

# Chapter 18

## Brain Aging as a Cause of Alzheimer's Disease

Toshiharu Suzuki, Ayano Kimura, Kyoko Chiba, Tadashi Nakaya, and Saori Hata

**Abstract** Alzheimer's disease (AD) is the most common form of senile dementia. Identification of genes causally associated with familial Alzheimer's disease (FAD) advanced our understanding of the molecular mechanisms of AD pathogenesis. However, FAD is much less common than sporadic Alzheimer's disease (SAD), which constitutes the majority of cases. Despite its similar pathology (albeit at a later age of onset), SAD is not linked to mutations in FAD-associated genes. In both FAD and SAD, the generation and oligomerization of amyloid  $\beta$  ( $A\beta$ ) peptide play central roles in neurotoxicity, but it remains unclear how qualitative and quantitative alterations in  $A\beta$  occur in SAD patients in the absence of causative mutations. The predominant risk factor for SAD is aging, suggesting that some as-yet-unknown alterations in the aged brain augment the amyloidogenic metabolism of APP and promote the neural toxicity of  $A\beta$  oligomers. In this chapter, we discuss potential biochemical changes in amyloid  $\beta$  precursor protein (APP) and proteins related to APP metabolism and function in the aged brain. APP axonal transport, membrane microlocalization and metabolism, including generation of  $A\beta$  in neurons, are regulated by interactions with several cytoplasmic proteins and phosphorylation of the APP cytoplasmic region. Age-related decline or aberration in the regulation of APP transport, localization and metabolism may induce generation of altered  $A\beta$ . Here, we focus on APP phosphorylation at threonine 668 in the cytoplasmic domain and the roles of APP regulatory proteins, including X11-like (X11L), JIP1, kinesin-1, and Alcadin, on the regulation of APP metabolism and intracellular trafficking.

**Keywords** Brain aging • Alzheimer's disease • APP • Amyloid  $\beta$  peptide • X11-like • JIP1 • Alcadin • Protein phosphorylation • Kinesin • p3-Alc

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## 18.1 Dementia and the Growing Population of Aged People

In almost all developed countries, the elderly population is increasing rapidly, and the relative size of the younger population is shrinking. Dementia is a major illness that critically impairs the health of older people, threatening their ability to live independently. The latest WHO report suggested that over 35 million people worldwide suffer from dementia, and this number is expected to triple by 2050 (WHO and Alzheimer's Disease International 2012). In countries with a growing aged population, the expanding costs of treating and caring for people with dementia has become a serious social and economic issue.

Alzheimer's disease (AD) is the most common form of senile dementia, accounting for 60–70 % of cases (Thies et al. 2013). Contributions by many AD researchers identified three causative genes involved in the pathogenesis of familial AD (FAD): amyloid  $\beta$  precursor protein (APP) and presenilins 1 and 2 (PS1 and PS2) (Haass and Selkoe 2007). Patients carrying pathogenic mutations in the APP or presenilin (PS) genes suffer from early-onset AD, as distinct from late-onset or sporadic AD (LOAD or SAD). Since their discovery of these genes in the late 1980s to early 1990s, the accumulated findings regarding their functions and the effects of causative mutations advanced our understanding of the molecular mechanisms of AD pathogenesis in both forms of the disease.

