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Mechanisms of Disease: new therapeutic strategies for Alzheimer's disease—targeting amyloid precursor protein processing in lipid rafts

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SUMMARY

Alzheimer's disease (AD) is the most common cause of age-related dementia. Pathologically, AD is characterized by the deposition in the brain of amyloid-β peptides derived from proteolysis of amyloid precursor protein (APP) by β-site APP cleaving enzyme 1 (BACE1) andγ-secretase. Growing evidence implicates cholesterol and cholesterol-rich membrane microdomains in amyloidogenic processing of APP. Here, we review recent findings regarding the association of BACE1, γ-secretase and APP in lipid rafts, and discuss potential therapeutic strategies of AD based on knowledge gleaned from the membrane environment that fosters APP processing.

Keywords

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

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease, and the leading cause of progressive dementia. The principal neuropathological characteristics of AD are the two lesions first described by Alzheimer, neurofibrillary tangles and senile plaques, which are found at significantly higher frequency in the cortex and hippocampus in individuals with AD than in other aged individuals.[1] Senile plaques are composed of dystrophic neurites displayed around extracellular deposits of 39–42 amino-acid-long amyloid-β (Aβ) peptides. Aβ is generated by sequential proteolytic processing of a large type I transmembrane protein, termed amyloid precursor protein (APP). It is becoming clear that changes in cholesterol homeostasis can markedly influence Aβ production, and studies in neuronal and non-neuronal cells implicate membrane microdomains rich in cholesterol, termed lipid rafts, in amyloidogenic processing of APP.

In this article, we review recent developments relating to factors that affect amyloidogenic processing by influencing compartmentalization of APP and secretase activities, and discuss potential strategies and advantages of targeting APP processing in lipid rafts.

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Competing interests

COMPARTMENTALIZATION OF AMYLOID PRECURSOR PROTEIN AND SECRETASES

Amyloid precursor protein secretases and processing pathways

APP is proteolytically cleaved through three enzyme activities. β-secretase and α-secretase cleave APP within the luminal domain, and a third activity, termed γ-secretase, cleaves APP within the transmembrane domain. Interestingly, all three APP secretases are transmembrane proteases: β-site APP-cleaving enzyme 1 (BACE1), the main neuronal β-secretase, is a transmembrane aspartyl protease;[2] α-secretase activity is associated with at least three members of the ADAM (a disintegrin and metalloprotease) family (ADAM 9, ADAM10 and ADAM17);[3] and γ-secretase is a multiprotein complex comprising four core subunits that are all transmembrane proteins—presenilins (PS1 or PS2), nicastrin, PEN2 and APH1.[4]

In the first step of amyloidogenic processing, BACE1 cleaves APP to generate a secreted ectodomain (APPsβ) and a membrane tethered-C-terminal fragment (β-CTF).[2] Then, γ-secretase cleaves β-CTF within the transmembrane domain to release Aβ into the extracellular milieu and the APP intracellular domain into the cytoplasm. Alternatively, APP can be cleaved within the Aβ domain by α-secretase to generate a secreted ectodomain (APPsα) and a truncated C-terminal stub (α-CTF) that can also be cleaved by γ-secretase. This nonamyloidogenic processing pathway precludes intact Aβ formation, however, because α-secretase cleavage truncates the N-terminus of Aβ.

The amyloidogenic pathway initiated by BACE1 cleavage appears to be the predominant mode of APP metabolism in neurons, which is probably a consequence of high levels of BACE1 expression.[6] In view of the fact that α-secretase and β-secretase cleavages of APP have opposite effects on Aβ generation, deciphering the signaling pathways and molecular events involved in the commitment of APP to these pathways has potential therapeutic value. Interestingly, the affinity of BACE1 towards APP is relatively low, which indicates that APP is not its sole physiological substrate. Indeed, recent studies identified additional BACE1 substrates including 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, and neuregulin.[5–11] 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.[13] Potential strategies to target BACE1 or γ-secretase activity will therefore have to consider—and incorporate rational means to avoid—adverse consequences resulting from inhibition of these diverse substrates.

