Molecular and Cellular Mechanisms for Alzheimer's Disease: Understanding APP Metabolism

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Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disease associated with aging. One important pathologic feature of AD is the formation of extracellular senile plaques in the brain, whose major components are small peptides called β-amyloid (Aβ) that are derived from β-amyloid precursor protein (APP) through sequential cleavages by β-secretase and γ-secretase. Because of the critical role of Aβ in the pathogenesis of AD, unraveling the cellular and molecular events underlying APP/Aβ metabolism has been and remains, of paramount importance to AD research. In this article we will focus on the regulation of APP metabolism leading to Aβ generation. We will review current knowledge of the secretases (α-, β-, and γ-secretases) involved in APP processing and various molecular and cellular mechanisms underlying intracellular trafficking of APP, which is a highly regulated process and whose disturbance has direct impacts on the production of Aβ.

INTRODUCTION

Alzheimer's disease (AD) is a prevalent aging-associated disorder, afflicting approximately 10% of the population over the age of 65 and 50% of the population over the age of 85. A subset of AD is classified as familial early-onset AD (FAD; onset in the fourth to sixth decade) and inherited as an autosomal dominant disorder. Mutations in the genes encoding β-amyloid precursor protein (APP) and presenilins (PS1 and PS2) are causative in the majority of FAD kindred [1-4]. Although documented FAD is rare (<10% of all AD), the characteristic clinicopathological features are apparently indistinguishable when FAD is compared with typical, common, "non-familial", or sporadic AD. Hence great efforts have been devoted to studying these FAD-linked genes and significant results have been generated to reveal mechanisms underlying AD pathogenesis.

AD is characterized clinically by an insidious onset and an inexorable progression of dementia, and pathologically by the abnormal accumulation of intracellular neurofibrillary tangles and extracellular neuritic plaques (or senile plaques) in vulnerable brain regions. The neurofibrillary tangles (NFTs) are composed largely of hyperphosphorylated twisted filaments of a microtubule-associated protein, tau [5]. The involvement of tau and NFTs in the pathogenesis of AD is beyond the scope of this review. Neuritic plaques consist of deposits of variously sized small peptides called β-amyloid (Aβ) [6,7], which are derived through sequential proteolytic processing of the β-amyloid precursor protein (APP) by β-secretase and γ-secretase. APP

can also be cleaved by α-secretase within the Aβ domain, therefore excluding Aβ generation. Data from humans, animal models, and cell studies all suggest that Aβ is the prime culprit for AD pathogenesis: overproduction of Aβ triggers a cascade of neurodegenerative steps resulting in formations of neuritic plaques and intra-neuronal fibrillary tangles and neuronal loss in AD [8,9]. It is the goal of this review to introduce various hypotheses regarding the metabolism of APP and the generation of Aβ, and to suggest how interrupting or delaying these early and invariable events in the pathogenesis of AD may be therapeutically feasible.

AN OVERVIEW OF APP

The gene encoding APP is located on chromosome 21 in humans [4]. Alternative splicing of this gene results in different APP isoforms. Three major APP isoforms found in humans are APP695, APP751 and APP770 (containing 695, 751, and 770 amino acids, respectively). Both APP751 and APP770 contain a 56 amino acid Kunitz Protease Inhibitor (KPI) domain within their extracellular regions and are expressed in most tissues, while APP695 lacks the KPI domain and is predominantly expressed in neurons [10,11]. However, although APP has been under great scrutiny since its identification, the physiological functions of APP and the significance of tissue-specific alternative splicing of APP remain largely undetermined. Recently APP has been suggested to play a role in transmembrane signal transduction, cell adhesion, calcium metabolism, neurite outgrowth and synaptogenesis, neuronal protein trafficking along the axon, etc., but those studies require corroboration with *in vivo* evidence (reviewed in [12]). In addition, the other metabolic fragments of APP than Aβ, namely soluble APPα and the intracellular domain of APP (AICD), have also been

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shown to have a variety of physiological functions that will be discussed below.