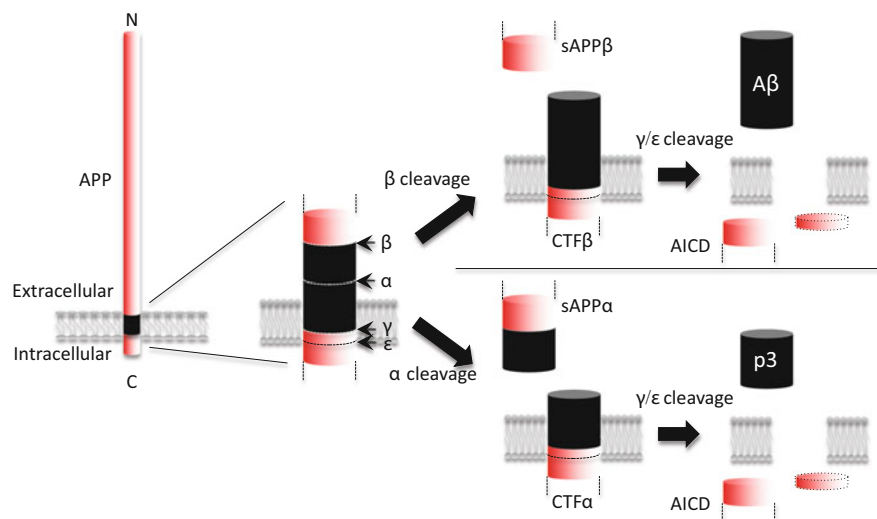
FAD is relatively rare; the majority of the AD patients have SAD, which is not linked to mutations in genes causally associated with FAD. Nonetheless, SAD exhibits pathology similar to that of typical FAD: neurodegeneration accompanied by extracellular senile plaques and intracellular neurofibrillary tangles. In both FAD and SAD, generation and oligomerization of amyloid  $\beta$  ( $A\beta$ ) peptide are believed to play central roles in the expression of neurotoxicity; thus,  $A\beta$  is thought to be a primary trigger of neurodegeneration (Haass and Selkoe 2007). However, it remains unclear how qualitative and quantitative alterations in  $A\beta$  generation occur in SAD patients in the absence of causative mutations. Studies aimed at identifying genes involved in SAD revealed the  $\epsilon 4$  allele of the ApoE gene as the strongest risk factor among many candidate genes (Kanekiyo et al. 2014). Although ApoE4 has been implicated in several aspects of AD pathogenesis, including  $A\beta$  deposition and clearance (Kok et al. 2009; Mawuenyaga et al. 2010), the molecular mechanisms and pathogenic roles of this ApoE4 isoform in SAD remain incompletely understood (Kanekiyo et al. 2014). A genomics analysis of AD-related genes affected by ApoE isoforms identified several genes related to intracellular protein trafficking, including the X11L gene (*APBA2*), as risk genes for AD (Rhinn et al. 2013), suggesting a role for intracellular trafficking defects in pathogenesis.

Notwithstanding the contributions of genetic factors, the predominant risk factor for SAD is aging itself. Therefore, it is important to understand what types of alterations in the aged brain augment the amyloidogenic metabolism of APP and promote the neurotoxicity of  $A\beta$  oligomers. In this chapter, we focus on potential biochemical changes in APP, as well as in proteins linked to APP metabolism or intracellular trafficking, in the aged brain. For almost three decades, we have sought

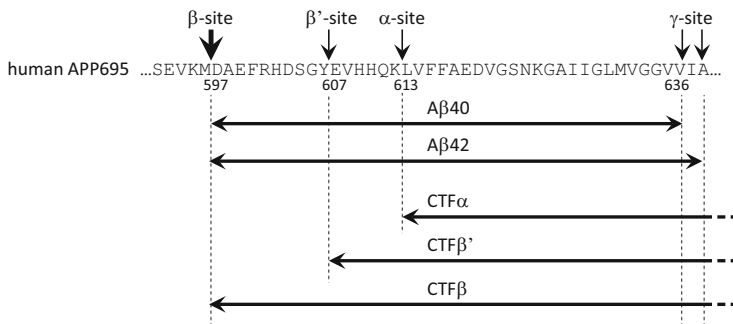
to understand the intracellular transport, metabolism, and function of APP (Suzuki et al. 2006; Suzuki and Nakaya 2008; Taru and Suzuki 2009). APP axonal transport, membrane microlocalization and metabolism, including A $\beta$  generation in neurons, is regulated by interactions with several cytoplasmic proteins and the phosphorylation of the APP cytoplasmic region. Age-related dysfunction in APP transport and metabolism may trigger quantitative or qualitative alterations in the generation of A $\beta$ , even in patients lacking pathogenic mutations in causative genes. This chapter describes neuron-specific APP phosphorylation at threonine 668 (Thr668) in the cytoplasmic region; APP-binding proteins that regulate intracellular metabolism, such as X11L and JIP1; and trafficking of APP. Furthermore, we describe alterations in substrate cleavage by  $\gamma$ -secretase, based on our analysis of the membrane proteins known as Alcadeins. Over the course of brain aging, changes in expression, localization, modification, and/or interaction of APP with regulators can augment amyloidogenic APP metabolism.

