Amyloid Precursor Protein Processing and Bioenergetics

Heather M. Wilkins[1,2] and **Russell H. Swerdlow**[1,2,3,4,*]

[1]Department of Neurology University of Kansas Medical Center, Kansas City, KS, USA

[2]University of Kansas Alzheimer's Disease Center, Kansas City, KS, USA

[3]Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, USA

[4]Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS USA

Abstract

The processing of amyloid precursor protein (APP) to amyloid beta (Aβ) is of great interest to the Alzheimer's disease (AD) field. Decades of research define how APP is altered to form Aβ, and how Aβ generates oligomers, protofibrils, and fibrils. Numerous signaling pathways and changes in cell physiology are known to influence APP processing. Existing data additionally indicate a relationship exists between mitochondria, bioenergetics, and APP processing. Here, we review data that address whether mitochondrial function and bioenergetics modify APP processing and Aβ production.

Introduction

Aβ associates with and is believed by many to cause Alzheimer's disease (AD), and thus is central to major therapeutic initiatives. Well before the advent of positron emission tomography (PET) – based amyloid imaging, Aβ plaques were known to deposit within brains of cognitively normal individuals (Bennett et al., 2006; Dickson et al., 1992; Price and Morris, 1999). PET-based Aβ imaging in living subjects advanced the field by enabling longitudinal measurements of Aβ plaque accumulation. These studies showed brain Aβ fibril deposition increases prior to the onset of cognitive decline, and profoundly slows during the clinically active stages (Burns and Swerdlow, 2013; Jack et al., 2013). Therefore, plaque deposition may precede cognitive decline by 1–2 decades and Aβ fibril accumulation essentially plateaus before overt clinical symptoms manifest. Why fibrillary Aβ accumulation begins, accelerates, and decelerates to the point that little additional accumulation occurs prior to symptom onset is unknown.

Studies depicting connections between mitochondrial dysfunction and Aβ suggest potential leads to these questions. Amyloid Precursor Protein (APP) and Aβ reportedly co-localize with mitochondria (Devi et al., 2006; Hansson Petersen et al., 2008). Aβ inhibits respiratory chain function, and Aβ toxicity appears reduced in cells that lack functional respiratory chains (reviewed in (Swerdlow, 2012)). Altering mitochondrial function also changes APP processing, and can increase or decrease the production of amyloidogenic derivatives (Canevari et al., 1999; Casley et al., 2002a; Gabuzda et al., 1994; Gasparini et al., 1997; Khan et al., 2000; Leuner et al., 2012; Onyango et al., 2010; Pereira et al., 1998; Webster et al., 1998). Here, we review evidence for the relationship between mitochondria, bioenergetics, and APP processing.

I. Amyloid Precursor Protein

In humans the *APP* gene is located on chromosome 21. Alternative splicing generates 8–11 APP protein isoforms of varying amino acid length (305, 639, 677, 695, 696, 714, 733, 746, 751,752, 770) (Matsui et al., 2007; Sandbrink et al., 1996). It is important to note APP splicing may vary between species (i.e. rodent versus human), and there may be additional unidentified splice variants. Of the currently known isoforms, APP 751 and 770 are expressed in glial cells, while APP 695 is the predominate form expressed in neurons (Matsui et al., 2007). APP is a type I transmembrane protein which is trafficked through both secretory and endocytic pathways.

Cellular localization of APP is dynamic. In the secretory pathway APP moves from the endoplasmic reticulum (ER) to the plasma membrane (Haass et al., 2012). During this process APP is post-translationally modified (phosphorylation, tyrosine sulphation, and N- or O- linked glycosylation) (Bhattacharyya et al., 2013; Lee et al., 2003; Pahlsson et al., 1992). APP cleaved at the plasma membrane is internalized (this requires a YENPTY motif), where it is endocytosed by either endosomes or lysosomes (Lai et al., 1995). Data from overexpression studies suggest the majority of APP is trafficked to the trans-Golgi network, while only 10% arrives at the plasma membrane (Haass et al., 2012). As mentioned above, the YENPTY domain is required for APP endocytosis. Mutations in this domain inhibit amyloidogenic APP processing (La Rosa et al., 2015; Perez et al., 1999). This domain contains a cytosolic adaptor in which proteins that contain a phosphotyrosine binding domain bind to APP. These adaptor proteins include but are not limited to, Fe65, Dab1, Mint 1, Mint 2, Mint 3, and JNK (Haass et al., 2012; Thinakaran and Koo, 2008).

APP Processing Pathways

APP processing is dependent on secretase enzymes, which yield products that are secreted into the extracellular space or which remain within or associated with the cell. APP processing is generally divided into two pathways, non-amyloidogenic and amyloidogenic. The non-amyloidogenic pathway begins with α-secretase-mediated cleavage of APP at amino acid 687 (in the APP 770 isoform) which releases the ectodomain, soluble APP α (sAPPα), into the extracellular space. As a result, a C-terminal fragment of APP that is 83 amino acids in length (CTF83) remains embedded in the plasma membrane. Cleavage of CTF83 by γ-secretase releases a small p3 fragment into the extracellular space and the APP

intracellular domain (AICD) into the cytoplasm (Figure 1). Conversely, amyloidogenic processing begins with β-secretase-mediated APP cleavage at amino acid 671 (in the APP 770 isoform). As a result, a smaller ectodomain, soluble APP β (sAPPβ), is released into the extracellular space. A larger APP C-terminal fragment that is 99 amino acids in length (CTF99) remains embedded in the plasma membrane. Finally, cleavage of CTF99 by γ-secretase releases Aβ into the extracellular space and the AICD into the cytoplasm (Figure 1). Numerous in depth reviews of APP processing pathways are available (Haass et al., 2012; Zhang et al., 2011).