APP, a type-I transmembrane protein, belongs to a protein family that includes APP-like protein 1 (APLP1) and 2 (APLP2) in mammals [13-15]. These proteins share several conserved domains such as the E1 and E2 domains in the extracellular sequences and the intracellular domain, and can be processed in a very similar way. However, the Aβ domain is unique to APP and Aβ can only be generated through APP processing. *APP* knockout mice are viable and fertile and show relatively subtle abnormal phenotypes [16,17], suggesting a functional redundancy of these APP homolog proteins that may be exerted by the conserved motifs rather than Aβ. Indeed, although *APLP1* and *APLP2* knockout mice are viable and fertile, *APP/APLP2, APLP1/APLP2* double null mice, and *APP/APLP1/ APLP2* triple null mice show early postnatal lethality [18-20]. Interestingly, the APP/APLP1 double null mice are viable [19], suggesting that the presence of *APLP2* is crucial when either *APP* or *APLP1* is absent.

Full-length APP is synthesized in the endoplasmic reticulum (ER) and transported through the Golgi apparatus to the trans-Golgi-network (TGN), the major site of APP residence in neurons at steady state [21-23]. APP can be transported in TGN-derived secretory vesicles to the cell surface if not first proteolyzed to Aβ or an intermediate metabolite. At the plasma membrane APP is either cleaved by α-secretase to produce a soluble molecule, sAPPα [24], or alternatively, re-internalized to an endosomal/lysosomal degradation pathway [25,26]. It has been proposed that Aβ can also be generated in the endosomal/lysosomal system [27,28]. In contrast to the neurotoxic Aβ species, sAPPα is neuroprotective [29-31]. Thus, the subcellular distribution of APP has a direct influence upon the relative generation of sAPPα versus Aβ. This phenomenon makes delineation of the mechanisms responsible for regulating the trafficking of APP relevant to understanding the pathogenesis of AD.

α-SECRETASE CLEAVES APP AT THE CELL SURFACE

In the non-amyloidogenic APP cleavage pathway, APP is cleaved by α-secretase within the Aβ domain (at the Lys16-Leu17 bond) and thus Aβ generation is precluded. Moreover, α-cleavage releases a large soluble ectodomain of APP (sAPPα) that has neuroprotective effects. sAPPα plays important roles in neuronal plasticity/survival and protects neurons against excitotoxicity [29]. Recently we also demonstrated that sAPPα can inhibit stress-induced CDK5 activation that is responsible for elevated tau phosphorylation in cells and brains derived from APP deficient mice [31]. APP is constitutively cleaved for sAPPα release. In addition, α-cleavage of APP can be regulated by reagents such as phorbol ester (see below for more information). An early study suggested that the α-secretase is a membrane-bound endoprotease which cleaves APP primarily at the plasma membrane [24]. Through proteinase

inhibitor profiling, it was found that α-secretase is a zinc metalloproteinase [32]. Further studies indicated that several members of the ADAM (a disintegrin and metalloproteinase) family possess α-secretase-like activity and may function as α-secretase. Similar to APP, ADAM family members are also type-I transmembrane proteins and one of them, tumor necrosis factor-α converting enzyme (TACE, or ADAM17), can be proteolytically cleaved to release its extracellular domain as soluble TGF-α [33]. Pharmacological manipulation of TACE leads to altered APP α-cleavage and Aβ generation, and the regulated APP α-cleavage is abolished in TACE-deficient cells, suggesting that TACE is likely the α-secretase responsible for the regulated APP cleavage [34]. An inhibitor against TACE has recently been shown to effectively prevent regulated α-secretase activity in human primary neurons [35]. Additional studies also show that TACE likely affects regulated but not constitutive α-cleavage of APP in various cells [36], indicating that although TACE is capable of cleaving APP, it is likely that additional members of the ADAM family are also involved in APP α-cleavage. Indeed, overexpression of another ADAM family member, ADAM10, increases α-cleavage of APP in several cell lines and a dominant-negative form of ADAM10 can inhibit endogenous α-secretase activity [37,38]. More significantly, the protein levels of ADAM10 are found to be dramatically reduced in platelets of sporadic AD patients together with significantly decreased sAPPα levels in platelets and cerebrospinal fluids [39], consistent with the observation that α-secretase activity is reduced in the temporal cortex homogenates from AD patients [40]. On the other hand, co-overexpression of another ADAM family member, ADAM9, with APP promoted the production of sAPPα upon phorbol ester treatment, suggesting that ADAM9 also functions as the α-secretase [41]. Together these results suggest that multiple ADAM family members are similarly involved in APP processing and there could be functional redundancy among them.

