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Membrane rafts in Alzheimer's disease beta-amyloid production

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Abstract

Alzheimer's disease (AD), the most common age-associated dementing disorder, is pathologically manifested by progressive cognitive dysfunction concomitant with the accumulation of senile plaques consisting of amyloid- β (A β) peptide aggregates in the brain of affected individuals. A β is derived from a type I transmembrane protein, amyloid precursor protein (APP), by the sequential proteolytic events mediated by β -site APP cleaving enzyme 1 (BACE1) and γ -secretase. Multiple lines of evidence have implicated cholesterol and cholesterol-rich membrane microdomains, termed lipid rafts in the amyloidogenic processing of APP. In this review, we summarize the cell biology of APP, β - and γ -secretases and the data on their association with lipid rafts. Then, we will discuss potential raft targeting signals identified in the secretases and their importance on amyloidogenic processing of APP.

Keywords

Alzheimer's disease; amyloid; amyloid precursor protein; cholesterol; palmitoylation; lipid rafts

1. Introduction

Alzheimer's disease (AD) is one of the major neurodegenerative diseases that is predominant among aged individuals. The principal pathological hall marks of AD, originally described by Alois Alzheimer a little over one hundred years ago, are the two lesions, neurofibrillary tangles and senile plaques, which are found at significantly higher frequency in the cortex and hippocampus in individuals afflicted with AD compared to age matched healthy individuals [1]. Eighty years later, the molecular composition of senile plaques was deciphered with the advent of advanced biochemical and genetic tools. Senile plaques consist of extracellular deposits of 39–42 amino acid-long amyloid- β (A β) peptides. Subsequent studies revealed that A β is released from a large type I transmembrane protein, termed amyloid precursor protein (APP), by the sequential proteolysis of a set of enzymes termed β - and γ -secretases. Identification of familial AD linked mutations in APP gene launched the exploration of the cell biology of APP and these secretases to modulate A β production to attenuate disease pathology. A growing body of evidence indicates that changes in cholesterol homeostasis can influence A β production, and studies in neuronal and non-neuronal cells implicate cholesterol-enriched membrane microdomains, termed lipid rafts, in amyloidogenic processing of APP. However, the connection between cholesterol, lipid rafts and APP processing has not been

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completely understood, and controversy still exists. In this review, we will first describe the cell biology of APP, β - and γ -secretases followed by the mechanistic details of amyloidogenic processing. Then, we will elaborate on the current status of research addressing the importance of raft association of BACE1 and γ -secretase, and discuss potential raft targeting signals in the secretases and their unanticipated redundant role on amyloidogenic processing of APP.

2. Cell biology of APP, secretases and APP processing

2.1. An overview of APP and secretases

The *APP* gene is mapped to chromosome 21 and different isoforms of *APP* exist as a result of alternative splicing of the nascent transcript. Predominant isoforms include APP695, 751 and 770 which differ by the absence (APP695) or presence (APP751 and 770) of an extracellular Kunitz protease inhibitor (KPI) domain. APP695 is the major neuronal isoform whereas APP770 isoform is expressed in most other cell types. Direct correlation of APP overexpression and AD pathology is evident in Down's syndrome in which trisomy of chromosome 21 results in an extra copy of the *APP* gene. Despite the fact that normal physiological function of APP is still unclear, many putative functions have been ascribed that include regulation of neurite outgrowth, cell adhesion, synaptogenesis and cell survival. Although APP knockout mice are viable, they develop impairments in spatial learning and long-term potentiation (LTP) [2].

A β is released from the precursor, APP by a two step cleavage process involving two proteases, which is referred to as the amyloidogenic processing of APP. β -secretase cleaves APP in the luminal domain proximal to the transmembrane segment and generates the N-terminus of A β . γ -secretase mediates the cleavage that generates the C-terminus of A β . In addition to these two secretases, a third enzyme activity termed α -secretase, initiates non-amyloidogenic processing of APP. Because the later enzyme activity cleaves within the A β domain, cleavage by α -secretase precludes the generation of intact A β . Interestingly, all three secretases are transmembrane proteases: β -site APP-cleaving enzyme 1 (BACE1) is a transmembrane aspartyl protease [3]; α -secretase activity is associated with at least three members of the ADAM (a disintegrin and metalloprotease) family (ADAM9, ADAM10 and ADAM17) [4]; and γ -secretase is a multiprotein complex comprising four core subunits that are each transmembrane proteins—presenilins (PS1 or PS2), nicastrin, PEN2 and APh1 [5].