## 18.2 Phosphorylation of APP

APP is subject to proteolytic cleavages, initially by  $\alpha$ - or  $\beta$ -secretase primarily and then by  $\gamma$ -secretase (Fig. 18.1). A combination of cleavages of APP by  $\alpha$ - and  $\gamma$ -secretase generates the p3 fragment, which consists of 24–26 amino acids. This is



**Fig. 18.1** Metabolism of APP. APP is cleaved at the juxtamembrane  $\alpha$ - or  $\beta$ -site to generate membrane-associated CTF $\alpha$  or CTF $\beta$ , accompanied by secretion of sAPP $\alpha$  or sAPP $\beta$ . CTFs are further cleaved at the  $\gamma$ -site, followed by the  $\epsilon$ -cleavage site. This intramembrane cleavages result in secretion of p3 or A $\beta$  and release of AICD into the cytoplasmic milieu. Amyloidogenic (*right upper*) and non-amyloidogenic (*right lower*) pathways are shown schematically



**Fig. 18.2** Cleavage site of APP by  $\alpha$ -,  $\beta$ -, and  $\gamma$ -secretases, and small peptide products of APP generated by secretase cleavages.  $\alpha$ -site, cleavage site by  $\alpha$ -secretase;  $\beta$ - and  $\beta'$ -sites,  $\beta$ -cleavage site by  $\beta$ -secretase;  $\gamma$ -site, major  $\gamma$ -cleavage sites by  $\gamma$ -secretase. Amino-acid sequences of A $\beta$  and N-terminal amino-acid sequence of CTFs are shown

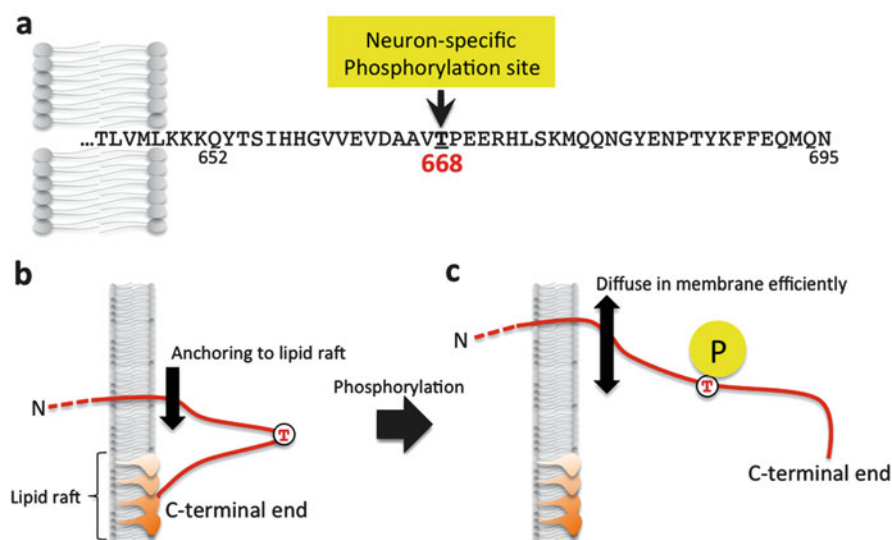
accompanied by secretion of sAPP $\alpha$ , a large extracellular domain, and the cytoplasmic release of the AICD fragment, followed by the generation of membrane-associated CTF $\alpha$  (C83), the 83 amino-acid C-terminal fragment. Because the  $\alpha$ -secretase, which is largely composed of ADAM10 and ADAM17, cleaves APP at the  $\alpha$ -site within the A $\beta$  domain on the cell membrane, this primary cleavage of APP is referred to as the non-amyloidogenic pathway or amyloidolytic processing. By contrast, the  $\beta$ -secretase, BACE, cleaves APP at the  $\beta$ -site and  $\beta'$  site to generate CTF $\beta$  (C99) and CTF $\beta'$  (C89), respectively. Although cleavage of the  $\beta'$ -site is also amyloidolytic, the major BACE cleavage occurs at the  $\beta$ -site. Therefore, cleavage by BACE is generally referred to as amyloidogenic processing (Cole and Vassar 2008) (Fig. 18.2). The cleavage of APP by  $\beta$ -secretase occurs in the acidic environment of early and/or recycling endosomes, into which APP molecules that escape from cleavage by  $\alpha$ -secretase on plasma membrane are incorporated (Thinakaran and Koo 2008).

