporine A which is an inhibitor of the mitochondrial permeability transition pore (mPTP). Interestingly, import of both $A\beta_{1–40}$ and $A\beta_{1–42}$ was prevented when import competent mitochondria were pre-incubated with antibodies directed towards proteins of the TOM complex, *i.e.* Tom20, Tom40, Tom70 (Fig. 2). Aβ import was not affected by the addition of valinomycin, an ionophore which cause depolarization of the mitochondrial inner membrane, indicating that the Aβ import was not dependent on the mitochondrial membrane potential. After import Aβ was mostly localized to mitochondrial cristae and associated with the inner membrane fraction. It was earlier reported that Aβ co-localizes with the mitochondrial matrix protein Hsp60 in mouse and human samples [87]. One explanation to this discrepancy might be that in our *in vitro* assay we studied Aβ localization after 30 min. of import, whereas Caspersen *et al.* report data from post-mortem AD brains and 8-month-old transgenic APP mice. However, our data from brain biopsies obtained from people, who display Aβ aggregates in the

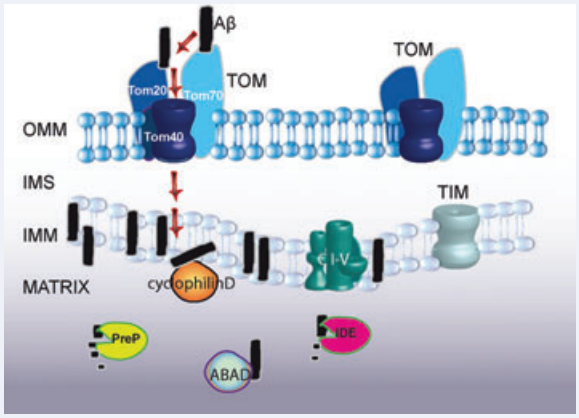

Fig. 2 $A\beta_{1-40}$ and $A\beta_{1-42}$ are taken up by mitochondria through the TOM machinery. After import most of the Aβ will reside in the inner mitochondrial membrane, where it possibly can inhibit the respiration chain (CI-V) resulting in an increased production of ROS. However, a fraction will also reach the matrix where it either can be degraded by proteases like PreP and IDE or interact with proteins such as CypD and ABAD.

neuropil, show Aβ immuno-gold labelling in association with mitochondrial inner membranes [90]. Still we cannot exclude that Aβ can be released or escapes from the membrane and also localizes to the matrix.

Aβ-binding alcohol dehydrogenase (ABAD) was identified as an Aβ-binding protein in a yeast two-hybrid screen [88]. ABAD is localized to the mitochondrial matrix and has an essential physiological role in mitochondria (Fig. 2). ABAD-Aβ complexes were detected in AD brain and in Tg mutantAPP/ABAD (Tg mAPP/ABAD) mice. Cortical neurons cultured from Tg mAPP/ABAD mice show increased production of ROS and decreased mitochondrial membrane potential, ATP levels and activity of respiratory chain complex IV. Consistently, these neurons displayed DNA-fragmentation and caspase-3 activity resulting in cell death by day 5–6 in culture [92]. ABAD uses NAD^+ and/or NADH as its cofactor and catalyses the reversible oxidation and/or reduction of alcohol group in its substrates [93]. The crystal structure of ABAD-Aβ complexes has been determined showing that the NAD^+ binding pocket is distorted, hindering NAD^+ from binding to ABAD in the presence of Aβ [88, 93]. Thus Aβ blocks ABAD activity causing mitochondrial dysfunction and ultimately cell death. Two stretches of ABAD residues in the L_D loop region (amino acids 95–113) have been shown to be important for Aβ binding. Cell permeable peptides (amino acids 92–120, ABAD-DP) protected cells from cytochrome c release, DNA fragmentation and attenuated cell death in neuronal cultures. ABAD-DP also blocked the production of ROS both in cultured neurons and in mouse brain tissue. Small-molecule inhibitors of the ABAD-Aβ interaction belonging to a class of benzothiazole ureas have been identified [94] and ABAD emerges as a new drug target for AD. Aβ has also been shown to specifically interact with cyclophilin D (CypD), a mitochondrial matrix protein that associates with the inner membrane during opening of the mPTP [95]. Cortical mitochondria from CypD-deficient mice are resistant to Aβ- and calcium-induced mitochondrial swelling and permeability transition. Moreover, Tg mAPP/CypD-null mice had improved learning and memory and synaptic function both in 12- and 24-month-old animals [95, 96].