ALTERNATIVE CLEAVAGE OF APP BY β-SECRETASE LEADS TO Aβ GENERATION

The first step for APP processing towards Aβ generation requires cleavage at the β-site by β-secretase. β-secretase was first identified independently by several groups and named as BACE, Asp2, or memapsin 2 [42-45]. BACE1, the now more common name for this protease, is a novel membrane-bound aspartyl protease and contains a characteristic single type I transmembrane domain near its C-terminus [42,43]. Overexpression or downregulation of BACE1 induced or inhibited cleavage of APP at the known β-site positions, Asp1 and Glu11, respectively. *In vitro* studies also show that BACE1 cleaves synthetic APP peptides at the β-site positions. These results provide convincing evidence to support BACE1 as the β-secretase [42-46]. BACE1 is synthesized as a larger precursor, pro-BACE1, which can be modified by glycosylation (and also phosphorylation) and cleaved by a furin-like endoprotease to produce mature BACE1 [47,48]. Optimal

BACE1 activity requires an acidic environment and as expected, the major cellular compartments, in various pre-mitotic cell lines overexpressing exogenous BACE1, include early Golgi, late Golgi/early endosomes, endosomes, and the cell surface [43,49-51]. However, mechanisms by which BACE1 trafficking and activity are regulated have not been substantially elucidated and deserve further investigation. BACE1 activity is thought to be a rate-limiting factor in generation of Aβ from APP. Original studies showed that BACE1 knockout mice do not produce detectable Aβ and otherwise have no severe phenotypic abnormalities [52,53]. BACE1 deficiency has also been shown to rescue memory deficits and cholinergic dysfunction in Tg2576 mice, which correlates with dramatic reduction in Aβ40/42 levels [54,55]. Significantly, several studies have found that the protein level and activity of BACE1 are elevated in regions of the brain affected by AD [56,57]. These results propose that BACE1 may be a valuable therapeutic targeting candidate as well as a prognostic marker for AD. However, recently Dominguez *et al.* [58] observed a variable but significant number of BACE1 null mice died in the first weeks after birth. The surviving BACE1 null mice were smaller than their littermate controls, presented a hyperactive behavior, and had subtle electrophysiological alterations in the steady-state inactivation of voltage-gated sodium channels. In addition, BACE1 null mice displayed hypomyelination of peripheral nerves and altered neurological behaviors such as elevated pain sensitivity and reduced grip strength, probably due to abolished neuregulin processing by BACE1 [59,60]. These results challenge the general idea of BACE1 as a safe drug target.

BACE2 is a homolog of BACE1 and maps to 21q22.3 [61], the Down's syndrome critical region, providing a logical link between this gene product and APP processing. *In vitro* enzymatic assays demonstrate that BACE2 cleaves β-secretase substrates, similar to BACE1, processing both the wild-type and Swedish mutant APP [62]. However, BACE2 expression in neurons is substantially lower than BACE1 [63]. In addition, cellular BACE2 has a limited effect on the β-secretase site but efficiently cleaves the sequences near the α-secretase site [64]. These results suggest that BACE1, rather than BACE2, is the major β-secretase. Nevertheless, the contribution of BACE2 to Aβ generation towards AD pathogenesis can not be excluded. Although *BACE2* knockout mice are overall healthy, a combined deficiency of BACE2 and BACE1 enhanced the *BACE1* null lethality phenotype, suggesting slight functional redundancy of BACE1 and BACE2 [58].

THE PS1/γ-SECRETASE COMPLEX CLEAVES APP AND OTHER TRANSMEMBRANE PROTEINS

After α- and β-cleavage, the remaining membrane-bound C-terminal fragments of APP (APP CTFα and CTFβ) are further cleaved by γ-secretase to release a P3 fragment (from CTFα) or Aβ (from CTFβ). Multiple