All secretases and their components for APP processing are membrane proteins. BACE and  $\alpha$ -secretase (ADAM 10/17) are type I membrane proteins with single transmembrane domains, like their substrate APP (Suzuki et al. 2006). By contrast, the  $\gamma$ -secretase is a complex of membrane proteins composed of PS, anterior pharynx-defective complex subunit (APH-1), presenilin enhancer-2 (PEN-2), and nicastrin (NCT). PS is subject to endoproteolytic cleavage by the active  $\gamma$ -secretase complex. Therefore, the regulation of microlocalization of APP and active secretases in the membrane are important determinants of whether APP is subject to amyloidogenic or non-amyloidogenic processing.

Active BACE and  $\gamma$ -secretase predominantly localize in lipid raft-like membrane microdomains. The microdomain containing BACE activity can be biochemically isolated as a 1 % Triton X-100-resistant membrane fraction (Riddle et al. 2001; Saito et al. 2008), whereas the fraction containing active  $\gamma$ -secretase complex is prepared using 1 % CHAPSO (Matsushima et al. 2012). Thus, active BACE may localize in a detergent-resistant membrane (DRM) fraction that is

biochemically distinct from the fraction containing the active  $\gamma$ -secretase complex. Alternatively, because  $\gamma$ -secretase consists of multiple subunits, it may be unstable in DRMs prepared with Triton X-100.

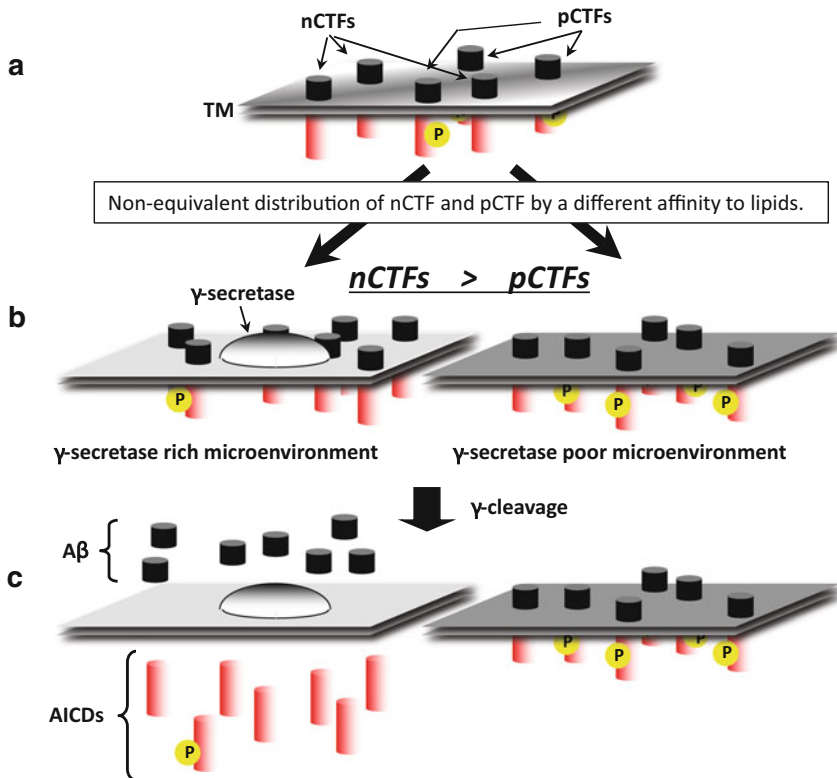
APP is a type I membrane protein, and its short 47 amino-acid cytoplasmic region is exposed to many factors that regulate the trafficking of APP-containing membrane vesicles and APP metabolism (Suzuki et al. 2006; Suzuki and Nakaya 2008; Taru and Suzuki 2009). Several APP isoforms/splicing variants with identical cytoplasmic regions exist, including APP770, APP751, and APP695; the APP695 isoform (composed of 695 amino acids) is exclusively expressed in neuronal cells. APP is subject to *N*-glycosylation in the ER to yield immature APP (imAPP) and *O*-glycosylation in Golgi to yield mature APP (mAPP). Therefore, mAPP with both *N*- and *O*-glycans is the true substrate of secretases, which act in the late secretory pathway. mAPP is further subject to neuron-specific phosphorylation at Thr668 (numbering of amino-acid positions is based on the APP695 isoform) (Iijima et al. 2000) (Fig. 18.3). The constitutive phosphorylation level of mAPP is low compared to CTFs, and in the brain almost 50 % of CTFs are phosphorylated at Thr668 (Matsushima et al. 2012). Phosphorylation induces a conformational change throughout the cytoplasmic region (Ramelot and Nicholson 2001; Ando et al. 2001), which regulates DRM localization of APP CTFs. The C-terminal end