2.2. APP processing

APP, β - and γ -secretases are the three principal players involved in A β production. Amyloidogenic processing is initiated by BACE1 cleavage of APP, which results in the release of the large soluble ectodomain (APPs β) and a membrane-tethered C-terminal fragment (β -CTF). The second cleavage is mediated by γ -secretase, which cuts β -CTF within the transmembrane domain to release A β into the extracellular milieu and the APP intracellular domain into the cytoplasm. γ -secretase cleaves at multiple sites within the transmembrane segment of APP due to its heterogeneous site preference, thus generating variable length (39-42 amino acid long) A β peptides. The longer forms of A β are prone to rapid aggregation, oligomerization and fibril formation, events that are thought to be critical for the development of AD pathology. Non-amyloidogenic processing of APP is initiated by α -secretases, which cut APP at the luminal domain at 16 amino acids downstream of BACE1 cleavage site, also releasing a soluble ectodomain of APP (APPs α) and generating a truncated CTF (α -CTF) that is then cleaved by γ -secretase. Because α -secretase cleavage truncates the N-terminus of A β , non-amyloidogenic processing pathway results in the generation of N-terminally truncated A β peptides, referred to as p3 peptides. Amyloidogenic and non-amyloidogenic processing of APP are mutually exclusive and commitment of APP into these pathways depends on the cellular levels of α - and β -secretases. While α -secretase processing is predominant in non-neuronal cells, APP is mainly channeled into the amyloidogenic pathway in neurons as a

consequence of high abundance of BACE1 in neuronal cells [6]. Proteolytic processing of APP *per se* is a highly regulated event and additional regulatory components of secretases have been identified. Given that α - and β -secretases compete with each other for APP processing and have opposing effects on A β generation, deciphering the signaling pathways and molecular events involved in the commitment of APP to these pathways has high therapeutic potential. APP is not the sole physiological substrate for BACE1 and γ -secretase. Additional BACE1 substrates have been identified by candidate approaches that include APP-like proteins (APLP1 and APLP2), β -galactoside α , 2,6-sialyltransferase, P-selectin glycoprotein ligand-1, low-density lipoprotein receptor-related protein (LRP), β subunits of voltage-gated sodium channels, interleukin-1 receptor II (IL-1R2) and neuregulin1 and 3 [6–13]. Recently, Hemming et al [14] identified 60 more BACE1 substrates based on unbiased proteomic approach. The majority of BACE1 substrates are type I membrane proteins. Interestingly, most of these substrates are involved in contact-dependent intercellular communications. Similarly, γ -secretase can mediate sequence-independent cleavage of a wide range of type I transmembrane proteins that undergo ectodomain shedding, including Notch receptors and ligands, the netrin receptor DCC, the receptor tyrosine kinase ErbB-4, and LRP, extending the physiological role of PS1 beyond the nervous system and AD pathogenesis [15]. Additional substrates of γ -secretase were identified recently by an unbiased proteomic study; dystroglycan, the Delta/Notch like EGF-related receptor, desmoglein-2, natriuretic peptide receptor-C, plexin domain containing protein 2, and vasorin [16]. Given the fact that the list of BACE1 and γ -secretase substrates continues to grow, potential strategies to target BACE1 or γ -secretase activity will have to consider—and incorporate rational means to avoid—potential adverse consequences resulting from total inhibition of BACE1 and γ -secretase processing of diverse substrates.