The α-secretase is a membrane bound zinc metalloproteinase consisting of the A Disintegrin And Metalloprotease (ADAM) family members ADAM9, ADAM10, ADAM19, and Tumor Necrosis Factor Converting Enzyme (TACE)/ADAM17 (Asai et al., 2003; Buxbaum et al., 1998; Lammich et al., 1999). ADAM10 appears to be the dominant α-secretase in neurons (Kuhn et al., 2010). Other substrates for α-secretase include NOTCH receptors, tumor necrosis factor α (TNFα), epidermal growth factor (EGF) receptor ligands, cadherins, and interleukin 6 (IL6) receptor (Haass et al., 2012). Cleavage of APP by α-secretase is dependent on both the α helical conformation of the cleavage site and the distance between the bond undergoing hydrolysis and the plasma membrane (Sisodia, 1992). Details about α-secretase function, structure, and regulation have been reviewed elsewhere (Allinson et al., 2003; Lichtenthaler, 2011)

The β-secretase 1 (BACE1) is required for APP cleavage and is rate limiting for the generation of Aβ from APP. Knockout of BACE1, but not BACE2, completely blocks the generation of Aβ (Cai et al., 2001; Haass et al., 2012). Beyond APP BACE1 also cleaves myelin, voltage dependent sodium channels, platelet selecting glycoprotein ligand 1, type II α 2,6 sialyltransferase, and interleukin like receptor type II (Haass et al., 2012). BACE1 is largely membrane bound with highest activity in an acidic pH environment (Haass et al., 1993a; Haass et al., 1993b; Selkoe et al., 1996). BACE1 structure, function, and cell trafficking are reviewed in depth elsewhere (Cole and Vassar, 2007).

The γ-secretase, an aspartyl protease, is comprised of four subunits. These include Presenilin 1 (PS1), Presenilin 2 (PS2), nicastrin, anterior pharynx defective (APH-1), and PS enhancer (PEN2) (Francis et al., 2002). PS1 and PS2 form the catalytic domain of γ-secretase while APH-1 may act as a stabilizer, PEN2 acts as a regulator/enhancer of activity, and nicastrin serves as a substrate receptor (Bolduc et al., 2016; Dries and Yu, 2008; Holmes et al., 2014). The cleavage event catalyzed by γ-secretase does not occur at a single site as there are three cleavage sites on APP separated by three amino acids; ε, γ, and δ. γ-secretase assembly, trafficking, and substrates have been reviewed elsewhere (De Strooper et al., 2012; Dries and Yu, 2008; Haapasalo and Kovacs, 2011).

The location of APP processing is still unclear. Data support APP processing at the plasma membrane, ER, trans-Golgi network, endosome vesicles, mitochondria, and lipid rafts (Choy et al., 2012; Devi and Ohno, 2012; Di Paolo and Kim, 2011; Haass et al., 2012; Hartmann et al., 1997; Hicks et al., 2012; Placido et al., 2014). Further uncertainty exists in the field regarding the existence of intracellular Aβ (Wirths et al., 2012), as some have argued this could represent an artifact of overexpressing a mutant human transgene in mice (Oakley et

al., 2006; Youmans et al., 2012). It is important to note, though, that studies depicting intracellular Aβ have also been performed in post-mortem human brains (Aho et al., 2010; Glabe, 2001; Gyure et al., 2001; Hashimoto et al., 2010; Takahashi et al., 2002).

The hypothesis that amyloidogenic APP processing occurs within endosomes is supported by discrete yet convincing studies (Koo and Squazzo, 1994). For example, endosomes contain the optimum pH for BACE1 activity, APP and BACE1 interact within the endosomal pathway, and APP CTFs are processed in endosomes. Furthermore, inhibition of endosomal signaling prevents amyloidogenic APP processing (Haass et al., 1993b; Haass et al., 2012; Selkoe et al., 1996). APP trafficking/recruitment to endosomes of the trans-Golgi network are both sites at which Aβ has been shown to be generated (Choy et al., 2012; Edgar et al., 2015; Morel et al., 2013).

Lipid rafts bring APP and BACE1 together, providing an interaction interface for amyloidogenic APP processing. Lowering cholesterol levels in N2a neuroblastoma cells reduces Aβ production. It is hypothesized that APP within lipid rafts undergoes amyloidogenic processing, while APP not found within lipid rafts is subjected to non-amyloidogenic processing (Ehehalt et al., 2003). γ-secretase may also exist within lipid rafts (Urano et al., 2005). Overall, cholesterol and lipid metabolism may play a pivotal role in determining where and how APP is processed (Di Paolo and Kim, 2011; Hicks et al., 2012).

