



The Physical Approximation of APP and BACE-1: A Key Event in Alzheimer's Disease Pathogenesis

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ABSTRACT: Alzheimer's disease (AD) is characterized by the accumulation of insoluble deposits of Amyloid β ($A\beta$) in brains. $A\beta$ is derived by sequential cleavage of the amyloid precursor protein (APP) by β -site secretase enzyme (BACE-1) and γ -secretase. Proteolytic processing of APP by BACE-1 is the rate-limiting step in $A\beta$ production, and this pathway is a prime target for AD drug development. Both APP and BACE-1 are membrane-spanning proteins, transported via secretory and endocytic pathways; and the physical interaction of APP and BACE-1 during trafficking is a key cell biological event

initiating the amyloidogenic pathway. Here, we highlight recent research on intracellular trafficking/sorting of APP and BACE-1, and discuss how dysregulation of these pathways might lead to enhanced convergence of APP and BACE-1, and subsequent β -cleavage of APP. © 2017

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Keywords: amyloid precursor protein; β -site secretase enzyme; APP; BACE-1; trafficking; protein–protein interaction; amyloid beta; fluorescence complementation; endocytosis

INTRODUCTION

The presence of amyloid β ($A\beta$) aggregates as “plaques” in brains is a neuropathologic hallmark of Alzheimer's disease (AD). $A\beta$ is generated by sequential cleavage of the amyloid precursor protein (APP) by β -site secretase enzyme (BACE-1) and γ -secretase, with BACE-1-cleavage as the rate-limiting step. Both APP and BACE-1 are membrane-spanning proteins synthesized via the ER→Golgi pathway. Thereafter, both proteins are transported in vesicles, eventually leading to convergence of these two proteins, and cleavage of APP by BACE-1. Thus, physical

proximity of APP and BACE-1 is a crucial and early step in $A\beta$ production; and it has been long recognized that deciphering the trafficking steps of APP and BACE-1—leading up to APP cleavage—is critical for designing therapies to perturb $A\beta$ production. In this article, we discuss the known intracellular itineraries of APP and BACE-1, focusing on the trafficking/sorting pathways that lead to their co-localization and APP processing.

APP/BACE-1 CONVERGENCE

Most studies on APP and BACE-1 trafficking have been done in non-neuronal cells. In these cells, APP is predominantly localized to the trans-Golgi network (TGN) and Golgi-derived vesicles, with low levels along the cell surface and endosomal compartments (Caporaso et al., 1994; Koo et al., 1996). BACE-1 is predominantly within the TGN and endosomal compartments, with relatively low levels along the

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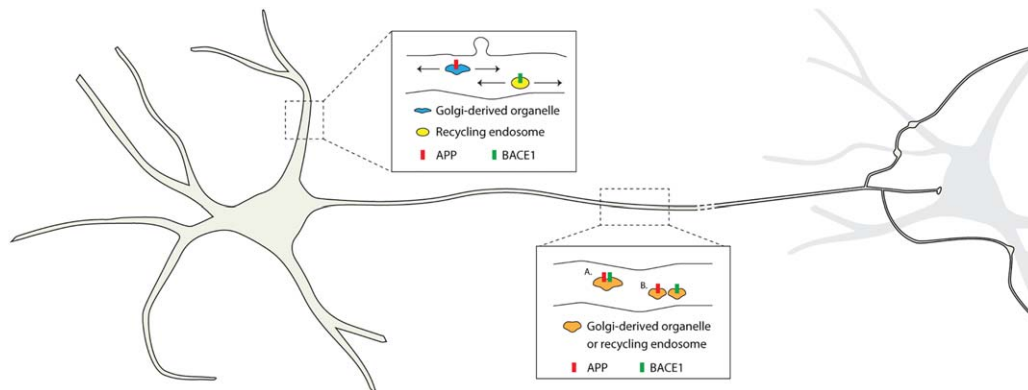