Recently, we have identified an additional Aβ target in mitochondria, namely a novel zinc-metalloendopeptidase called PresequenceProtease, PreP that has been shown to be the protease responsible for degradation of Aβ in mitochondria [97]. PreP was originally found and characterized in *Arabidopsis thaliana* [98] as a protease degrading targeting peptides that are cleaved off in mitochondria after completed protein import as well as other unstructured peptides up to 65 amino acid residues in length, but not small proteins [99, 100]. Crystal structure of *At*PreP has been solved and refined at 2.1Å [101]. The structure revealed a unique totally enclosed large peptidasome cavity and a novel catalytic mechanism involving hinge bending motions of the enzyme in response to peptide binding.

A human homologue of PreP, hPreP, originally identified as hMP1 [102] is a 1037 amino acids protein (AAH05025) that belongs to M16C pitrilysin oligopeptidase family. Its gene is located on chromosome 10. The protein has a predicted mitochondrial targeting peptide of 29 amino acids and its mitochondrial localization has been confirmed by proteomic studies of human mitochondria [103]. We have showed that the recombinant hPreP completely degraded both Aβ40 and Aβ42 as well as Aβ Arctic protein (42, E22G) at unique cleavage sites including several sites in a very hydrophobic C-terminal $A\beta_{29-42}$ segment that is prone to aggregation. Interestingly, PreP is an organellar functional analogue of the human insulin degrading enzyme (IDE), implicated in AD as it cleaves Aβ before insoluble amyloid fibres are formed [104–106]. IDE is also located on chromosome 10 (10q) and genetic association between single nucleotide polymorphisms (SNPs) in IDE and late onset of AD has been reported [107]. In contrast to IDE, hPreP does not cleave insulin despite the fact that the overall 3D structure of IDE is highly similar to PreP [108]. However, there exist differences that may explain varying cleavage specificity [109]. 3D structural model of hPreP generated from the 3D structure of *At*PreP [97, 101] revealed the presence of two cysteine residues in close proximity to each other. These residues form a disulphide bridge under oxidizing conditions locking the enzyme in a closed conformation and hindering substrate binding. This finding might be of physiological importance as it implies a possible inhibition of hPreP under conditions of elevated ROS production in mitochondria. An increased ROS production has been reported upon accumulation of Aβ in mitochondria [88]. Therefore, the degradation of the mitochondrial Aβ by hPreP may potentially be of importance in the pathology of AD. Furthermore, we make the assumption that hPreP may prevent Aβ–ABAD and Aβ–CypD interactions by clearance of the Aβ peptides that may provide a new strategy against AD.

One important question is how Aβ can reach the mitochondrial surface. Aβ is generated in the lumen of the endoplasmatic

reticulum/intermediate compartment, *trans*-Golgi network and endosomal/lysosomal pathway as well as released from the plasma membrane [110]. Intracellular Aβ42 has been shown to accumulate in intracellular multivesicular bodies [111] and it is possible that Aβ leaking from these vesicles could reach the mitochondria. Moreover, in our confocal microscopy analysis fluorescent $A\beta_{1–40}$ applied extracellulary is taken up by the cells and later partly localized to mitochondria [90]. Accordingly, Saavedra *et al.* have recently shown that $A\beta_{1–42}$ is internalized by primary neurons in the absence of apolipoprotein E [112]. These data suggest that secreted Aβ can be re-internalized into cells either itself or through some kind of vesicular transport and come in contact with mitochondria. These mechanisms require further investigation.

Conclusions

Mitochondria have a central role in AD pathogenesis and maintenance of mitochondrial functions arises as a novel approach for AD treatment. As we have discussed here, both APP and Aβ accumulate in AD mitochondria leading to impairment of mitochondrial functions and cell toxicity. We have recently shown that Aβ is imported into mitochondria through the TOM complex and that PreP can degrade Aβ inside mitochondria. Other studies have shown that Aβ causes toxicity inside mitochondria by interactions with, *e.g.* ABAD or CypD or by inhibiting complex IV activity in the respiratory chain. Accumulation of APP in the TOM complex prevents import of other proteins and thus it impairs both mitochondrial and cellular functions. Avoiding APP targeting to mitochondria or clearance of the import pore by, *e.g.* HtrA2/Omi would abolish such toxicity. Taken all together, prevention of mitochondrial Aβ and APP uptake, enhancing Aβ degradation while inside mitochondria and prevention of Aβ-protein interactions are all worthwhile novel strategies for AD-drug development.

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