Secretases cleave amyloid precursor protein in multiple subcellular compartments

Nascent APP is post-translationally modified by *N*-glycosylation and *O*-glycosylation, phosphorylation, and tyrosine sulfation en route to the plasma membrane in the secretory pathway (Figure 1, step 1). At steady state, the majority of APP is localized in the Golgi apparatus, trans-Golgi network (TGN), and post-TGN vesicles. APP trafficking in neurons acquires an extra layer of complexity, as APP is axonally transported via the fast anterograde transport machinery. As a consequence, one documented source of amyloid deposits is the synaptically released Aβ pool.[12,13]

In cultured cells, it is estimated that only about 10% of nascent APP molecules arrive at the plasma membrane. A fraction ofAPP is cleaved by α-secretases on arrival at the cell surface or during late steps in the transit to the surface, resulting in the shedding of APPsα ectodomain.[14] This is consistent with the known function of ADAM proteases in shedding ectodomain

fragments of various transmembrane proteins, including tumor necrosis factor, the p75 tumor necrosis factor receptor, Notch receptors, the Notch ligand Delta, transforming growth factor-α, L-selectin, and the interleukin-6 receptor α subunit. Moreover, ADAM family metalloproteases are type I transmembrane proteins that become active when their pro-domain is removed by proteolytic processing as they traffic through the secretory pathway. Activation of protein kinase C increases APPsα secretion (refered as "regulated" α-secretase processing) by mechanisms involving the formation and release of secretory vesicles from the TGN, thereby enhancing APP (and possibly α-secretase) trafficking to the cell surface.

Unlike many cell surface receptors, full-length APP does not dwell at the cell surface for extended periods of time. It is estimated that roughly two thirds of surface-bound APP is internalized within minutes of arriving at the plasma membrane (Figure 1, step 2). A 'YENPTY' internalization motif located near the C-terminal tail of APP is responsible for its efficient endocytosis. Following endocytosis, APP is trafficked to late endosomes and a fraction of endocytosed molecules are recycled to the cell surface (Figure 1, step 3). Studies using pharmacological inhibitors suggest that appreciable amounts of endocytosed APP can also undergo degradation within lysosomes. Data from analysis of transfected cell lines indicate that Aβ is mainly generated in the TGN as APP is trafficked through the secretory and recycling pathways. This conclusion is consistent with the subcellular sites where BACE1 and γ-secretase reside predominantly in cultured cell lines. 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 compartment and endosomes.[15] BACE1 is most active at acidic pH *in vitro*, which also implicates acidic organelles such as endosome as major sites of BACE1 cleavage of APP. Indeed, there has been compelling early evidence to suggest that BACE1 cleaves wild-type APP during transit in the endocytic pathway.[16] By contrast, APP harboring the Swedish double K670N–M671L mutation associated with familial early-onset AD (FAD) is more readily cleaved by BACE1 in the secretory pathway, as early as during transit of nascent APP though the Golgi apparatus.[17] A significant amount of BACE1 is localized in lipid raft microdomains of cellular organelles (see below). The localization of γ-secretase has been a somewhat controversial issue, because subunits of this enzyme have been found in multiple organelles including the endoplasmic reticulum (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 components reside in cholesterol- and sphingolipid-rich detergent-resistant lipid raft microdomains of post-Golgi, TGN and endosome membranes (see below).[18]

LIPID RAFT LOCALIZATION OF BACE1 AND γ-SECRETASE

Lipid rafts

Specialized organelle membrane microdomains rich in cholesterol and sphingolipids, termed lipid rafts, participate in a variety of important biological functions.[19] Initially, 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 at 4°C.[20] Most researchers now agree, however, that there are serious limitations in the use of detergents to study lipid raft localization of proteins in biological membranes, and other methods such as fluorescence visualization at nanoscale resolution are essential to corroborate biochemical results.[21] On the basis of a consensus between biophysicists, biochemists and cell biologists, 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".[22] Lateral segregation of cholesterol and sphingolipids is thought to initially occur in the Golgi, where nascent sphingolipids mature and are present on the plasma membrane, and in the membranes of the Golgi, TGN and endocytic compartments. Rafts are heterogeneous in size and lifetime, and they are suggested as a means to form ordered platforms, which float around in the liquid-disordered matrix of the cellular membranes and represent versatile devices to compartmentalize membrane-associated signaling processes.[19,23]