APP function

Overall, the function of APP is unclear. It has been assumed to relate to several processes, including cell adhesion and trophic support. APP has been implicated in cell adhesion due to its interaction with laminin and collagen and it co-localizes with integrins, specifically within neuronal axons that are sites of cell adhesion (Yamazaki et al., 1997; Young-Pearse et al., 2008). APP was also found to support cell growth. In fibroblasts, genetic deletion of APP led to slow cell growth and division. This consequence was rescued by restoring sAPPα levels (Saitoh et al., 1989). Furthermore, sAPPα may stimulate the differentiation of neuronal stem cells into an astrocytic phenotype (Caille et al., 2004). sAPPβ may have a similar function to sAPPα, but may be less potent (Chasseigneaux et al., 2011). Data also support an autocrine/paracrine function for APP. The structure of APP may also provide some insight into its possible function. APP contains metal binding domains (for copper and zinc), extracellular matrix components (such as heparin, collagen, and laminin), and a protease inhibitor domain (Kunitz, only in isoforms 751 and 770) (Dawkins and Small, 2014). Comparisons of data depicting functions of full length APP, sAPPα, and sAPPβ can be found elsewhere (Chasseigneaux and Allinquant, 2012).

Independent functions for smaller APP processing fragments have also been reported. While the APP ectodomains may be pivotal for cell growth and adhesion, the intracellular domains may play different roles. For example the AICD, when bound to its cofactor, Fe65, can regulate gene expression (Muller et al., 2008). Most studies focus on how and why Aβ forms aggregates, however limited studies suggest Aβ may also have a physiological function. Inhibition of Aβ production (which also limits sAPPβ production) induces neuronal cell death (Pearson and Peers, 2006; Plant et al., 2003). Further, data support a role for Aβ as an

antimicrobial protein that protects distinct organisms (worms and mice) from microbial infections (Kumar et al., 2016; Soscia et al., 2010).

Substantial data support a role for APP and its fragments (including the AICD and Aβ) in modulating mitochondrial function. As we will discuss below, studies completed over several decades indicate a relationship exists between APP processing, APP (and its fragments), mitochondria, and bioenergetics. While Aβ and APP influence mitochondrial function and bioenergetic pathways, the converse is also true; mitochondrial function and bioenergetics modulate APP processing and trafficking pathways.

II. APP, Aβ, and Mitochondria

APP is known to localize to mitochondria, where it appears to impede protein import. In particular, APP arrests and forms a "super-complex" with the translocase of the outer mitochondrial membrane 40 (TOMM40) and the translocase of the inner mitochondrial membrane 23 (TIM23). Data suggest this transmembrane arrest of APP inhibits the import of nuclear-encoded mitochondrial proteins (Anandatheerthavarada et al., 2003; Devi et al., 2006). Observations of this interaction have been observed both *in vivo* and *in vitro*. This transmembrane arrest of APP induces mitochondrial dysfunction, including disruption of mitochondrial ATP synthesis, mitochondrial membrane potential, and reductions in cytochrome oxidase (COX) activity (Anandatheerthavarada et al., 2003). APP localization to mitochondria is dependent on three positively charged amino acid residues, as mutation of these residues to neutral amino acids prevents mitochondrial APP accumulation (Anandatheerthavarada et al., 2003).

More recent evidence suggests a 12 amino acid stretch located within the C-terminal domain of APP is important for its mitochondrial localization. Deletion of this 12 amino acid stretch (which contains a transmembrane domain) decreased APP mitochondrial localization, while also inducing mitochondrial dysfunction. *In vitro*, this APP deletion mutant led to decreased cell viability, increased ROS, decreased ATP, and depolarized mitochondrial membrane potential (Wang et al., 2016). More studies are warranted to understand the consequences of mitochondrial localized APP.

In addition to mitochondrial APP localization, mitochondria also reportedly contain a functional γ-secretase. The mitochondrial γ-secretase cleaves the CTF83 of APP (of which has been shown to associate with the mitochondrial outer membrane-in addition to full-length APP protein). The topology of the CTF83 of APP is likely to generate the AICD and Aβ within the mitochondria (Pavlov et al., 2011). This is important because Aβ is found within mitochondria where it interacts with mitochondrial proteins and alters mitochondrial function (Du et al., 2008; Lustbader et al., 2004). Data support the accumulation of APP C-terminal fragments and full-length APP within mitochondria, particularly within the inner membrane and matrix of 5X Familial Alzheimer's disease (5XFAD; mice expressing 5 human transgenes including 3 mutations in APP and 2 mutations in PS1) mice. Heterozygous genetic depletion of BACE1 prevented the mitochondrial accumulation of both CTF99 and full length APP protein in an age-dependent manner (i.e. at 6 months of age, but not 12 months of age). This partial deletion of BACE1 also rescued mitochondrial

dysfunction within this model as illustrated by reduced oxidative damage and preserved complex II activity (Devi and Ohno, 2012).

Mitochondrial-localized APP is also cleaved by the serine protease HtrA2. This cleavage event generates a C-terminal fragment that is 161 amino acids in length (CTF161). CTF161 was found accumulated within the cytosol, under physiological conditions *in vitro* and *in vivo*. HtrA2 processing of mitochondrial-localized APP is hypothesized to prevent the accumulation of full-length APP and downstream mitochondrial dysfunction (Park et al., 2006).

Overall, the mechanism by which Aβ accumulates within mitochondria is hypothesized to occur via several plausible mechanisms. These include Aβ/ABAD interaction, local APP processing within mitochondria, or aberrant intracellular Aβ accumulation due to increased Aβ production or failed Aβ degradation. The effect of Aβ on mitochondrial function are discussed below.