Figure 1 APP and BACE-1 transport in neurons. In dendrites (upper inset), APP is mainly transported in Golgi-derived organelles, while BACE-1 moves in recycling endosomes. The initial segregation of APP and BACE-1 appears to occur in the soma after synthesis, where BACE-1 is sorted into recycling endosomes. The internalized fraction of BACE-1 in dendrites move in a persistently retrograde manner, while internalized dendritic APP is not very mobile (not shown). In axons (lower inset), moving APP and BACE-1 are co-localized, suggesting that they are either in a single vesicle (A), or in a closely spaced vesicle that cannot be resolved by conventional light microscopy (B). The reason for this differential trafficking of APP and BACE-1 in axons and dendrites is unknown, but may be related to transcytosis. The precise identities of the axonal organelles in anterograde and retrograde transport are also unclear. [Color figure can be viewed at wileyonlinelibrary.com]

cell surface (Huse et al., 2000; Yan et al., 2001). Thus the TGN, plasma membrane, and endosomes are the main sorting stations for APP and BACE-1, and likely sites where APP and BACE-1 converge. Co-localization studies show that APP and BACE-1 are present in the TGN and endosomal compartments (Greenfield et al., 1999; Prasad and Rao, 2015).

Few studies have directly looked at the physical convergence of APP and BACE-1. Using fluorescence resonance energy transfer (FRET), Kinoshita et al. (2003) found that APP and BACE-1 interact along plasma membrane and early endosomal compartments in H4 neuroglioma cells. Recently, we developed an assay to visualize the physical convergence of APP and BACE-1 using fluorescence complementation (the optical convergence of APP and BACE-1, or OptiCAB assay; Das et al., 2016). Using this assay in cultured hippocampal neurons, we found that APP and BACE-1 interact in both ER/Golgi and endocytic compartments, particularly in recycling zones such as dendritic spines and presynaptic boutons. In other studies, we also found that after synthesis in the ER/Golgi, BACE-1 is selectively sorted into recycling endosomes (Das et al., 2013). Collectively, the data support a model where APP and BACE-1 are sorted into distinct vesicles after biogenesis. However, under conditions that promote amyloidogenesis, APP undergoes endocytosis and converges with BACE-1 in recycling endosomes, where β -cleavage occurs (Figure 1). Using the OptiCAB assay, Ferguson et al. recently reported an increase in APP/BACE-1 convergence in axons upon disruption of

lysosomal transport (Gowrishankar et al., 2017). Also, blockage of BACE-1 recycling to the axonal plasma membrane by BIN1, or blockage of APP sorting in dendrites by CD2AP enhances APP/BACE-1 co-localization (Ubelmann et al., 2017). Interestingly, unlike dendrites, APP and BACE-1 were found to be co-localized in axons (Das et al., 2016), supporting the view that they are differentially regulated in these two domains. Collectively, the data indicate that APP/BACE-1 sorting events play key roles in their convergence, and further emphasize the importance of endosomes for APP/BACE-1 interaction and initiation of the amyloidogenic pathway.

INTRACELLULAR ITINERARY OF APP

APP is ubiquitously expressed in all tissues, with particularly high levels in the brain. It is a single-pass transmembrane protein with a large extracellular N-terminus and a short cytosolic C-terminus (Reinhard et al., 2005). Biogenesis and trafficking of APP has also been mostly studied in non-neuronal cells. After protein synthesis in ER-associated polysomes, APP is N-glycosylated in the ER and then transported to the Golgi for O- and N-glycosylation, phosphorylation, and sulfonation at tyrosine sites (Lai et al., 1995). At steady state, most of the APP is found in the Golgi and trans-Golgi network (TGN), while a small portion is sorted to the plasma membrane (Kuentzel et al., 1993).