Association of β-secretase with lipid rafts

Mounting evidence suggests that lipid rafts might be the principal membrane platforms in which amyloidogenic processing of APP occurs. As mentioned above, BACE1 undergoes *S*-palmitoylation, a post-translational modification responsible for targeting a variety of cytosolic and transmembrane proteins to lipid rafts.[24] Indeed, biochemical fractionation and antibody co-patching methods reveal that a significant fraction of BACE1 is found in lipid rafts, and targeting the BACE1 lumenal domain to lipid rafts by the addition of a glycophosphatidylinositol anchor increases APP processing at the β-cleavage site.[25–27] Elegant studies by Simons and colleagues showed antibody-mediated co-patching of cell surface APP and BACE1, as well as amyloidogenic processing of APP, in raft microdomains.[28] Interestingly, however, Abad-Rodriguez *et al*. recently 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.[29] Unlike BACE1, α-secretases have not been linked to raft microdomains on the basis of cholesterol depletion and loading studies.[28]

Association of γ-secretase with lipid rafts

Each of the four core subunits of the γ-secretase complex (PS1-derived N-terminal and C-terminal fragments, nicastrin, APH-1, and PEN-2) is localized in DIM fractions enriched in the lipid raft markers flotillin-2 and prion protein (Figure 2A).[18] Association of γ-secretase components with DIM domains is sensitive to acute cholesterol depletion using the cholesterol-sequestering agent methyl-β-cyclodextrin, fulfilling a stringent criterion for determining lipid raft localization. The paucity of γ-secretase at the plasma membrane makes cell surface co-patching studies unsuccessful in visualizing membrane raft localization of endogenous γ-secretase. Nevertheless, detailed biochemical fractionation studies, including magnetic immunoisolation, indicate that the γ-secretase complex co-resides in lipid raft microdomains with APP CTFs and SNARE proteins such as VAMP-4 (TGN), syntaxin 6 (TGN and vesicles) and syntaxin 13 (early endosomes).[18] These studies strongly implicate lipid raft microdomains of intracellular organelles as the preferred sites of γ-secretase processing of APP CTFs. Interestingly, the cell-surface raft-associated protein SNAP-23 does not co-reside with mature components of the γ-secretase complex, raising the possibility that the relatively small amount of active γ-secretase complex present at the cell surface could be residing in non-raft membrane domains.[18] Such spatially distinct localization of the γ-secretase complex probably allows intramembranous processing of diverse substrates. Indeed, our recent studies indicate that APP CTFs in adult brain and cultured cells preferentially accumulate in raft microdomains (Figure 2A), whereas several other substrates such as CTFs derived from Notch1, Jagged2, N-cadherin and DCC remain in non-raft membranes.[30] Taken together, these findings are consistent with the prediction that γ-secretase cleavage of APP occurs in lipid rafts. Further investigations are needed to clarify the mechanisms regulating γ-secretase distribution between raft and non-raft microdomains.

TARGETING AMYLOID PRECURSOR PROTEIN PROCESSING IN LIPID RAFTS

Therapeutic strategies aimed at modulation of lipids have been proposed for the treatment of several diseases, including cancer, cardiovascular disease, obesity, metabolic disorders, infectious diseases, inflammation, and Alzheimer's disease.[31] The organization and maintenance of lipid rafts can be potentially modulated by the levels of cholesterol and sphingolipids. Targeting amyloidogenic processing in lipid rafts is a relatively unexplored area in Alzheimer's disease therapeutics. Nevertheless, a wealth of information is available implicating cholesterol levels with AD pathogenesis, and cholesterol has been considered as a major candidate for targeting amyloidogenic processing of APP in lipid rafts.

Cholesterol and amyloid-β production

Cholesterol has attracted particular interest in the context of AD pathogenesis in recent years. Levels of total cholesterol and LDL in serum have been found to correlate with Aβ load in the brains of patients with AD.[32] Epidemiological evidence suggests that elevated cholesterol levels during mid-life increase the risk of developing AD.[33] In retrospective studies, patients treated with statins (inhibitors of the hydroxymethyl glutaryl-coenzyme A reductase, the rate-limiting enzyme in cholesterol synthesis) to lower their cholesterol showed significantly reduced prevalence and incidence of AD.[34,35] In agreement with these results, elevated dietary cholesterol uptake has been found to increase amyloid plaque formation in rabbits.[36] Furthermore, 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 Aβ production and deposition.[37–40] For example, depletion of cholesterol by 70% (measured by equilibrium labeling of the cholesterol pool with [^{3}H] cholesterol for 16 h) in cultured hippocampal neurons through treatment with a combination of the lipophilic statin lovastatin and the cholesterol-extracting agent methyl-β-cyclodextrin markedly lowered Aβ production.[37] In guinea pigs, as well as in an APP/PS1 double-transgenic mouse model, treatment with cholesterol-lowering drugs markedly reduced Aβ deposition, demonstrating a positive correlation between plasma cholesterol levels and cerebral Aβ load.[38,40]