2.3. Secretases cleave APP in diverse cellular compartments

The efficiency of APP processing to A β is greatly affected by its subcellular localization, and therefore the regulators of intracellular trafficking and subcellular localization of APP and the secretases have been extensively examined. APP is synthesized in the endoplasmic reticulum (ER) and is trafficked through the secretory pathway in a constitutive manner although only a small fraction of APP (~10%) arrives at the plasma membrane. APP is modified by the addition of N- and O-linked oligosaccharides, tyrosine sulfation and phosphorylation during the transit in the secretory pathway en route to the plasma membrane [17–22]. In cultured cells, at steady state, the majority of APP is localized in the Golgi apparatus, trans-Golgi network (TGN), and post-TGN vesicles. APP has a relatively short residence time at the cell-surface as it either undergoes α -secretase cleavage or becomes internalized into endosomes (Fig. 1) [23,24]. A ‘YENPTY’ internalization motif located near the C-terminal tail of APP is responsible for its efficient endocytosis. Several adaptor proteins including X11/Mint, Fe65, Dab1, JIP family of proteins and Sorting nexin bind to the ‘NPTY’ motif within the cytoplasmic domain of APP and regulate its trafficking and, ultimately modulate APP processing to A β [25–30]. In addition to cytosolic adaptors, several transmembrane proteins that include LDLR family members (LRP1, LRP1B and SorLA) also interact with APP, modulate its trafficking, and affect A β production [31–33]. In neurons, APP is axonally transported via the fast anterograde transport machinery. As a consequence, one documented source of amyloid deposits is the synaptically released A β pool [15,34]. The subcellular localization of APP processing to A β has been a topic of central importance. The majority of studies on localization of BACE1, γ -secretase and APP processing were performed in cultured cells, especially in non-neuronal cells such as CHO and HEK293 cells. In transfected cells, A β is mainly generated in the TGN and endosomes as APP is trafficked through the secretory and recycling pathways. This is consistent with the predominant localization of BACE1 and γ -secretase in these organelles. BACE1 is synthesized as a preproenzyme, the pro-domain of which is cleaved by furin-like protease as it is trafficked to the plasma membrane through the secretory pathway. BACE1 then cycles between the cell surface and endosomes, and at steady-state, the majority of BACE1 is found in the late Golgi–

TGN compartments and endosomes [35]. The C-terminus of BACE1 has an acidic dileucine motif (⁴⁹⁵DDISLLK⁵⁰¹) that targets BACE1 from plasma membrane to endosomes [35], and phosphorylation at Ser⁴⁹⁸ is implicated in trafficking between early endosomes and the TGN/late endosomes [36]. However, mutation of both of these trafficking signals did not influence BACE1 activity or A β production in overexpression studies [37]. Optimal activity of BACE1 at pH 4.5 *in vitro* implicates acidic organelles such as endosome as major sites of BACE1 cleavage of APP. Indeed, endocytosis of APP has been shown to be critical for A β production both in cultured cells and *in vivo* [38,39]. BACE1 cleaves wild-type APP during transit in the endocytic pathway [38]. Interestingly, APP bearing mutations associated with familial early-onset AD in a Swedish kindred (APP_{Swe}) is more readily cleaved by BACE1 in the secretory pathway, as early as during transit of nascent APP through the Golgi apparatus [40]. Cholesterol-enriched membrane microdomains termed lipid rafts has been implicated in BACE1 cleavage of APP (discussed below).

γ -secretase is a multiprotein complex made of four integral membrane proteins that include PS1, nicastrin, APH1 and PEN2. The assembly of γ -secretase complex starts with the stabilization of nascent PS1 by nascent nicastrin and APH1. Subsequently, PEN2 enters this trimeric complex to complete the assembly process [5]. Gene knockout, knockdown and mutational studies have established that PS1 is the catalytic subunit of γ -secretase [41–43]. Nicastrin has been proposed as the substrate binding subunit, but this notion has not yet gained wide acceptance. The subcellular localization of γ -secretase and its activity still remains controversial because subunits of this enzyme have been found in multiple organelles including ER, ER–Golgi intermediate compartments, Golgi apparatus, endosomes, lysosomes, phagosomes, plasma membrane, and mitochondria. By combining fractionation with non-ionic detergent extraction analysis, we found that γ -secretase subunits reside in cholesterol- and sphingolipid-rich detergent-resistant lipid raft microdomains of post-Golgi, TGN and endosome membranes (discussed below) [44].