In neurons, tubulovesicular structures carrying APP are seen (Szodorai et al., 2009) in both dendrites and axons, but the transport behavior is very different in these two compartments. While the movement of dendritic APP vesicles is very interrupted—with short bursts of transport and frequent reversals—APP transport in axons is mostly persistent, with rare reversals (Kaether et al., 2000; Tang et al., 2012; Das et al., 2016). Axonal transport of APP is mediated by kinesin-1 (Ferreira et al., 1993; Kamal et al., 2001), and the retrograde transport is presumably dynein dependent. Though the motors involved in dendritic transport are not established, kinesin-I may be involved, as the instantaneous velocity of moving APP vesicles is similar in axons and dendrites (compare APP velocity parameters in dendrites (Supporting Information, Table 1 of Das et al., 2013) to axons (Supporting Information, Table 1 of Das et al., 2016)). APP transport is regulated by the scaffold protein c-Jun N-terminal kinase-interacting protein 1 (JIP1), which links APP to kinesin slight chain-1 (Klc-1; Matsuda et al., 2003). JIP1 is also reported to regulate kinesin heavy chain—via phosphorylation of the S421 site in JIP1—thereby controlling the directionality of APP transport (Fu and Holzbaur, 2013).

Through a pulse-chase analysis of newly synthesized APP, Toh et al. (2017) recently found that APP can be directly transported from Golgi to early endosomes through secretory pathway. Only a small portion of APP is detected at plasma membrane because the cell surface APP is rapidly internalized (Koo and Squazzo, 1994). Along the plasma membrane, APP is cleaved by α -secretase via a “non-amyloidogenic” process (Sisodia, 1992; De Strooper and Annaert, 2000). Importantly, the α -secretase enzyme cuts APP within the A β sequence, thereby precluding the BACE-1-mediated amyloidogenic pathway; thus α -cleavage is thought to be protective (Haass et al., 2012). APP internalization from the plasma membrane is mediated by clathrin-dependent endocytosis, which requires the canonical NPXY motif (Lai et al., 1995; Das et al., 2013). Though the precise fate of this internalized APP is still unclear, it is thought to return to the cell surface, degraded in lysosomes, or transported back to the TGN via a retromer-dependent pathway (Vieira et al., 2010). We found that in neurons, a substantial fraction of surface APP is routed into recycling endosomes, the latter enriched in BACE-1 (Das et al., 2013). However, a portion of the internalized APP is also routed into LAMP-1-positive late endosomes (Das et al., 2013). As BACE-1 is not enriched in this compartment, we speculate that this might be a pathway by which excessive β -cleavage is avoided. Alternatively, this

may also reflect postendosomal sorting/retrieval of APP (see later).

INTRACELLULAR ITINERARY OF BACE-1

BACE-1 is a transmembrane aspartyl protease with its catalytic domain at the extracellular site (Hong et al., 2000), and the enzyme is optimally active at acidic pH (Vassar et al., 1999). BACE-2, a homolog of BACE-1, has minimal effect on β -cleavage, though it may affect α -cleavage (Farzan et al., 2000). Importantly, BACE-1 knockout completely blocks β -cleavage and A β generation (Cai et al., 2001), indicating that this is a critical rate-limiting enzyme in the production of A β . BACE-1 is promiscuous, with many other substrates in addition to APP, such as voltage-gated sodium channel β 2-subunit (Wong et al., 2005), neuregulin 1 (Willem et al., 2006), sialyltransferase ST6GAL1 (Kitazume et al., 2001), and P-selectin glycoprotein ligand-1 (Lichtenthaler et al., 2003). BACE-1 knockout mice show abnormal myelination (Hu et al., 2006; Willem et al., 2006) and reduced spine density in hippocampal pyramidal neurons (Savonenko et al., 2008), attesting to the roles of this enzyme outside the amyloidogenic pathway. Thus, enzymatic inhibition of BACE-1—an approach pursued by most pharmaceutical companies—may not be the best strategy, and other avenues such as inhibition of APP/BACE-1 protein–protein interaction, or interference with APP/BACE-1 trafficking pathways before convergence, may be better alternatives.