**Fig. 18.3** Neuron-specific phosphorylation site in APP cytoplasmic region, and the conformational change of the cytoplasmic tail of APP that occurs upon phosphorylation. (a) Amino-acid sequence of the APP cytoplasmic region and the position of Thr668, which is subject to phosphorylation in neurons. (b) The C-terminal tail of APP CTFs exhibits lipid-binding ability. This binding decreases the membrane fluidity of APP CTFs. (c) Phosphorylation of APP CTFs alters the overall conformation of the cytoplasmic region, resulting in translocation of the tails of APP CTFs into the cytoplasm

of the APP cytoplasmic region tends to be anchored to membrane lipids, restricting CTF fluidity in the membrane (Beel et al. 2008). CTF phosphorylated at Thr668 loses its lipid-binding ability due to the conformational change, and thereby acquires greater mobility in the plasma membrane (Matsushima et al. 2012). Although phosphorylated and non-phosphorylated CTFs are kinetically equivalent substrates of  $\gamma$ -secretase, phosphorylated CTF can escape more easily from lipid raft-like membrane microdomains enriched in active  $\gamma$ -secretase. The phosphorylation of CTF $\beta$  is not affected for  $\gamma$ -cleavage of CTF $\beta$ ; nevertheless, phosphorylated CTF $\beta$  is a poorer source of A $\beta$  than non-phosphorylated CTF $\beta$  due to its freer mobility and ability to escape from lipid raft-like membrane microdomains (Matsushima et al. 2012) (Fig. 18.4).

The phosphorylation level of APP CTF decreases with brain aging in monkey, indicating that more APP CTF is available for cleavage by  $\gamma$ -secretase in the aged



**Fig. 18.4** Membrane localization of APP CTFs. (a) In brain neurons, phosphorylated (pCTF) and non-phosphorylated (nCTF) forms of APP CTFs are equally abundant, but nCTFs are preferentially cleaved by  $\gamma$ -secretase. (b, c) Because the C-terminal tails of nCTFs are prone to be anchored in membrane lipids (see Fig. 18.3), more nCTFs are localized in the lipid-rich membrane microdomain where  $\gamma$ -secretase is active. pCTFs are also localized in the microdomain, but without a lipid-binding C-terminal tail they are more mobile and can escape quickly

brain. This phenomenon may arise due to elevated generation of neurotoxic A $\beta$ . In fact, deposition of A $\beta$  peptide and Tau phosphorylation in the brain both increase over the course of aging in cynomolgus monkeys (Matsushima et al. 2012; Oikawa et al. 2010). Thus, it is possible that phosphorylation of APP CTFs at Thr668 plays an important role in regulation of membrane microlocalization of CTFs. However, it remains unclear which protein kinase phosphorylates APP at Thr668 in the brain in vivo; candidate kinases include CDK5, JNK, and GSK3 $\beta$ . Likewise, the protein phosphatase that dephosphorylates phosphorylated APP CTFs in neurons has not been identified. Prevention of APP CTF phosphorylation represents a therapeutic strategy to overcome A $\beta$  generation.