3. Lipids and Alzheimer's disease connection

3.1. The role of cholesterol in AD pathogenesis

Cholesterol has long been clinically associated with AD pathogenesis and this connection attracted many research groups to explore the underlying causal role of cholesterol on APP processing for therapeutic intervention. In fact, the brain is the most cholesterol-rich organ in our body, which can sequester 25% of total cholesterol even though it contributes to 2% of total body weight [45,46]. Since, cholesterol is the main constituent of lipid rafts, it is imperative to understand the importance of lipid rafts in the central nervous system. Indeed, there is an increasing number of neurological functions attributed to lipid rafts that include neuronal cell signaling, adhesion and axon guidance [47–49]. The importance of cholesterol in AD pathogenesis became evident from the following studies. First, the levels of total cholesterol and LDL in serum correlate with A β load in the brains of patients with AD [50]. Second, epidemiological evidence suggests that individuals with elevated cholesterol levels during mid-life tend to develop AD pathology [51]. Third, in retrospective studies, patients treated with statins, inhibitors of the hydroxymethyl glutaryl-coenzyme A (HMG) reductase (the rate-limiting enzyme in cholesterol synthesis), to lower their cholesterol showed significantly reduced prevalence and incidence of AD [52,53]. In support of these results, elevated dietary cholesterol uptake has been found to increase amyloid plaque formation in rabbits [54]. Recently, however, the benefits of statins with respect to the incidence of AD or cognitive decline in patients with AD have been challenged [55–57]. Furthermore, recent studies demonstrated that the commonly used cholesterol-lowering agent lovastatin is known to have cholesterol-independent effects on APP trafficking and processing [58,59]. Fourth, cholesterol loading and depletion studies in cultured cells and in transgenic mouse models of AD found a correlation between cholesterol levels and the efficiency of A β production and

deposition [60–63]. Finally, in guinea pigs, as well as in transgenic mouse models of AD, treatment with cholesterol-lowering drugs markedly reduced A β deposition, demonstrating a positive correlation between plasma cholesterol levels and cerebral A β load [61,63].

3.2. Lipids modulate amyloidogenic processing of APP

Abnormality in cellular distribution and transport of cholesterol have been causally linked to many neurodegenerative diseases that include Alzheimer's disease, Smith–Lemli–Opitz syndrome, Huntington's, and Niemann–Pick Type C diseases [64–66]. In cell culture and animal experiments, alteration in subcellular cholesterol distribution has been found to modulate APP processing. Both secreted and intracellular A β were significantly reduced in neuronal cells when cholesterol transport from late endocytic organelles to the ER was blocked by the cholesterol transport inhibiting drug, U18666A [67]. Increased cholesterol efflux mediated by ATP-binding cassette transporter A1 decreases A β production by reducing BACE1 and γ -secretase cleavage of APP [68]. Cholesteryl esters, derived from free cholesterol by acyl-coenzyme A:cholesterol acyl transferase, have also been shown to modulate A β production [69]. Another study carefully examined the effect of statins, which lower the levels of cholesterol and nonsterol isoprenoids such as farnesyl pyrophosphate, and geranylgeranyl pyrophosphate, and showed that lowering cellular isoprenoid levels increased the intracellular pool of APP metabolites and A β [70]. Interestingly, supplementing mevalonate at a low level was sufficient to rescue statin-induced blockade of isoprenoid synthesis and prevent the increase in intracellular A β levels. These results suggest that cellular cholesterol and isoprenoid levels have independent effects on APP metabolism and A β production [70]. Sphingolipids, another major constituent of membrane raft domains are also involved in regulation of APP processing. Lowering sphingolipid levels either by inhibiting serine palmitoyltransferase, which is involved in the early step of sphingolipid biosynthesis, or by mutating one of the serine palmitoyltransferase enzyme subunits, elevates α -secretase cleavage [71]. Furthermore, secretion of A β ₄₂, but not A β ₄₀, was markedly elevated under these conditions, suggesting additional modulation of γ -secretase cleavage.

4. Amyloidogenic processing in lipid rafts

4.1 Lipid rafts

Lipid rafts are dynamic and highly ordered membrane microdomains rich in cholesterol and sphingolipids that are distinct from surrounding membranes of unsaturated phospholipids. The average size of lipid rafts is estimated to be 50 nm in diameter, although several distinct raft domains can exist in a cell that are heterogeneous in size and life time [72,73]. Lipid rafts concentrate select proteins and serve as a platform for cellular processes such as cell signaling, pathogen entry, cell adhesion, motility, protein sorting and trafficking [72,74]. At first, lipid rafts were biochemically defined as detergent-insoluble membrane (DIM) domains that resisted extraction with certain non-ionic detergents such as Triton X-100 and Lubrol WX at 4°C [75]. Now, there are several concerns about the use of detergent to study lipid raft localization of proteins in biological membranes, and other methods such as fluorescence visualization at nanoscale resolution are necessary to substantiate biochemical results [76]. A working definition of lipid rafts was developed at the 2006 Keystone Symposium on Lipid Rafts and Cell Function: “membrane rafts are small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein–protein and protein–lipid interactions” [77]. Although lipid rafts are highly abundant at plasma membrane, they are first assembled in the Golgi and are found in the anterograde vesicles trafficking from the Golgi to the plasma membrane in the biosynthetic pathway [75,78]. On the other hand, retrograde vesicles from Golgi to ER have very little sphingolipid and cholesterol content

[78]. Rafts are constantly endocytosed from plasma membrane through the endocytic pathway and either recycled back to plasma membrane or returned to Golgi apparatus [79,80].