Immature BACE-1 is synthesized in the ER with a short prodomain; and after transport to the Golgi, furin or furin-like proteases removes the prodomain (Creemers et al., 2001). Removal of the prodomain allows proper folding of the protease domain of BACE-1 and enhances its activity by twofold (Shi et al., 2001). In neurons, a large proportion of BACE-1 is localized to recycling endosomes (Das et al., 2016). Cell surface BACE-1 is quickly internalized, and a dileucine motif at the cytoplasmic tail of BACE-1 is thought to facilitate this internalization (He et al., 2005). Thus, there are two routes by which BACE-1 is sorted to endosomal compartments—direct transport from TGN—or internalization from plasma membrane. After internalization, the dendritic BACE-1 undergoes retrograde transport, regulated by the Eps-15-homology-domain-containing (EHD) proteins, a family of endocytosis regulators (Buggia-Prevot et al., 2013). In addition, axonal BACE-1 is also retrogradely transported by dynein and adaptor

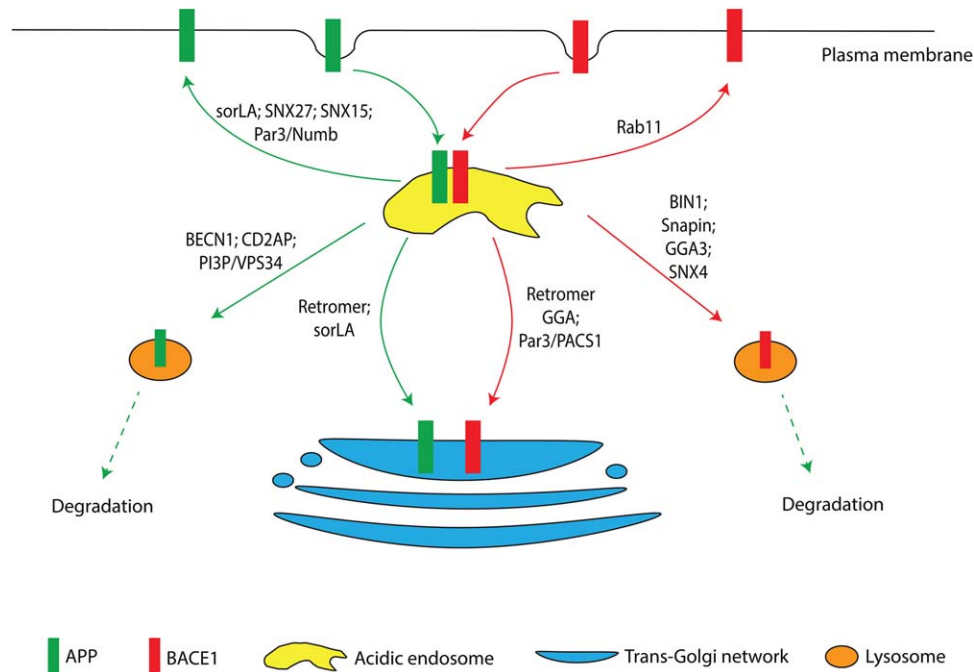


Figure 2 Sorting routes of endosomal APP and BACE-1. APP and BACE-1 interact in acidic endosomes after internalization. Further sorting/retrieval pathways help degrade and/or recycle these proteins, limiting excessive APP β -cleavage in acidic endosomes. The retromer complex binds to the adaptor protein sorLA, which in turn binds to APP and mediates its retrieval to the trans-Golgi network. APP recycling back to plasma membrane is mediated by sorLA, SNX27, SNX15, and Par3/Numb. BECN1, CD2AP, and PI3P/VPS34 modulate APP turnover and stability by sorting it into lysosomes. The retrieval pathway of BACE-1 is mediated by the retromer complex and sorting proteins GGA and PACS1. The recycling pathway of BACE-1 may be regulated by Rab11, and sorting of BACE-1 to lysosome for degradation is thought to be mediated by BIN1, Snapin, GGA3, and SNX4. [Color figure can be viewed at wileyonlinelibrary.com]

Snapin, which is important for BACE-1 degradation (Ye and Cai, 2014; Ye et al., 2017).