Statins are clinically safe drugs that pass through the blood–brain barrier, so they could potentially be used as an effective means of reducing Aβ burden. Recently, however, the beneficial effect of statins with respect to the incidence of AD or cognitive decline in patients with AD have been challenged.[41–43]. Furthermore, it has been suggested that the primary action of statins in humans with AD might be to reduce inflammation attributable to microglial activation, rather than to decrease Aβ load.[44] Decisions regarding the clinical use of statins as a therapy for AD pathogenesis are therefore likely to depend on the outcomes of additional larger clinical trials.

Certain aspects of cholesterol metabolism seem to regulate Aβ generation by directly influencing amyloidogenic processing of APP by BACE1 and γ-secretase. This is expected, given the association of BACE1, γ-secretase and APP CTFs with lipid rafts. In addition to modulating BACE1 and γ-secretase processing, lowering of cholesterol levels leads to marked elevation of nonamyloidogenic processing of APP by increasing α-secretase expression and activity, and through other mechanisms including accumulation of APP at the cell surface and altered membrane fluidity.[45] The results of a recent study by Dotti and colleagues, however, raised concerns in pursuing a therapeutic strategy involving drugs to lower cholesterol: in contrast to the drastic decrease in cholesterol levels achieved in many studies, moderate reduction (<25% loss) of membrane cholesterol in cultured hippocampal neurons paradoxically increased Aβ production by promoting β-secretase cleavage of APP.[29] The authors found that moderate loss of cholesterol in hippocampal neurons facilitates spatial proximity of APP and

BACE1 by displacing BACE1 from lipid rafts. They did, however, show that >35% loss of membrane cholesterol (quantified using enzymatic oxidation of cholesterol by cholesterol oxidase) leads to a marked decrease in Aβ production, confirming the earlier data on cholesterol depletion and APP processing. This study therefore indicates that the extent of cholesterol loss is a critical factor that determines the outcome on Aβ production.

Another important issue that confounds proper interpretation of statin studies on Aβ production is the pleiotrophic effects of cholesterol depletion on Golgi morphology and vesicular trafficking, which are expected consequence of changes in both membrane fluidity and curvature.[46,47] Furthermore, lovastatin has been shown to decrease cholesterol levels in the exofacial membrane leaflet and to reduce membrane bulk fluidity.[48] The precise interplay between cholesterol levels, APP trafficking and processing at the subcellular level might therefore be much more complicated than was previously understood.

In addition to total cholesterol level, subcellular distribution and transport of cholesterol have also been found to be critical for APP metabolism. Inhibition of intracellular cholesterol transport from late endocytic organelles to the endoplasmic reticulum by class-2 amphiphiles such as U18666A causes dose-dependent reduction in levels of both secreted and cellular Aβ in cultured neurons and neuroblastoma cells.[49] Overexpression of ATP-binding cassette transporter A1, which mediates cholesterol efflux, decreases Aβ production by reducing BACE1 and γ-secretase cleavage of APP.[50] In other studies, a correlation between cellular cholesteryl-ester levels and Aβ production was uncovered.[51] A more recent study carefully assessed the effects of statins, which lower the levels of cholesterol and nonsterol isoprenoids such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate, and found strong evidence that lowering cellular isoprenoid levels increased the intracellular pool of APP metabolites and Aβ. Addition of mevalonate at a low level was sufficient to rescue statin-induced blockade of isoprenoid synthesis and prevented the increase in intracellular Aβ levels. These results raise the intriguing possibility that cholesterol and isoprenoid levels have independent effects on APP metabolism and Aβ production, highlighting the importance of exercising caution when interpreting experiments where statins were used as a means of lowering cholesterol levels in the absence of exogenous mevalonate.[52]