lines of biochemical evidence demonstrated that γ-secretase activity resides in a high molecular weight complex that consists of at least four components: presenilin (PS, PS1 or PS2), nicastrin (Nct), anterior pharynx-defective-1 (APH-1), and presenilin enhancer-2 (PEN-2) [65,66]. There are two *PS* homologs in mammals, *PS1* and *PS2* [1,3]. Mutations in the two genes, especially PS1, are causative in the majority of pedigrees with FAD. PSs are multitransmembrane proteins, spanning the membrane different times according to different studies [67,68]. Nascent PSs undergo endoproteolytic cleavage to generate an amino-terminal fragment (NTF) and a carboxyl-terminal fragment (CTF) to form a functional PS heterodimer [69]. Based on the fact that PSs possess two highly conserved aspartate residues that are indispensable for γ-secretase activity and that specific transition-state analogue γ-secretase inhibitors bind to PS1 NTF/CTF heterodimers [65,70], PSs are generally believed to be the catalytic component of the γ-secretase complex. Nct, a type I transmembrane glycoprotein, is the first identified protein cofactor of PS and regarded as the scaffolding protein within the γ-secretase complex. Recently it has been shown that the ectodomain of Nct can bind to APP and Notch and recruit them into the γ-secretase complex, suggesting that Nct serves as the γ-secretase receptor [71]. APH1 and PEN2 were identified through genetic screening in *Caenorhabditis elegans* [72,73]. APH-1 has been shown to interact with Nct to form a stable intermediate in the early stages of γ-secretase complex assembly [65]. PEN-2 is incorporated into the γ-secretase complex at a late stage and facilitates PS endoproteolysis [71]. Each of the four γ-secretase components is necessary for the enzymatic activity and deficiency of any of them will dramatically impair the γ-secretase activity. Coexpression of the four components reconstitutes γ-secretase activity in the yeast *Saccharomyces cerevisiae* that arguably lacks endogenous γ-secretase activity, suggesting that the four components are essential γ-secretase components [74,75]. More recently another two proteins, CD147 [76] and p23/TMP21 [77,78], have been suggested to be additional γ-secretase complex components. However, such a perception requires further validation and the exact roles of these two proteins in the γ-secretase complex remain undetermined.

The γ-secretase-mediated cleavage is unique in that the substrates are cleaved within the transmembrane domain. However, the exact γ-cleavage site seems to be variable. It is widely known that γ-cleavage yields Aβ40 and Aβ42, with Aβ40 as the majority species but Aβ42 as the more amyloidogenic species. Recent data show that PS/γ-secretase also mediates the ζ-site cleavage (Aβ46) [79,80] and ε-site cleavage (Aβ49) [81,82], suggesting a sequential cleavage model in which APP is first cleaved at the ε-site, followed by cleavages at the ζ-site and the γ-sites.

Because γ-cleavage is the final step for Aβ generation and determines the relative amounts of various Aβ species, inhibiting γ-secretase activity has been considered as a key approach for AD intervention. How-

ever, in addition to cleaving APP to generate Aβ and release the intracellular domain of APP (AICD), γ-secretase also cleaves a series of functionally important transmembrane proteins including Notch [2], cadherin [77], ErbB4 [53], CD44 [49], etc. to release their intracellular domains (ICDs) (also see review [83]). Notch intracellular domain (NICD) is well known to translocate into the nucleus and regulate the expression of genes important for development [84,85]. Other ICDs may also have transactivation activities. For example, AICD has been suggested to regulate the expression of several genes such as *APP, KAI1, BACE1* and *p53* [77,86-89]. We have also recently demonstrated that AICD binds to *EGFR* promoter and regulates *EGFR* expression [90]. In addition, ErbB4 ICD has been shown to bind to astrocytic gene promoters and suppress their expression [91]. Thus, a universal inhibition of γ-secretase activity to prevent Aβ generation would also block other important cellular pathways and cause unwanted results. It is therefore important to develop drugs that specifically inhibit the cleavage of PS/γ-secretase on APP but not on other substrates.

There is strong evidence to suggest that the PS1/γ-secretase resides principally in the ER, TGN and intermediate compartments that are not very consistent with the major subcellular localizations of APP [92,93]. Interestingly, the PS1/γ-secretase cleavage of variant substrates seems to occur at different subcellular compartments. For example, APP is mainly cleaved at the trans-Golgi Network (TGN) and early endosomal domains, whereas Notch is predominantly cleaved at the plasma membrane [21,23,94]. Hence on one hand, disturbance of proper intracellular trafficking of PS1/γ-secretase may result in more interaction between PS1/γ-secretase and APP, contributing to abnormal Aβ generation and AD pathogenesis. On the other hand, there is a new novel strategy for AD therapeutics, i.e., to alter the subcellular localization of PS/γ-secretase so that it has less accessibility to APP.