4.2. The role of lipid rafts in amyloidogenic processing of APP

Multiple lines of evidence implicates lipid rafts in amyloidogenic processing of APP. A subset of BACE1 and full length APP (APP FL) associates with lipid raft domains [81,82]. Targeting the BACE1 luminal domain to lipid rafts by the addition of a glycosphosphatidylinositol anchor increases APP processing at the β -cleavage site [83]. Elegant work utilizing antibody-mediated co-patching of cell surface APP and BACE1 demonstrated that processing of APP into A β can be induced in raft microdomains [84]. Interestingly, however, Abad-Rodriguez *et al.* [85] reported that displacement of BACE1 from raft domains by moderate reduction of cholesterol promotes membrane proximity of BACE1 and APP in non-raft domains and increases β -cleavage of APP [85]. Each of the four core subunits of the γ -secretase complex are enriched in DIM fractions that are positive for *bona fide* lipid raft markers, flotillin-2 and prion protein [44]. More importantly, raft association of γ -secretase subunits is sensitive to acute cholesterol depletion, fulfilling a stringent criterion for determining lipid raft localization. Involvement of lipid rafts in amyloidogenic processing of APP by γ -secretase is also demonstrated by the increased accumulation of APP CTFs in lipid raft microdomains either by inhibition or absence of γ -secretase activity [44]. Studies using a combination of biochemical fractionation and magnetic immunoisolation, indicate that the γ -secretase complex co-resides in lipid raft microdomains with APP CTFs and SNARE proteins such as VAMP4 (TGN), syntaxin 6 (TGN and vesicles) and syntaxin 13 (early endosomes) [44,82]. These studies indicate that γ -secretase processing of APP CTFs may be preferentially localized in lipid raft microdomains of post-Golgi and endocytic organelles. Interestingly, mature components of the γ -secretase complex are excluded from cell surface raft domains that are positive for SNAP-23. These results suggest that the relatively small amount of active γ -secretase complex present at the cell surface could be residing in non-raft membrane domains [44]. Interestingly, our studies also showed that spatial segregation of the γ -secretase complex in membrane rafts of intracellular organelles might limit the access to some of its diverse substrates [82]. For example, APP CTFs in adult brain and cultured cells preferentially enriched in raft microdomains, whereas several other substrates such as CTFs derived from Notch1, Jagged2, N-cadherin and DCC reside in non-raft membranes [82]. These findings reiterate the prediction that γ -secretase might preferentially cleave APP in lipid rafts.

5. Approaches to target raft associated amyloidogenic processing of APP

5.1. Cholesterol depletion

Selective targeting of BACE1 and γ -secretase processing of APP in lipid rafts is considered as an elegant therapeutic strategy to modulate A β production. Cholesterol is the main constituent of lipid rafts and depletion of cellular cholesterol by sequestering agent such as methyl- β -cyclodextrin disturb the integrity of lipid raft domains. Therefore, cholesterol depletion was used as an approach to displace secretases and APP from raft domains. As a consequence, APP processing to A β was strongly inhibited with a concomitant increase in α -secretase processing of APP, which does not involve lipid rafts [84]. These results however are difficult to interpret because the increase in α -secretase processing of APP may not be a direct consequence of loss of raft integrity, but possibly the end result of other mechanisms including accumulation of APP at the cell surface and altered membrane fluidity [86]. Further studies using cultured hippocampal neurons showed that depending on the extent of depletion, loss of cholesterol can either have a positive (<25% loss) or negative (>35% loss) effect on amyloidogenic processing of APP and therefore raised concerns on the use of cholesterol lowering drugs such as statin as a therapeutic approach to lower A β production [85]. Another critical issue that confounds proper interpretation of statin studies on A β production is the pleiotropic effects of cholesterol

depletion on Golgi morphology and vesicular trafficking, which are an unexpected consequence of changes in both membrane fluidity and curvature [87,88]. In addition, lovastatin has been shown to decrease cholesterol levels in the exofacial membrane leaflet and to reduce membrane bulk fluidity [89]. Taken together these studies necessitate better approaches for the displacement of APP or its secretases from raft domains in an attempt to reduce A β production.