As another major component of membrane raft domains, sphingolipids are also involved in regulation of APP processing. Lowering sphingolipid levels either by inhibiting serine palmitoyltransferase, which is involved in the early step of sphingholipid biosynthesis, or by mutating one of the serine palmitoyltransferase enzyme subunits, elevates α-secretase cleavage.[53] In addition, secretion of $A\beta_{42}$, but not $A\beta_{40}$, was markedly elevated under these conditions, suggesting additional modulation of γ-secretase cleavage. Recently, Hartmann and colleagues reported an intriguing link between APP processing and the regulation of cholesterol and sphingomyelin metabolism: $A\beta_{42}$ directly decreases sphingomyelin levels by activating neutral sphingomyelinases, whereas $A\beta_{40}$ reduces *de novo* cholesterol synthesis by inhibition of hydroxymethylglutaryl-CoA reductase activity. Expression of FAD-linked PS1 mutants in fibroblasts increases cholesterol levels and reduces sphigomyelin levels, providing strong support for a physiological role for endogenous Aβ in regulating cholesterol and sphingomyelin metabolism. Nevertheless, the possibility that metabolites derived from γ-secretase cleavage of other type I membrane receptors might also influence lipid homeostasis has not been formally ruled out.[54]

Displacing BACE1, γ-secretase or amyloid precursor protein from lipid rafts

The reason why some proteins preferentially associate with lipid rafts is not completely understood, but certain lipid modifications have been found to be critical for the association of specific proteins with lipid rafts. One modification, attachment of a glycophosphatidylinositol group, is found on proteins that are located on the extracellular face

of the plasma membrane. Other modifications, such as *N*-myristoylation and *S*-palmitoylation, are found on transmembrane proteins and cytosolic proteins that associate with the cytoplasmic face of intracellular membranes. Targeting proteins to lipid rafts through *N*-myristoylation and *S*-palmitoylation relies on the ability of the saturated fatty acid to fit well in an ordered lipid environment, rather than the hydrophobic property of the lipids.[55] 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.[56] Protein–protein interactions also facilitate raft targeting of certain proteins, such as in the case of raft localization of glutamate receptor-interacting protein 2 (GRIP2) through interaction with the C-terminus of the raft-resident transmembrane protein ephrin B1.[57]

As described above, BACE1 undergoes *S*-palmitoylation at three cysteine residues within its transmembrane domain and cytosolic tail.[24] Mass spectrometry analysis of BACE1 purified from transfected HEK293 cells revealed that the extent of *S*-palmitoylation is markedly reduced by treatment with the cholesterol inhibitor lovastatin.[58] Whether this decrease in *S*-palmitoylation is attributable to altered intracellular trafficking of BACE1 or a consequence of pleiotropic effects of statins on cellular physiology has not been assessed. Nevertheless, it is possible to develop strategies that would compromise *S*-palmitoylation of BACE1, the rationale being that exclusion of BACE1 from lipid rafts might lower Aβ production. Overexpression of palmitoylation-deficient BACE1, however, resulted in an increase in $A\beta_{11–40}$ peptide levels with no significant change in $A\beta_{1–40}$ levels.[24] Displacing BACE1 from raft domains might therefore promote BACE1 processing of APP by removing the spatial barrier that separates raft-localized BACE1 from non-raft-localized APP. This interpretation is entirely consistent with the enhanced Aβ production observed in studies with moderate cholesterol extraction, and with findings in mice heterozygous for the cholesterol-synthesizing enzyme seladin-1.[29,59]

As mentioned above, γ-secretase and APP CTFs co-reside in raft microdomains of late endosomes, the TGN, and TGN-derived vesicles.[30] In theory, displacing either γ-secretase or APP CTFs from raft domains should prove effective in reducing Aβ production, by compromising the spatial proximity of the enzyme and the substrate. The mechanisms regulating raft association of γ-secretase complex are not, however, fully understood. Similarly, the signals within the transmembrane domain or cytosolic tail of APP that promote preferential segregation of APP CTFs into lipid rafts following ectodomain shedding of APP remain to be identified. The available data indicate that APP association with lipid rafts is enhanced during endocytic trafficking. [28] It remains to be determined whether proteins such as Mint, Fe65, LRP and GGA, which modulate APP trafficking and processing and Aβ production, affect raft versus non-raft distribution of APP. Displacement of full-length APP from lipid rafts might inhibit the amyloidogenic pathway initiated by BACE1 by shifting of APP ectodomain shedding to the nonamyloidogenic pathway mediated by α-secretases in non-raft domains. Finally, it might be possible to modify BACE1 or γ-secretase inhibitors to increase their affinity for cholesterol-containing membrane domains in an attempt to selectively target amyloidogenic processing of APP in lipid rafts. In view of the fact that a number of γ-secretase substrates are spatially segregated in non-raft domains,[30] selective inhibition of raft-localized γ-secretase might obviate potential side effects derived from complete inhibition of γ-secretase.