Aβ and Mitochondria

In numerous model systems Aβ impairs mitochondrial function. In neuroblastoma cells APP overexpression lead to elevated $A\beta_{1-40}$ with reductions in cellular respiration, ATP levels, and COX activity; complex III activity, though, was elevated (Rhein et al., 2009). In PC12 cells, exogenous Aβ depolarizes the mitochondrial membrane potential and decreases activities of the mitochondrial electron transport chain complexes I, III, and IV while also reducing oxygen consumption (Pereira et al., 1998). Shorter fragments, such as $A\beta_{25-35}$, reduce cellular ATP production, levels of antioxidants (glutathione, GSH), mitochondrial membrane potential, and electron transport chain activities (CI-IV) in primary cortical neurons (Casley et al., 2002b). However, in isolated rat brain mitochondria $A\beta_{25-35}$ reduced the activity of COX but did not affect complex I, II, or III activities (Canevari et al., 1999; Casley et al., 2002a). $A\beta_{25-35}$ impairs anterograde transport of mitochondria to synapses in mouse hippocampal neurons (Calkins and Reddy, 2011). Aβ may alter mitochondrial dynamics by interacting with a mitochondrial fission protein, dynamin related protein 1 (DRP1) (Manczak et al., 2011). Both oligomeric and monomeric forms of Aβ were shown to bind to DRP1. Additional further mechanisms have also been suggested.

In transgenic mice and brains from AD subjects, Aβ interacts with Aβ binding alcohol dehydrogenase (ABAD), an interaction which inhibits the binding of NAD+ (Lustbader et al., 2004). Neurons from mice transgenic for mutant APP and wild-type ABAD display elevated levels of apoptosis and reactive oxygen species (ROS) production, with reduced COX activity. Whole brain preparations from these mice revealed decreased glucose utilization, lower COX activity, and reduced ATP levels (Takuma et al., 2005). Inhibiting the interaction between ABAD and Aβ improves mitochondrial function and reduces Aβ accumulation (Yao et al., 2011).

Aβ toxicity appears in some studies to be mediated through its effects on the mitochondrial respiratory chain. In N2a neuroblastoma cells $A\beta_{25-35}$ increased caspase activation, ROS production, and cytochrome c release. However, in ρ0 cells that lack a functional respiratory chain due to an absence of mitochondrial DNA (mtDNA), $A\beta_{25-35}$ failed to induce ROS

production, caspase activation, or cytochrome c release (Cardoso et al., 2002). This indicates the damaging effects of Aβ work through mitochondria, particularly through direct or indirect interactions with the respiratory chain.

Other APP fragments, including the AICD, also alter mitochondrial function. For example, overexpression of the AICD and its Fe65 cofactor in neuroblastoma cells decreased mitochondrial membrane potential, ATP levels, and superoxide production. The AICD/Fe65 complex is known to induce genes which disrupt actin dynamics. Overexpression of the actin binding protein, transgelin, recapitulated these results; Transgelin is a downstream target gene of AICD/Fe65 (Ward et al., 2010).

Overall APP and its fragments, and in particular Aβ, interact with, localize to, and alter the function of mitochondria. Studies showing these effects have utilized various *in vitro* and *in vivo* model systems. We will next discuss how mitochondria and bioenergetics influence APP processing and sorting pathways.

III. APP Processing and Bioenergetics

In vitro studies

The first studies describing a bioenergetic influence on the fate of APP processing were reported in the 1990s. Initial studies in which COS cells were exposed to a COX inhibitor (sodium azide) and a mitochondrial un-coupler (CCCP) noted the formation of an 11.5 kDa APP CTF that contained the Aβ motif (possibly the CTF99 fragment). Bioenergetic perturbations produced by these mitochondrial toxins also lead to APP accumulation in the Golgi. The production of this CTF was also increased when protein transport in the secretory pathway was inhibited (Gabuzda et al., 1994). Further studies in COS cells showed glucose deprivation, in combination with a glycolysis inhibitor (2-Deoxy-D-glucose), reduced sAPPα levels. Inhibition of COX by sodium azide also decreased sAPPα levels. These effects were reversed by an antioxidant, GSH (Gasparini et al., 1997).

The effects observed by Gasparini et. al. were recapitulated in a separate study using both COS and PC12 cells. It was further found that inhibition of glycolysis by 2-Deoxy-D-glucose resulted in reduced APP glycosylation. The changes observed could be inhibited by phorbol 12-myristate 13-acetate, a protein kinase C (PKC) activator, and increased co-localization was observed between APP and protein phosphatase 1 α (PP1α). This study suggests altered bioenergetics may influence signal transduction pathways which regulate APP processing (Henriques et al., 2005). Other studies have provided evidence that upon glycolysis and COX inhibition (i.e. treatment of cells with 2-Deoxy-D-glucose and sodium azide), APP accumulates in the ER and Golgi, a process also dependent on phosphorylation and signal transduction (Domingues et al., 2007).

The influence of bioenergetics on APP processing has been depicted in various cell types. In PC12 cells, secretion of APP-like peptides was reduced by glycolysis and/or mitochondrial respiration inhibition (either through glucose starvation, 2-Deoxy-D-glucose treatment, or ATP synthase inhibition using oligomycin) (Webster et al., 1998). In fibroblasts derived from control and sporadic AD subjects, glucose deprivation (i.e. reduced glycolysis flux)

lowered sAPP production. COX inhibition induced by sodium azide, in addition to glucose deprivation, further reduced sAPP production in sporadic AD fibroblasts. Unlike in the prior studies, antioxidants did not abolish these observations (Gasparini et al., 1999).