5.2. Raft targeting signals in BACE1

A more realistic approach will be to identify the raft targeting signals in secretases and APP. The attachment of a glycosylphosphatidylinositol (GPI) group is responsible for raft targeting of proteins that are located on the extracellular face of the plasma membrane. Post-translational acyl modifications of proteins such as *S*-palmitoylation, *N*-myristoylation target variety of cytosolic and transmembrane proteins to lipid raft microdomains due to the high affinity of acyl chains for the ordered lipid environment within raft domains [90]. BACE1 undergoes *S*-palmitoylation at four Cys (Cys^{474/478/482/485}) residues near the transmembrane and cytoplasmic boundary (Fig. 2) [91,92]. Experimental mutation of these residues completely abolishes palmitoylation of BACE1 and prevents raft association of BACE1. Importantly, unlike the case in many other proteins, the lack of palmitoylation does not affect protein stability or subcellular localization of BACE1. Surprisingly however, displacement of BACE1 by abolishing *S*-palmitoylation neither affected BACE1 processing of APP nor the secretion of A β in cultured cell lines. These results indicate that *S*-palmitoylation-dependent raft targeting of BACE1 is dispensable for APP processing and the palmitoylation-deficient mutant of BACE1 can process APP as efficiently as wild type BACE1 [92]. To substantiate this conclusion, biochemical fractionation showed that there is a considerable shift in the distribution of APP CTFs into non-raft fractions in cells stably expressing palmitoylation-deficient BACE1 mutant, consistent with BACE1 processing of APP in non-raft domains.

5.3. Raft targeting signals in γ -secretase

Our earlier studies demonstrated that γ -secretase and APP CTFs co-reside in raft microdomains of late endosomes, the TGN, and TGN-derived vesicles and processing of other γ -secretase substrates is segregated from the APP processing in the raft domains [82]. As discussed above, displacement of BACE1 from lipid rafts by substitution of palmitoylation sites did not affect A β production. Irrespective of BACE1 processing of APP in raft (BACE1 wild type) or non-raft domains (BACE1 palmitoylation-deficient mutant), BACE1 cleaved APP CTFs eventually accumulate in lipid raft domains [92], and similarly α -secretase cleaved fragments (APP α -CTFs) in *BACE1*^{-/-} fibroblasts also accumulate in lipid raft domains (our unpublished results). Furthermore, absence or inhibition of γ -secretase activity results in the accumulation of APP CTFs in raft domains [82,93]. Together, these results strongly suggest that γ -secretase cleavage of α -CTF and β -CTF occurs in raft domains. Therefore, displacing either γ -secretase or its immediate substrate, APP CTFs from raft domains should theoretically be an effective strategy to reduce A β production by separating the enzyme away from the substrate. We recently identified potential raft targeting signals in γ -secretase subunits, nicastrin and APH1 [94]. Nicastrin is *S*-palmitoylated at cysteine residue (Cys⁶⁸⁹) in the transmembrane domain and APH1 undergoes *S*-palmitoylation at Cys¹⁸² and Cys²⁴⁵ that are oriented towards the cytosol (Fig. 2). Unlike the case of BACE1, *S*-palmitoylation contributes to the protein stability of nascent nicastrin and APH1. Interestingly, assembly of nicastrin and APH1 into the γ -secretase complex does not require palmitoylation, and the stability of palmitoylation-deficient nicastrin and APH1 that are already assembled in the γ -secretase complex is indistinguishable from that of the wild type subunits. Furthermore, palmitoylation of nicastrin and APH1 is required for raft association of these nascent subunits but did not affect the raft localization of PS1 and PEN2 or the assembled γ -secretase complex. Therefore, these studies imply the presence of additional dominant signals or interacting proteins that may target the fully

assembled γ -secretase complex to rafts. In addition to lipid attachment, hydrophobic transmembrane domain residues in contact with the exoplasmic leaflet of the membrane have been implicated in raft association of proteins [95]. Protein–protein interactions also facilitate raft targeting of certain proteins, such as in the case of raft localization of glutamate receptor-interacting protein 2 through interaction with the C-terminus of the raft-resident transmembrane protein ephrin B1 [96]. Like in the case of BACE1, stable overexpression of these palmitoylation-deficient mutant subunits of nicastrin and APH1 did not affect amyloidogenic processing of APP and as well as other γ -secretase substrates. Thus the physiological significance of S-palmitoylation of APP secretases has not been completely understood.