CONCLUSIONS

Numerous recent investigations have focused on the molecular mechanisms underlying the pathogenesis of AD, and the development of novel therapeutic strategies that selectively inhibit Aβ production in recent years. Multiple lines of evidence suggest that amyloidogenic processing of APP is associated with membrane raft microdomains. Targeting APP processing in lipid rafts, as opposed to the standard strategy of inhibiting BACE1 and γ-secretase by

blocking their active sites, has the potential to selectively reduce Aβ burden without the adverse effects associated with overall inhibition of the actions of these enzymes on a variety of other substrates. Identification of the elusive mechanisms responsible for raft localization of APP CTFs and γ-secretase could provide a strong impetus for the development of novel therapeutics based on the targeting of APP processing in lipid rafts.

KEY POINTS

Cholesterol has been an important focus of research into Alzheimer's disease (AD), but controversy has emerged regarding the therapeutic value of lowering cholesterol as a protective measure against AD

Certain aspects of cholesterol metabolism regulate amyloid-β production through direct modulation of amyloid precursor protein (APP) processing by secretases

β-site APP cleaving enzyme 1, γ-secretase and APP C-terminal fragments are localized in lipid rafts, which are membrane microdomains rich in cholesterol and sphingolipids

Amyloidogenic processing of APP occurs in lipid-raft domains, and experimental manipulations that disturb the integrity of lipid rafts affect amyloid-β production

Compartmentalization of APP C-terminal fragments and secretases offers unique opportunities to selectively target APP processing

REVIEW CRITERIA

PubMed was searched for articles published up to February 2007, including electronically released publications. Search terms included "Alzheimer's disease", "lipid rafts", "APP", "β-secretase", "BACE", "γ-secretase", "cholesterol" and "Aβ production". Relevant articles were retrieved and prioritized for inclusion in this review and their references were checked for additional material when appropriate. In addition, the authors used their own files of references.

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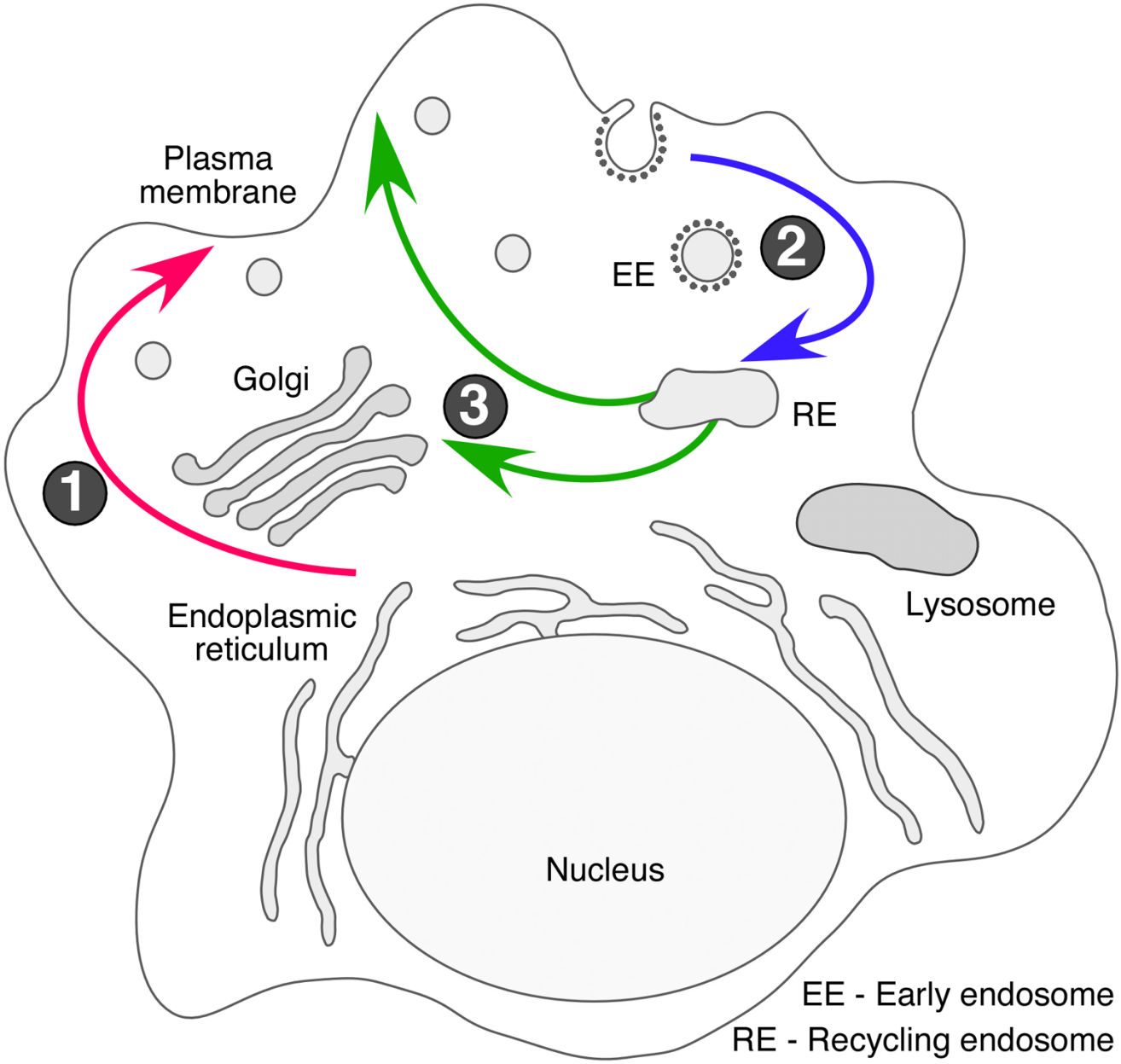