KEY REGULATORS OF APP/BACE-1 SORTING/RETRIEVAL IN AMYLOIDOGENIC PATHWAY

Internalization from the plasma membrane, and subsequent convergence of APP and BACE-1 is regulated by various adaptor/linker proteins (Figure 2). For example, APP interacts with the adaptor protein X11 through its YENPTY motif (Borg et al., 1996). This interaction retains APP in detergent-sensitive membranes; separating it from BACE-1, which is largely present in detergent-resistant membranes (Saito et al., 2008). Inhibition of this “APP sequestering” mechanism by knockout or phosphorylation of X11 causes a shift of APP into BACE-1-containing microdomains and $A\beta$ overproduction (Sano et al., 2006; Saito et al., 2008; Sakurai et al., 2008). APP endocytosis is also regulated by the adaptor protein Dab2, via

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interaction with the APP NPXY endocytosis motif (Nordstedt et al., 1993; Lee et al., 2008). In contrast to APP, internalization of BACE-1 is thought to be regulated by ADP ribosylation factor 6 (Arf6) and AP-2 complex (Sannerud et al., 2011; Prabhu et al., 2012).

After APP and BACE-1 convergence in endosomes, they are further sorted/retrieved into various cellular compartments. Understanding these sorting/retrieval pathways could also offer new therapeutic avenues to decrease APP-cleavage products. Under physiological conditions, APP and BACE-1 can be further sorted via three trafficking pathways: (1) retrieval to TGN; (2) recycling back to the plasma membrane; and (3) trafficking into lysosomes for degradation. Abnormal residence time of APP and BACE-1 in endosomes can lead to increased cleavage of APP, and this might be the pathological mechanism of sporadic AD (Gowrishankar et al., 2017). Studies have begun to shed light into the sorting/retrieval pathway in AD. A key regulator of APP retrieval pathway is sorLA (sorting protein-related receptor with A-type repeats) (Andersen et al., 2005),

a scaffold protein linking APP to the retromer complex (Fjorback et al., 2012). Overexpression of sorLA leads to the accumulation of APP in Golgi and reduction in A β production (Andersen et al., 2005). Genome-wide association studies (GWAS) also show an association of sorLA with sporadic, late-onset AD (Lambert et al., 2013). In mammals, the retromer complex is essential for retrieval transport, and it has been implicated in APP endosomal sorting in AD as well (Muhammad et al., 2008; Vieira et al., 2010).

Fjorback et al. (2012) found that the c-terminus FANSHY motif of sorLA interacts with the VPS26 subunit of the retromer complex, linking APP to the retromer. Disruption of this interaction redistributes APP to the endosomal network and increase amyloid processing. Knockdown of VPS35, the core element of the retromer complex, leads to accumulation of APP in endosomes along the processes of neurons and increases co-localization of APP and BACE-1 (Bhalla et al., 2012). In addition, the retrograde transport of the retromer complex is driven by motor protein dynein. Dysfunction of dynein blocks retrograde transport of retromer and APP, resulting in endosomal accumulation of APP and increased β -cleavage (Kimura et al., 2016). Endosomal APP can also recycle with the cell surface. The sorting nexins and the polarity protein Par3 play important roles in this process. Specifically, sorting nexin-27 (SNX27) positively regulates APP endosome-to-cell surface recycling by forming a ternary complex with APP and sorLA through interaction with c-terminus of sorLA (Huang et al., 2016). Similarly, SNX15 also increases cell surface level of APP by accelerating APP recycling (Feng et al., 2016). Par3 competes with the endocytic adaptor protein Numb to associate with APP, accelerating APP sorting to recycling endosomes, and recycling it back to plasma membrane (Sun et al., 2016). Acceleration of APP sorting to recycling endosomes and its recycling back to plasma membrane may protect APP from BACE-1-cleavage, reducing A β production. APP localized in early endosomes is further sorted into intraluminal vesicles (ILV) for degradation. Key molecules—CD2AP and phosphatidylinositol-3-phosphate (PI3P)/VPS34—were found to regulate this process (Morel et al., 2013; Ubelmann et al., 2017). Downregulation of these two pathways is thought to constrain APP at the limiting membrane of early endosomes, inhibiting APP sorting to ILV and increasing A β generation. The APP lysosome targeting is regulated by autophagy regulatory protein Beclin-1 (BECN1; see Swaminathan et al., 2016). BECN1 targets the internalized APP to lysosomes for degradation, while BECN1 phosphorylation at Ser295 inhibits BECN1/