THE GENERATION OF Aβ

In the early days, Aβ was regarded as an abnormal and toxic species that is restricted to the brains of aged or demented humans. This concept was soundly refuted by the finding of soluble Aβ species in the body fluids of various species [95] and in the conditioned medium of cultured cells [96]. Most of the Aβ peptide produced is Aβ40, whereas a small proportion (approximately 10%) is Aβ42. The Aβ42 variant is more hydrophobic and more prone to fibril formation than Aβ40 [97]. Studies on FAD-linked PS mutations show that these mutations invariably increase the ratio of Aβ42/40 [69,98], suggesting that elevated levels of Aβ42 relative to Aβ40 is critical for AD pathogenesis, probably by providing the core of the amyloidogenic plaques [99,100].

The generation of Aβ is through sequential cleavage of APP by β-secretase and γ-secretase and hence requires the interaction of APP and the two secretases.

Because APP, β-secretase and γ-secretase distribute in overlapping subcellular compartments, it is likely that Aβ generation can occur in several localizations. It has been shown that optimal BACE1 activity requires an acidic environment. In addition, treatment of APP-expressing cells with Bafilomycin A (Baf A), a vacuolar-type H+-ATPase inhibitor, abolished Aβ generation [101]. These results suggest that Aβ production requires maturation of APP through the Golgi and processing in an acidic compartment.

Consistent with this hypothesis, we and others have found that the TGN, the major site of APP residence in neurons at steady state with an ideal acidic environment for maximal secretase activity, is also a major site for the generation of secreted Aβ peptides [21-23]. Using a cell-free system, we found that Aβ can be generated from the TGN in the obvious absence of vesicle formation [21]. Supporting evidence also comes from the presence of β-secretase and PS/γ-secretase in the TGN. Moreover, the TGN is a known sorting station for many lysosomal enzymes and plasma proteins; physiologically relevant events, such as prohormone processing, occur here as well [102]. Generated Aβ in the TGN may be subjected to the secretory pathway and released into the extracellular fluid. Uncleaved APP can be transported in TGN-derived secretory vesicles to the cell surface. At the plasma membrane APP is either cleaved by α-secretase to produce sAPPα [24] or reinternalized within clathrin-coated vesicles to an endosomal/lysosomal degradation pathway [25,26]. It has been proposed that Aβ can also be generated in the endosomal/lysosomal system, which also provide acidic environments [27,28].

APP is initially synthesized and cotranslationally inserted into the ER. It has been shown that retention of APP in the ER blocks production of Aβ40 but not Aβ42, suggesting that Aβ42 can be produced in the ER [22,23,103]. Using a cell-free assay to investigate the subcellular compartments within which distinct Aβ species are produced and those from which they are secreted, we determined that Aβ40 (Aβ1-40 and various N-terminally truncated Aβx-40 species) is generated exclusively in the TGN and secreted in post-TGN secretory vesicles [23]. In addition, we found that while the Aβx-42 produced and retained in the ER in an insoluble state, both Aβ1-42 and Aβx-42 species are generated in the TGN and packaged into secretory vesicles which accounts for the secreted pool of Aβ42.

Intracellular generation of Aβ imply that Aβ can accumulate within the neurons and contribute to disease pathogenesis. Indeed, intraneuronal Aβ immunoreactivity has been reported in the hippocampal and the entorhinal cortical regions that are prone to the development of early AD pathology in patients with mild cognitive impairment (MCI) [104]. Accumulation of intracellular Aβ also has been found to precede extracellular plaque formation in DS patients [105], and the levels of intraneuronal Aβ decrease as extracellular plaques accumulate [106]. Studies on transgenic mouse models also provide consistent results, showing that intracellular Aβ accumulation is an early event in

the progression of the neuropathological phenotype and its levels decrease as extracellular plaques start to build up [107-109].

REGULATION OF APP INTRACELLULAR TRAFFICKING AND PROCESSING

Because APP can be subjected to two different processing pathways that are mutually exclusive and likely occur at different subcellular compartments, alterations in APP intracellular trafficking and localization directly impact Aβ production. Available evidence has demonstrated that intracellular trafficking of APP may be regulated by multiple factors such as signal transduction compounds, steroid and peptide hormones, PS1, etc. Hence disturbance of these factors may dramatically affect the intracellular trafficking and thus the proteolytic processing of APP.