Figure 1.

Schematic diagram of intracellular amyloid precursor protein (APP) trafficking in a model cell. Nascent APP matures through the constitutive secretory pathway (1). Once APP reaches the cell surface, it is rapidly internalized and subsequently trafficked through endocytic (2) and recycling compartments (3) back to the cell surface, or is degraded in lysosomes. Nonamyloidogenic processing occurs mainly at the cell surface, where α-secretase activity is abundant. Amyloidogenic processing involves transit through the endocytic organelles, where APP encounters β-site APP-cleaving enzyme 1 (BACE1) and γ-secretase. Abbreviations: EE, early endosome; RE, recycling endosome.

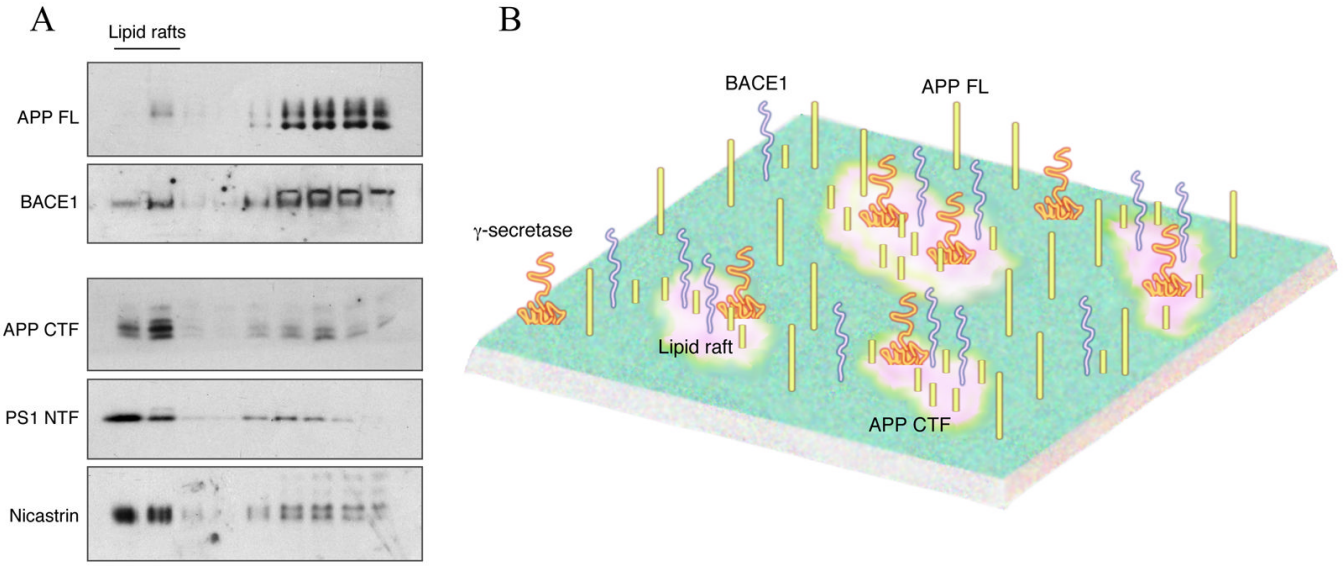

Figure 2.