In astrocytes, glycolysis flux influences APP processing pathways. In human fetal astrocytes inhibition of the glycolysis regulating enzyme 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (PFKFB3) through genetic or pharmacological approaches lead to increased Aβ accumulation and toxicity. It was further found that PFKFB3 is upregulated in astrocytes from 12 month old TgCRND8 AD mice, another transgenic AD mouse model (Fu et al., 2015).

It is clear from these studies that glycolysis flux and mitochondrial function (particularly respiration) influence APP processing pathways. It is important to note, however, that changes in glycolysis flux also affect mitochondrial respiration, and vice versa. For example, in SH-SY5Y neuroblastoma cells, inhibition of glycolysis flux using either 2-Deoxy-D-glucose, glucose deprivation, or iodoacetate increases mitochondrial respiratory flux (Swerdlow et al., 2013). An inverse relationship can thus exist between glycolysis and mitochondrial respiratory flux. Changes in the flux of these bioenergetic pathways will further alter other bioenergetic pathways and intermediates, mitochondrial membrane potential, ROS production rates, ATP/ADP, and ROS scavengers (i.e. NAD+/NADH, NADP+/NADPH, GSH/GSSG) (Graham et al., 2012; Jiang et al., 2013; Korenic et al., 2014; Russell et al., 2002; Shutt et al., 2010; Swerdlow et al., 2013). Such changes influence the fate of APP processing (Figure 2).

BACE1 transcription is influenced by the same pathways that regulate mitochondrial biogenesis. Peroxisome proliferator activated receptor-γ (PPARγ) regulates the transcription of BACE1 and peroxisome proliferator activated receptor coactivator 1-alpha (PGC1α). PGC1α modulates mitochondrial biogenesis pathways (Scarpulla, 2011). Overexpression of PGC1α in N2a neuroblastoma cells decreased levels of Aβ secretion, while increasing sAPPα. Overexpression of PGC1β also decreased Aβ secretion. The authors attributed these findings to a decrease in BACE1 mRNA and protein expression, and to a decrease in BACE1 activity. Upon genetic ablation of PGC1α expression, BACE1 expression was elevated (Katsouri et al., 2011).

Mitochondrial DNA from sporadic AD subjects also influences APP processing pathways. In cytoplasmic hybrid (cybrid) cell lines in which mtDNA from sporadic AD subjects is transferred into an identical nuclear background, changes in mitochondrial function were observed. In these cells the $A\beta_{1-40}/A\beta_{1-42}$ ratio was increased with elevated Congo red reactive Aβ deposits as compared to cybrids prepared from non-demented, age-matched subjects (Khan et al., 2000). In a separate study, cybrid cells generated from sporadic AD donors displayed elevated ROS production, reduced ATP production, and decreased COX activity. Upon exposure of these AD cybrid cells line to exogenous $A\beta_{1-40}$ mitochondrial function further declined to a greater extent than it did in cybrids generated from non-demented, age-matched subjects. Mitochondrial membrane potential depolarization and cell death pathway activation was also observed (Cardoso et al., 2004b).

In vivo studies

In AD animal models bioenergetic manipulations alter APP processing. For example, in Tg2576 transgenic mice overexpression of a mitochondrial targeted catalase, which contributed to the conversion of hydrogen peroxide to water and oxygen, lead to a reduction in brain APP protein levels and reduced levels of BACE1, CTF99, $A\beta_{1-40}$, and $A\beta_{1-42}$. Conversely, this increased CTF83 and sAPPα levels. Beyond apparent reductions in amyloidogenic APP processing, these mice also had reduced Aβ deposits (Mao et al., 2012).

In Tg19959 AD mice overexpression of manganese superoxide dismutase (MnSOD, also called SOD2), a mitochondrial antioxidant enzyme that converts superoxide into hydrogen peroxide, reduced total levels of oxidized proteins (protein carbonyls) and cortical plaque burden. No change was observed in soluble or insoluble Aβ oligomer levels (Dumont et al., 2009). A different effect was found in Tg19959 AD mice with heterozygous knockout of MnSOD, as these mice displayed increased Aβ levels and plaques (Li et al., 2004). Together, these *in vivo* studies support a role for mitochondrial-derived ROS in driving amyloidogenic APP processing.

While *in vitro* studies found PGC1α overexpression decreased Aβ secretion, *in vivo* studies report opposite outcomes. PGC1α overexpression in the Tg19959 AD mouse model increased Aβ and tau accumulation. Changes in Aβ included a larger Aβ plaque area, an increased Aβ plaque number, an increased $A\beta_{1-42}/A\beta_{1-40}$ ratio, and increased prefibrillar Aβ. As part of a separate yet interesting finding, these mice also displayed increased tau phosphorylation. Finally, these mice also showed decreased citrate synthase, complex I, and complex II activities (Dumont et al., 2014).

In another study mtDNA mutator mice, which harbor a D257A polymerase G mutation, were crossed with APP/Ld mice. D257A mice accumulate mtDNA mutations at an elevated rate, including 3–5 fold more mtDNA point mutations, and increased levels of short-linearized mtDNA fragments (Trifunovic et al., 2004). D257A/APP/Ld mice displayed increased $A\beta_{1-42}$ plaques and levels, but no change in BACE1, PS1, or CTF99/CTF83 fragments. In this study increased Aβ accumulation was attributed to decreased clearance by insulin degrading enzyme (IDE) (Kukreja et al., 2014).