5.4. Raft targeting signals in APP

Analysis of APP has revealed an interesting characteristic with reference to lipid raft association. Less than 10% of APP FL associate with lipid raft domains, whereas APP CTFs accumulate predominately in these domains. Therefore, the signals within the transmembrane domain or cytosolic tail of APP CTFs that promote raft association following ectodomain shedding of APP FL, remain to be identified. The available data indicate that APP association with lipid rafts is enhanced during endocytic trafficking [84]. APP interacting proteins which modulate A β production such as cytosolic adaptor proteins, Mint and Fe65 or transmembrane receptor proteins, LRP and SorLA may have a role on the segregation of APP CTFs into the rafts or the exclusion of APP FL from lipid raft domains. Interestingly, the cytoplasmic domain of LRP has been shown to facilitate the association of APP and BACE1 and enhances the delivery of APP in lipid rafts through the endocytic pathway [97]. Given the fact that a number of γ -secretase substrates are spatially segregated in non-raft domains [82], the rational design of inhibitors that target raft-localized γ -secretase seems to be promising and possibly preclude at least some of the potential side effects associated with the complete inhibition of γ -secretase.

6. Conclusion

The role of cholesterol in amyloidogenic processing of APP came under close scrutiny following the publication of epidemiological studies that correlated cellular cholesterol and AD pathogenesis. Given that cholesterol is one of the major constituent of lipid rafts, the involvement of raft membrane microdomains in APP processing was investigated. Indeed, multiple lines of evidence suggest that amyloidogenic processing of APP is associated with membrane raft microdomains. Spatial segregation of γ -secretase processing of APP from that of other substrates suggested targeting of APP processing in lipid rafts as a novel approach to selectively modulate A β production. Surprisingly, displacement of BACE1 by mutating raft targeting signals in this secretase did not affect the APP processing in cell culture studies. Similarly, raft targeting signals identified in γ -secretase complex subunits, nicastrin and APH1 neither affected the raft association of mature γ -secretase complex nor APP processing to A β . Thus the targeting signals that are primarily responsible for lipid raft association of γ -secretase and APP CTF await identification. Further investigation in this area could possibly provide a rational approach to pharmacologically target APP processing in lipid rafts.

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Abbreviations

APP amyloid precursor protein

PS	presenilin (s)
BACE1	β -site APP cleaving enzyme 1
A β	β -amyloid
CTF	C-terminal fragment
TGN	trans-Golgi network
DIM	detergent-insoluble membrane
VAMP	Vesicle-associated membrane protein

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Fig. 1.

Schematic illustration of intracellular itinerary of amyloid precursor protein (APP). Synthetic APP is trafficked through the constitutive secretory pathway to the plasma membrane (blue arrows). From the cell surface, a fraction of APP is internalized and trafficked through endocytic and recycling compartments (red arrows) to reach the cell surface, the TGN, or sorted to the lysosomes for degradation (dotted red arrows). Non-amyloidogenic processing occurs mainly at the cell surface, where α -secretase activity is abundant. Amyloidogenic processing likely occurs in the endocytic pathway as APP encounters BACE1 and γ -secretase in the endosomes and recycling organelles. γ -secretase subunits and APP CTFs are enriched in lipid raft microdomains isolated from these compartments (highlighted by a circle). EE, Early endosome; For simplicity, the constitutive secretory trafficking pathway is not indicated by arrows.

Fig. 2.

Raft targeting signals identified in BACE1 and γ -secretase subunits nicastrin and APh1. BACE1 is S-palmitoylated at four Cys residues (Cys474/478/482/485) located at transmembrane and cytoplasmic boundary. (B) S-palmitoylation of γ -secretase subunits nicastrin and APh1 at indicated Cys residues were identified by mutagenesis; Nicastrin is S-palmitoylated at Cys 689 and APh1 is at Cys 182/245.