Distribution of amyloid precursor protein (APP) and secretases in membrane microdomains. (**A**) The brain of a 12-month-old mouse was homogenized and solubilized in a buffer containing 0.5% Lubrol WX at 4°C for 30 min. The lysates were then subjected to flotation sucrose density gradient centrifugation to separate cholesterol-rich detergent-insoluble membranes (enriched in lipid raft markers) from detergent-soluble proteins.[30] An equal volume of each fraction was analyzed by Western blotting with antibodies against the APP C-terminus,β-site APP-cleaving enzyme 1 (BACE1), presenilin 1 (PS1) and nicastrin. (**B**) A model depicting compartmentalization of APP C-terminal fragments and secretases in lipid rafts. Full-length APP (APP FL) is predominantly localized in non-raft regions of membranes, whereas APP C-terminal fragments are highly abundant in lipid rafts. BACE1 appears to be distributed evenly between raft and non-raft domains, whereas γ-secretase preferentially associates with lipid rafts. α-secretases are excluded from lipid rafts, and are not shown here for the sake of clarity. Abbreviations: APP CTF, amyloid precursor protein C-terminal fragments; APP FL, full-length amyloid precursor protein; BACE1, β-site APP-cleaving enzyme 1; PS1 NTF, presenilin 1 N-terminal fragments.

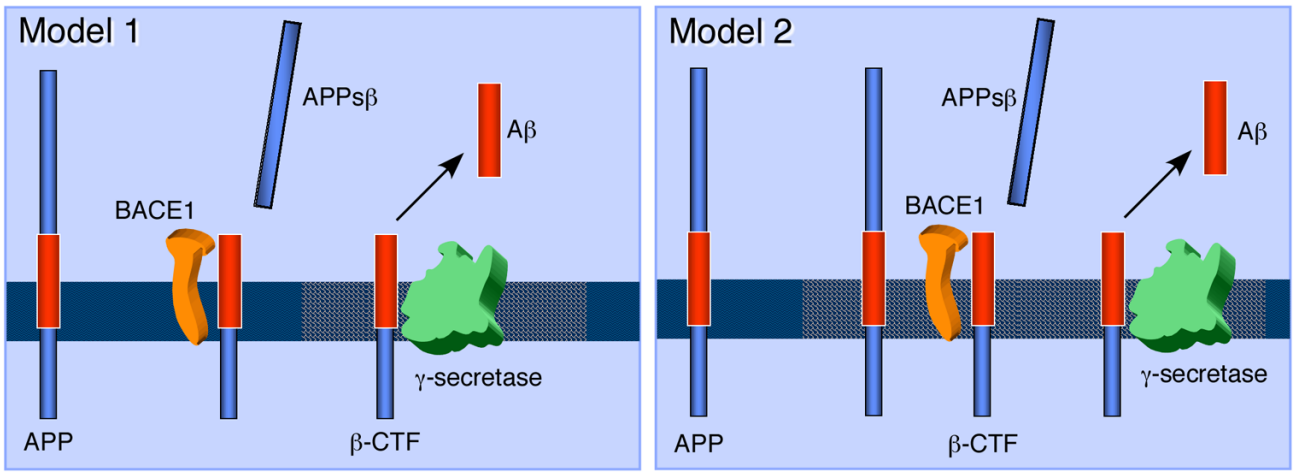

Figure 3.

Models of amyloid precursor protein (APP) processing. (**A**) Model 1 is deduced from raft and non-raft distribution of BACE1 and paucity of full-length APP (APP FL) in membrane microdomains. BACE1 cleaves a subset of APP FL in non-raft regions. The resulting APP β-C-terminal fragment becomes associated with lipid rafts and is processed by raft-associated γ-secretase. (**B**) Model 2 depicts an alternative interpretation of the data in Figure 1A. A subset of APP FL becomes associated with lipid rafts, and is sequentially processed by β-site APP-cleaving enzyme 1 (BACE1) and γ-secretase. The models differ with respect to the microdomain localization of BACE1 cleavage, which has not been fully resolved on the basis of cholesterol depletion studies.[28,29,37] Abbreviations: APP, amyloid precursor protein; Aβ, amyloid-β; APPsβ, BACE1-generated amyloid precursor protein ectodomain; BACE1, β-site APP-cleaving enzyme 1; β-CTF; amyloid precursor protein β-C-terminal fragment.