An additional study established mitochondrial congenic mouse lines by crossing females of different strains to male APP transgenic mice. Strains with varying mtDNA on a consistent nuclear background were specifically generated by crossing female FVB/N, AKR/J, or NOD/LtJ mice to male C57BL/6 APP transgenic mice and then back-crossing the female progeny to the male C57Bl/6 mice for a minimum of ten generations. The resulting unique mtDNA strains displayed changes in Aβ burden, including increased plaque numbers and size. The authors also assessed a variety of bioenergetic-related parameters, and concluded high levels of ATP and ATP release increased microglial activation and phagocytosis, which resulted in reduced Aβ plaque numbers and size (Scheffler et al., 2012).

In HEK293 and SH-SY5Y neuroblastoma cells, inhibition of complex I and III by rotenone and antimycin A, respectively, increased the production of Aβ and also ROS. These effects were mitigated by the addition of an antioxidant. Further, mice transgenic for mutant APP

(KM670/671NL and V717I) and treated with a complex I inhibitor or crossed with complex I deficient mice (*Ndufs4* knockout mice) displayed elevated Aβ levels (Leuner et al., 2012).

In AD transgenic mice reductions in COX activity due to reduced COX holoenzyme limits Aβ production. In a seminal experiment *Cox10* knockout mice were crossed with an AD mouse model transgenic for mutant APP and PS1. *Cox10* knock-out reduced the ability to generate the COX holoenzyme (Diaz et al., 2006). In the *Cox10* knockout/AD mouse model cross, Aβ plaque accumulation, $Aβ_{1-42}$, and β-secretase activity were reduced (Fukui et al., 2007). Additional studies crossed AD transgenic mice (expressing mutant APP and PS1) with mice expressing a mitochondrial-targeted endonuclease (PstI). This cross resulted in lower mtDNA levels, reduced Aβ plaque burden, and lower $Aβ_{1-42}$ levels (Pinto et al., 2013).

Under *in vivo* conditions accumulation of high amounts of mtDNA mutations and alterations in mitochondrial ROS production, as well as reductions in COX activity, complex I activity, or mtDNA levels change the function of APP processing pathways. These alterations may also influence tau phosphorylation and accumulation. Whether the genetic and/or chemical manipulations used in these AD mouse models primarily affect bioenergetics in neurons, glial cells, or both remains undetermined. Further, how bioenergetic interactions between neurons and glial cells influence APP processing pathways has not been addressed. However, based on *in vitro* data, bioenergetics in both neuronal cells and primary glial cells can alter APP processing pathways.

Conclusions

Mitochondrial function and bioenergetics are perturbed in AD subjects; these changes are not brain-limited. Brain glucose metabolism is reduced in AD subjects (De Santi et al., 2001; Rapoport et al., 1991; Swerdlow et al., 1994). Reduced COX activity in AD subject platelets, brains, and fibroblasts has been reported (Cardoso et al., 2004a; Mancuso et al., 2003; Swerdlow, 2012; Wilkins et al., 2014). Spectral analysis of the COX enzyme from AD subject brains report the enzyme is kinetically altered and therefore structured differently than in control subject brains (Parker and Parks, 1995). Electron microscopy and molecular studies of AD brains also show that despite an overall reduction in the number of intact mitochondria, a heterogeneous picture exists in which amounts of mitochondrial components, such as mtDNA, can vary from markedly reduced (in abnormal appearing neurons) to increased (in relatively healthy-appearing neurons) (Brown et al., 2001; Hirai et al., 2001; Swerdlow, 2012). Moreover, the amount that a component is necessarily increased or decreased can depend on what intracellular compartments are included in the analysis, as autophagosomes that contain the contents of degrading mitochondrial material may contain large amounts of mtDNA and mitochondrial protein (Hirai et al., 2001; Swerdlow, 2012). Overall, these studies support the view that mitochondrial mass varies during the course of AD, with mitochondrial mass increasing in relatively preserved neurons, and decreasing in relatively more damaged neurons.

Consistent with this, brain mtDNA content reportedly increases during aging in cognitively intact individuals (Barrientos et al., 1997; Hirai et al., 2001). This is consistent with the

possibility that increased Aβ production may occur in the run-up to clinical AD while neurons are still able to compensate for age-related declines in mitochondrial function, and decline later on in the symptomatic stages as cells can no longer compensate for impaired aerobic function and begin to actually downregulate their aerobic infrastructure.

It is important to note APP localizes to synapses and increased synaptic activity associates with increased processing of APP to Aβ (Brody et al., 2008; Cirrito et al., 2005; Groemer et al., 2011). This may partly arise from a synaptic activity-related retargeting of APP from the plasma membrane to intracellular structures that are more likely to produce Aβ, but based on extensive existing literature it seems likely that another synaptic activity-associated physiologic parameter, aerobic metabolism, also contributes to variations in Aβ production (Cavallucci et al., 2013; Du et al., 2010; Reddy et al., 2012).

In vivo and *in vitro* studies support a major role of mitochondria and bioenergetics in the fate of APP processing and cellular trafficking. Future studies are warranted to elucidate mechanisms surrounding the relationships between APP (and its fragments), mitochondria, and bioenergetic pathways.