### 18.3 Intracellular Trafficking of APP in Neurons

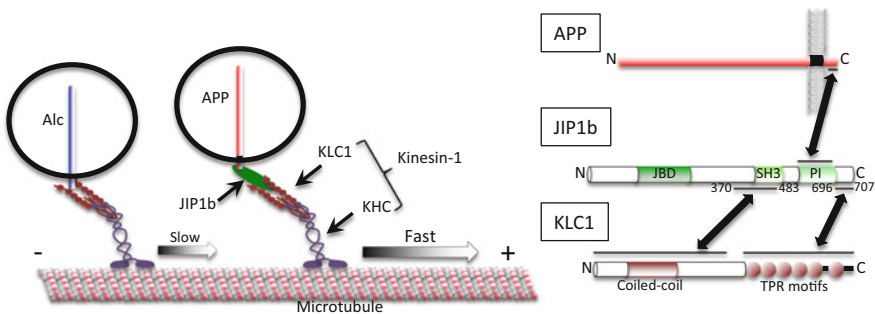
Neurons have highly specialized, polarized shapes consisting of a long axon and highly branched dendrites. In these cells, transport systems for proteins, RNA, and organelles are organized by a bidirectional motor system (Hancock 2014). In particular, anterograde transport in axon plays an essential role in supplying various materials to nerve termini (synapses); consequently, perturbation of axonal transport system might induce neurodegenerative diseases (Millecamps and Julien 2013). APP is subject to axonal anterograde transport by the kinesin-1 motor (Kamal et al. 2000; Araki et al. 2007), which is composed of two light chains (KLC) and two heavy chains (KHC) (Verhey and Hammond 2009; Hirokawa et al. 2009). Amyloidogenic processing, including neurotoxic A $\beta$  generation and axonopathy, are facilitated when anterograde transport of APP cargo is disturbed (Araki et al. 2007; Stokin et al. 2005).

In the presence of JNK-interacting protein 1 (JIP1), APP predominantly associates with kinesin-1 motor. This interaction is mediated by JIP1b (Araki et al. 2007; Chiba et al. 2014); among JIP1a, JIP1b, and JIP2, the JIP1b isoform has the highest affinity for APP (Taru et al. 2002). In *Drosophila*, APP-like protein (APPL) is transported in an anterograde direction in axons, as is APP in mammals, and *Drosophila* APPL-interacting protein (APLIP1) is an ortholog of JIP1 (Taru et al. 2002; Horiuchi et al. 2005). Human APP expressed in *Drosophila* is transported to fly synapses in a manner resembling axonal transport in mammals (Yagi et al. 2000). Thus, APP cargo anterograde transport by kinesin-1, mediated by the JIP1 association, is conserved across widely divergent animals. Together, these observations indicate that APP is the cargo receptor for kinesin-1, rather than a simple cargo that is transported within vesicles. Although kinesin-1 was the first anterograde motor identified in squid giant axon (Vale et al. 1985), the cargo receptors of kinesin-1 and the mechanisms of regulation of cargo transport by this protein remain unknown. In addition to APP, apolipoprotein receptor 2 (ApoER2) and Alcadin  $\alpha$  (Alc $\alpha$ ) have been identified as kinesin-1 cargo receptors. Alcadin forms a tripartite complex with APP, mediated by its association with X11-like (X11L) (Araki et al. 2003). Complex formation stabilizes the intracellular

metabolism of APP and Alca; however, both APP and Alca cargoes are transported largely independently (Araki et al. 2004, 2007).