APP interaction and subsequent APP degradative sorting.

Regarding BACE-1 retrieval trafficking, the retromer component SNX6 is thought to interact with BACE-1, regulating its retrograde transport (Small et al., 2005). Disruption of other retromer components, VPS26 and VPS35, blocks BACE-1 retrograde transport (He et al., 2005; Wen et al., 2011). Also, the sorting proteins GGA and PACS1, which mediate protein trafficking between TGN and endosomes, interacts with cytosolic dileucine motif of BACE-1 for its retrieval trafficking (Wan et al., 1998; Bonifacino, 2004; He et al., 2005; Sun and Zhang, 2017). The interaction between BACE-1 and these sorting proteins requires the phosphorylation of BACE-1 at Serine 498 by casein kinase 1 and aPKC/Par3 complex (Walter et al., 2001; von Arnim et al., 2004; Sun and Zhang, 2017).

Regulators of the BACE-1 recycling pathway were recently identified by a Rab-GTPase screen, where the authors found that Rab11 could specifically regulate recycling of the endosomal BACE-1 back to the plasma membrane, and thus control A β generation (Udayar et al., 2013). Pathways of BACE-1 degradation are also becoming clear. BIN1, a genetic risk factor of late-onset AD found in GWAS studies, binds to BACE-1, regulating its lysosomal targeting (Lambert et al., 2013; Miyagawa et al., 2016). Depletion of BIN1 retains BACE-1 in early endosomes and reduces BACE-1 lysosomal degradation, leading to increased A β production. The SNARE-associated protein snapin is also thought to regulate BACE-1 trafficking from late endosomes to lysosomes (Ye and Cai, 2014). In addition, GGA3 and SNX4 were also found to regulate BACE-1 stability by sorting it to lysosome for degradation (Tesco et al., 2007; Kim et al., 2017). However, the cooperation between these pathways to regulate BACE-1 degradation is still unclear.

SUMMARY

Pathways related to biogenesis and trafficking of APP and BACE-1 have been long recognized as important, and there has been significant progress. Yet many of the pathways are not firmly established, and their roles in α and β cleavage of APP are unclear. Cellular trafficking pathways are deeply intertwined, and the interrelationship between APP/BACE-1 biogenesis, trafficking, endocytosis and sorting is still uncertain. There are also some discrepancies in the literature that need to be resolved as we move forward. For instance, APP β -cleavage is

thought to occur in early endosomes, and yet at steady state, most of the BACE-1 in neurons appears to be in recycling endosomes.

One important issue is that a vast majority of the trafficking experiments have been done in non-neuronal cells that lack the unique morphology and polarization of neurons. For example, neurons have axons and dendrites that are several orders of magnitude longer than their minuscule cell bodies—the latter is presumably the anatomic correlate of a non-neuronal cell—and neurons also have pre- and postsynaptic specializations, where vesicle trafficking is known to be distinct from other compartments. While the utility of non-neuronal cells in unraveling the basic trafficking pathways of APP and BACE-1 is unquestionable, concepts emerging from these cells cannot simply be transferred to neurons. Furthermore, unbiased, discovery-based studies that look at the entirety of APP/BACE-1 trafficking pathways are also needed. The translational importance of APP/BACE-1 convergence in AD, the many unanswered questions surrounding this key phenomenon, and the lack of any disease modifying agent to date is a perfect trifecta for the coming generations of scientific explorers.

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