Signal Transduction

It has been known for a long time that phorbol esters can stimulate the α-secretase pathway by increasing sAPPα secretion and reducing Aβ generation through activating protein kinase C (PKC) [110-112]. However, although PKC can directly phosphorylate APP Ser655 [113], PKC still stimulates sAPPα secretion even when the phosphorylation sites on APP are mutated or when the entire cytoplasmic domain has been deleted [114], suggesting that PKC actually exerts its effects on APP metabolism by phosphorylating a different target other than APP per se. One possibility is that PKC phosphorylates a TGN phosphoprotein, resulting in a re-distribution of APP from the TGN to the cell surface. This possibility is supported by our study showing that PKC increases formation of secretory vesicles containing APP from the TGN in a cell-free system [115]. Evidence on this is also provided in that protein kinase A (PKA) can similarly reduce Aβ generation and stimulate the release of APP-containing vesicles from the TGN [116]. However, the effects of PKC and PKA are additive, suggesting that although PKC and PKA converge on the level of formation of APP-containing vesicles from the TGN, the regulatory mechanisms involved are independent [116].

Hormones

Estrogen belongs to a family of sex steroids that function as a sex hormone [117]. Epidemiological evidence suggests that elderly women with reduced levels of circulating estrogen have an increased incidence of AD and post-menopausal women receiving estrogen replacement therapy (ERT) have both a delayed onset and reduced risk for AD development [118-120]. Although the Women's Health Initiative's (WHI) studies found little improvement on cognitive performance following 2-15 months of trials of estrogen in women with clinically diagnosed AD [121], the overall benefits of ERT may not be discounted for the following reasons: (1) in the WHI studies the human subjects consisted of women of age 65 or older with dementia that probably has already irreversibly developed; and (2) prolactin was supplemented in the treatment which could have compromised estrogen's effect. Nevertheless, it is still unclear how estrogen protects against AD development. Several possible mechanisms have been proposed in this regard: (1) estrogen may act on interlukin 6 to antagonize inflammation [122]; (2) the phenolic structure of estrogen may contribute to its antioxidant effects in both cells and rat models [123]; and (3) estrogen may reduce the levels of appolipoprotein E (ApoE) that is a risk factor for AD development [124]. In addition, we have found that estrogen may reduce the levels of Aβ by affecting APP metabolism through stimulating the α-secretory pathway of APP and inhibition of Aβ generation, i.e., estrogen can stimulate the formation of APP-containing vesicles from the TGN in cell-free systems derived from both neuroblastoma cell and primary neurons, which precludes the maximum generation of Aβ [125-127]. Interestingly, stimulation of sAPPα secretion by estrogen can be blocked by a PKC inhibitor, suggesting that the action of PKC-dependent pathway may be involved in estrogen-induced pathway [128]. In addition, Rab11, a member of the GTP-binding protein family of membrane trafficking regulators implicated in protein transport along the biosynthetic and endocytic pathways, has been found to mediate estrogen regulated APP trafficking, and estrogen facilitates binding of Rab11 to the TGN membrane. A dominant negative Rab11 mutant abolishes estrogen-regulated stimulation of APP trafficking from the TGN leading to increased Aβ formation [125].

Similar to estrogen, testosterone, another sex steroid, also decreases with age in older men and in postmenopausal women. Studies in animal models show neuroexcitatory and neuroprotective properties of testosterone and improved cognitive performance after testosterone treatments. Several but not all studies in humans also show positive effects of testosterone supplementation (see review [129]). Preliminary mechanistic studies suggest that testosterone can also promote sAPPα secretion and reduce Aβ peptides and this event is regulated *via* aromatase-mediated conversion of testosterone to estrogen [130,131].