Alca is a cargo receptor that can directly bind KLC (Araki et al. 2007; Konecta et al. 2006; Kawano et al. 2012), whereas APP and ApoER2 require scaffold and/or adaptor proteins, such as JIP1, to associate with KLC. APP and ApoER2 contain the NPXY motif, to which JIP1 binds, whereas Alca contains an NP motif, to which X11L but not JIP1 can bind. The velocity of Alca cargo transport by kinesin-1, measured in live axons, closely matches the speed of kinesin-1 on microtubules in vitro. Intriguingly, anterograde transport of APP by kinesin-1 is almost twice as fast as transport of Alca cargo (Araki et al. 2007). Efficient APP cargo transport with increased velocity and higher frequency in the anterograde direction suggests the importance of cargos that APP carries to the nerve terminus, and implies that neuronal function would deteriorate if the APP cargo transport system were impaired.

In JIP1-deficient neurons, the velocity of anterograde transport of APP cargo is reduced, but the speed can be restored by expression of exogenous JIP1b. Efficiency of anterograde transport of APP cargos is also reduced in the absence of JIP1 (Chiba et al. 2014). Therefore, JIP1b is an essential factor for rapid and efficient anterograde transport of APP cargo. The JIP1 carboxyl-terminal region, consisting of 11 amino acids (C11), interacts with the tetratricopeptide repeat (TPR) motifs of KLC1, and the amino-terminus of KLC1 binds to KHC (Verhey et al. 2001). However, recent analysis revealed that the interaction between JIP1b and KLC is more complex than previously thought. A tyrosine residue in C11 is essential for high-velocity transport, but a more complex interaction regulates the association of JIP1b with KLC, resulting in efficient anterograde transport of APP cargo (Chiba et al. 2014) (Fig. 18.5). Although it remains unknown at the molecular level how JIP1b increases the velocity of APP cargo transport by kinesin-1, it is clear that JNK binding to JIP1b and the phosphorylation of APP by JNK are not involved in



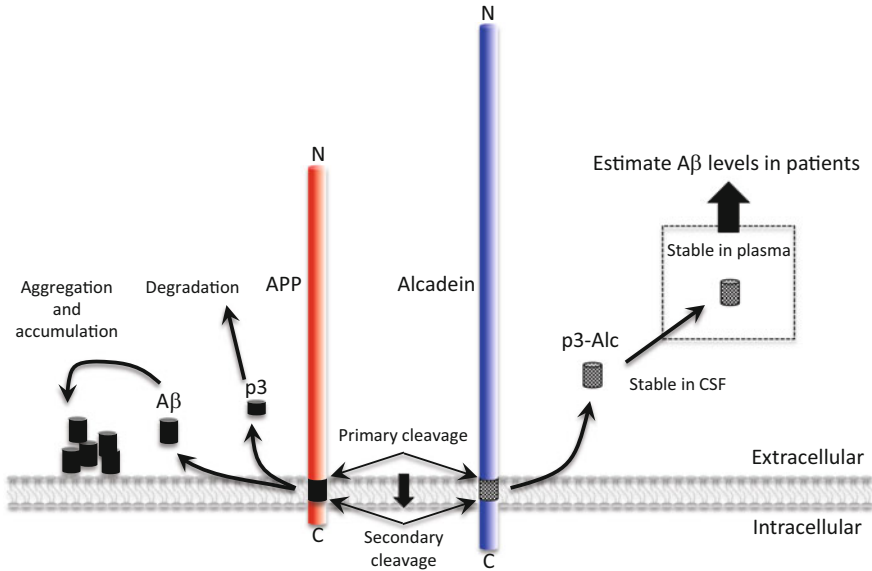
**Fig. 18.5** Schematic picture of Alca and APP cargos transport by kinesin-1. Alca associates with kinesin-1 directly, whereas APP associates with KLC by mediation of JIP1. JIP1 mediates efficient anterograde transport of APP cargo at high velocity. Schematic interaction between JIP1b and KLC1 is also shown at *right*. Complex interactions regulate the transport activity of APP cargos. *JBD* JNK-binding domain, *SH3* Src homology domain 3, *PI* phosphotyrosine interaction domain, *TPR* tetratricopeptide repeat. KLC1 is the major KLC isoform expressed in neurons