Acknowledgments

Abbreviations

ABAD	Aβ binding alcohol dehydrogenase
AD	Alzheimer's disease
ADAM	A Disintegrin and metalloproteinase
AICD	APP intracellular domain
APH1	anterior pharynxdefective 1
APP	amyloid precursor protein
ATP	adenosine triphosphate
Aβ	amyloid beta
BACE1	β-secretase 1
BACE2	β secretase 2
CCCP	Carbonyl cyanide m-chlorophenyl hydrazine
COX	cytochrome oxidase or complex IV
CTF	C-terminal fragment

CTF83	C-terminal fragment 83
CTF99	C-terminal fragment 99
cybrid	cytoplasmic hybrid
DRP1	dynamin related protein 1
EGF	epidermal growth factor
ER	endoplasmic reticulum
GSH	glutathione
IDE	insulin degrading enzyme
IL6	interleukin 6
MnSOD/SOD2	manganese superoxide dismutase
mtDNA	mitochondrial DNA
PEN2	Presenilin enhancer 2
PET	positron emission tomography
PFKFB3	6-phosphofructo-2-kinase-fructose-2,6-biphosphatase 3
PGC1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PGC1β	Peroxisome proliferator-activated receptor gamma coactivator 1-beta
PKC	protein kinase C
PPARγ	Peroxisome proliferator-activated receptor gamma
PP1α	protein phosphatase 1 α
PS1	Presenilin 1
PS2	Presenilin 2
ROS	reactive oxygen species
sAPPα	soluble APP α
sAPPβ	soluble APP β
TACE	tumor necrosis factor-alpha converting enzyme
TIM23	translocase of the inner mitochondrial membrane 23
TNFα	tumor necrosis factor α
TOMM40	translocase of the outer mitochondrial membrane 40

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- A relationship between bioenergetics, mitochondria, and APP exists.

- APP and amyloid beta alter bioenergetic pathways and mitochondrial function.

- Bioenergetics and mitochondrial function may determine how APP is processed.
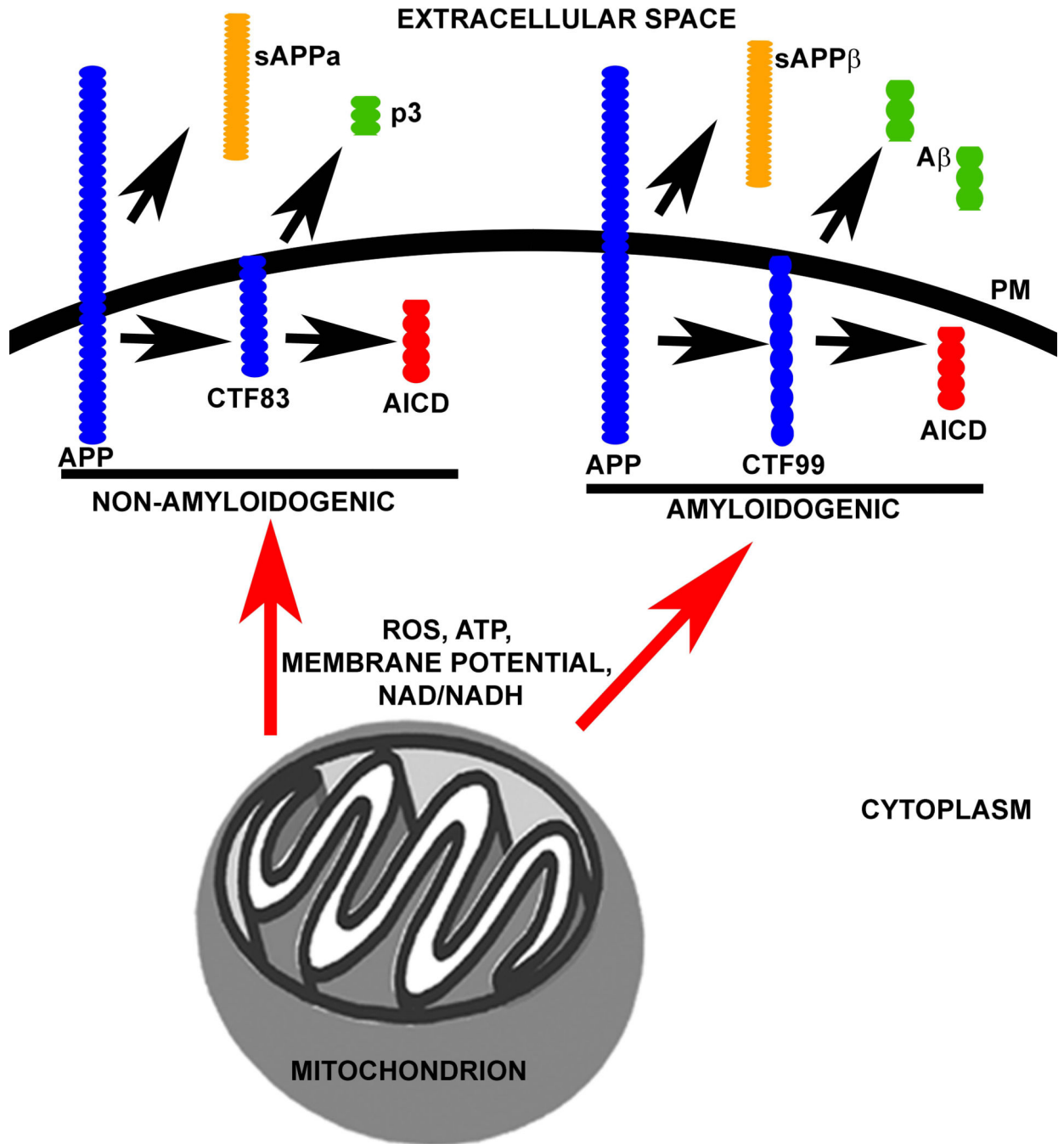
Figure 1. APP Processing Pathways