PS1

Presenilins (PS1 and PS2) are critical components in the γ-secretase complex [65,66]. In addition to its participation in the γ-secretase activity, PS1 has been shown to regulate intracellular trafficking of multiple membrane proteins including other γ-secretase components (nicastrin, APH-1 and PEN-2), TrkB, and ICAM-5/telecephalin [83]. Moreover, we and others have shown that PS1 can also regulate the intracellular trafficking of APP: absence of PS1 or the expression of a loss of function PS1 variant resulted in increased budding/generation of APP-containing vesicles from both the ER and the TGN with a concomitant increase in complex glycosylation and cell surface appearance of APP. In contrast, FAD-linked PS1 variants significantly reduced budding of APP-containing vesicles

from both the ER and the TGN, resulting in decreased delivery of APP to the cell surface [132]. These findings raise the possibility that FAD-linked PS1 variants may influence APP processing by increasing the time of APP residing at the TGN, consequently prolonging their availability for cleavage by β- and γ-secretases within the TGN. PS1 has been found to interact with cytosolic factors such as Rab11, Rab6 and Rab GDI that are involved in regulation of vesicular transport, suggesting that PS1 may regulate protein trafficking *via* its interaction with protein trafficking factors [133-135]. Indeed, modulation of Rab6-mediated transport has been shown to affect APP processing. Recently we have found that PS1 interacts with phospholipase D1 (PLD1), a phospholipid-modifying enzyme that regulates membrane trafficking events. The results demonstrated that this PS1-PLD1 interaction recruits PLD1 to the Golgi/TGN and thus possibly modulates APP trafficking, since overexpression of PLD1 promoted generation of APP-containing vesicles from the TGN and increased the cell surface levels of APP [136,137].

Other Factors

Furthermore, other proteins interacting with APP may also regulate its intracellular trafficking. For example, APP C-terminus has been found to interact with several adaptor proteins such as Fe65, Tip60, and all three mint (X11) family members (mint1, mint2, and mint3, also called X11, X11L, and X11L2, respectively) [138-140]. It has been shown that interaction of mint proteins with APP participates in the regulation of APP processing by stabilizing cellular APP, which affects both sAPPα and Aβ secretions [141]. More and more of such type of trafficking factors have been implicated in regulating APP trafficking and Aβ generation. SorLA/LR11 is a type I membrane protein expressed in neurons. Although the functions of SorLA/LR11 is not known, its homology to sorting receptors that transport between the plasma membrane, endosomes, and the Golgi suggests a similar protein trafficking function [142,143]. The expression of SorLA/LR11 is reduced in the brain of AD patients [144]. Recently, it has been found that SorLA/LR11 interacts with APP and overexpression of SorLA/LR11 causes redistribution of APP to the Golgi and decreased Aβ generation, whereas sorLA/LR11 knockout mice have increased levels of Aβ in the brain similar to the situation in AD patients [145]. In addition, inherited variants in the *SorLA/LR11* gene have been found to associate with late-onset-AD [146]. Moreover, a SorLA/LR11-related protein, low-density lipoprotein receptor-related protein (LRP), also binds to APP through the cytoplasmic adaptor protein Fe65 [147]. LRP has already been shown to mediate the clearance of Aβ by binding to Aβ either directly or indirectly [148,149]. Antagonizing the extracellular interaction between cell surface APP and LRP causes an increase in cell surface APP and a decrease in Aβ generation [150], whereas expression of a functional LRP minireceptor in neurons of an AD mouse model deteriorated memory deficits with increased Aβ levels in aged mice [151]. Similarly, an LRP-related protein 1B

(LRP1B) also binds APP at the plasma membrane and prevents APP internalization, leading to decreased Aβ production and increased sAPPα secretion [152].

Finally, APP can be delivered to the cell surface in where APP is either cleaved by α-secretase to produce sAPPα or reinternalized within clathrin-coated vesicles for endosomal/lysosomal degradation. Clathrin-mediated endocytosis is a tightly controlled process requiring the participation of AP-2, dynamin I, and many other factors [153-155]. It has been shown that when the endocytic pathway is impaired by overexpressing a dominant-negative form of dynamin I, APP is accumulated in the cell surface and Aβ levels in conditioned media is increased [156,157].

CONCLUDING REMARKS

Overproduction and accumulation of Aβ in the brain are key pathogenic events in AD. Aβ is derived from APP by various secretase cleavages but the identity and characteristics of these secretases are only recently revealed. In this review we have discussed the secretases involved in APP processing. In addition, we have addressed several possible mechanisms by which APP trafficking and processing are regulated by various factors. Since APP trafficking is a highly regulated process, a disturbance of APP intracellular trafficking/processing has direct impacts on Aβ generation. As such, a thorough understanding of APP trafficking regulation is an important step toward uncovering new therapies to reduce Aβ and its associated dementia.

ACKNOWLEDGEMENTS

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