The non-amyloidogenic pathway begins with cleavage of full-length APP (membrane bound) by α-secretase, yielding sAPPα (ectodomain; released to extracellular space) and CTF83 (membrane bound). CTF83 is then cleaved by γ-secretase (three consecutive cleavage sites) to generate p3 (released to extracellular space) and AICD (released to cytoplasm). The amyloidogenic pathway begins with cleavage of full-length APP (membrane bound) by β-secretase, yielding sAPPβ (ectodomain; released to extracellular space) and CTF99 (membrane bound). CTF99 is then cleaved by γ-secretase (three

consecutive cleavage sites) to generate Aβ (released to extracellular space) and AICD (released to cytoplasm).
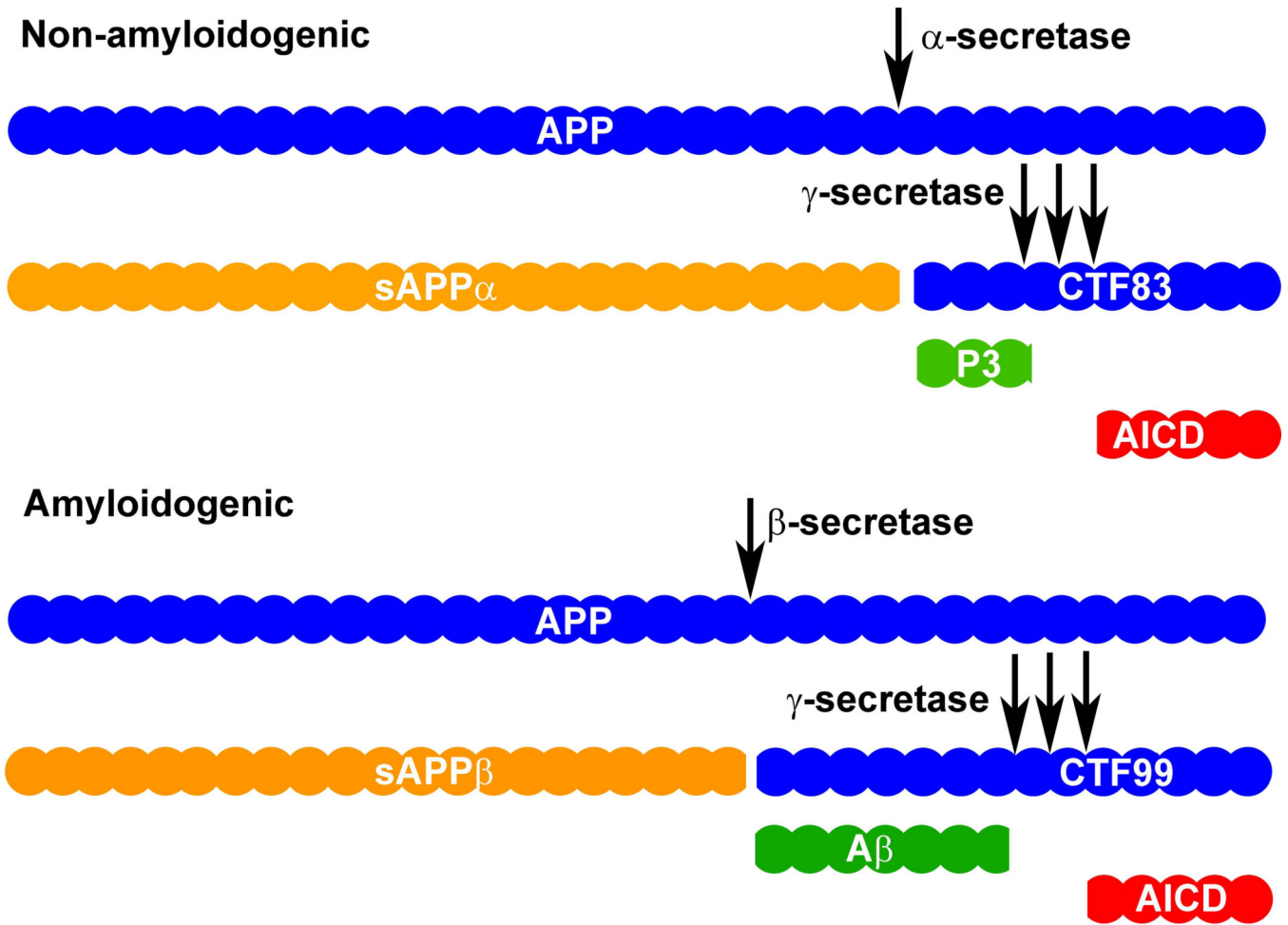
Figure 2. Mitochondrial function and bioenergetic intermediates influence APP Processing Pathways

Mitochondrial function and bioenergetic pathways (probably through intermediates, such as ROS, ATP, membrane potential, and NAD/NADH for example) can increase or decrease amyloidogenic and non-amyloidogenic APP processing. PM=plasma membrane.