regulating the interaction between APP and JIP1b or the association of JIP1b with KLC (Chiba et al. 2014). In the absence of JIP1 expression, the efficiency of APP cargo transport is slower, and the frequency of anterograde transport is reduced. If the efficiency of APP anterograde transport is impaired in the neurons of aged brain, as in JIP1-deficient neurons, this may affect neuronal function. The reduced efficiency of APP cargo transport could also affect the transport of contents in cargo and/or induce metabolism of APP itself, including alterations in A $\beta$  generation. In fact, kinesin-1 levels are reduced in the brains of AD patients (Morel et al. 2012), and a specific KLC isoform modifies A $\beta$  accumulation in brain (Moriyama et al. 2014). Although it is impossible to observe alteration of APP axonal transport in living neurons in vivo, biochemical changes in cargo receptors, adaptor proteins, and motor molecules suggest that the cargo transport system is altered in the aged brain. Preservation of axonal transport system may be important for maintenance of neural function in the aged brain, and for protecting the brain against neurodegenerative diseases.

## 18.4 Alcadin in AD

Alcadesins (Alcs), also called calstentins, are evolutionarily conserved neural membrane proteins of the cadherin superfamily. The family has three members in mammals: Alc $\alpha$ , Alc $\beta$ , and Alc $\gamma$  (Araki et al. 2003; Vogt et al. 2001). Alcs are largely similar to APP in regard to function, metabolism, and localization. Metabolically, Alcs are subject to a primary cleavage by APP  $\alpha$ -secretase, resulting in secretion of a large extracellular domain fragment (sAlc) and generation of membrane-associated carboxyl-terminal fragments (Alc CTFs). Alc CTFs are further cleaved by  $\gamma$ -secretase, as is APP, resulting in secretion of a small peptide (p3-Alc) and release of the cytoplasmic-domain fragment (Alc ICD) into the cytoplasm (Araki et al. 2003, 2004, 2007; Hata et al. 2009; Takei et al. 2015). In contrast to A $\beta$  from APP, secreted p3-Alc peptide is not prone to aggregation; consequently, unlike p3 peptide of APP, p3-Alc is sufficiently metabolically stable to be detected in cerebrospinal fluid (CSF) and blood (Fig. 18.6). Therefore, qualitative and quantitative alterations of p3-Alc can reflect metabolic changes in APP, including A $\beta$  generation, in the aged brain (Hata et al. 2011, 2012; Konno et al. 2011; Kamogawa et al. 2012; Omori et al. 2014). Because APP  $\gamma$ -cleavage is altered in the brains of SAD patients (Kakuda et al. 2012), as are the  $\gamma$ -cleavage sites of p3-Alc $\alpha$  in the CSF of SAD patients (Hata et al. 2011), dysfunction in  $\gamma$ -cleavage is as obvious a contributor to the pathogenesis of SAD as PS pathogenic mutations are to the pathogenesis of FAD. Recently, the molecular mechanisms by which  $\gamma$ -secretase cleaves APP and Alc have been revealed (Takami et al. 2009; Piao et al. 2013). Both APP and Alcs are surrogate substrates for detecting dysfunctions in  $\gamma$ -secretase and/or changes in the membrane environment where  $\gamma$ -cleavage occurs.



**Fig. 18.6** Comparison of Alcadein proteolysis with APP proteolysis. Alcadein is cleaved by  $\alpha$ - and  $\gamma$ -secretase, as is APP. The cleavages generate a small secreted peptide, p3-Alc, which is not prone to aggregation. Therefore, quantitative and qualitative alterations of p3-Alc are detectable in body fluids. Alterations of  $\gamma$ -cleavage appear on the C-terminal amino-acid sequence of p3-Alc as an endophenotype as can be seen in A $\beta$  generation

The magnitudes of carboxyl-terminal alterations generated by altered cleavages of CTFs by  $\gamma$ -secretase are not equivalent among APP, Alc $\alpha$ , Alc $\beta$ , and Alc $\gamma$  (Hata et al. 2009; Piao et al. 2013). This suggests that some alteration for  $\gamma$ -secretase or its environment in a specific brain area appears on the alteration of  $\gamma$ -cleavage of APP, such as an increase of A $\beta$ 42 generation, whereas other alteration is apparent in Alc but not APP. Even if we cannot detect alterations in A $\beta$  generation by analyzing CSF and/or blood, the underlying alteration in  $\gamma$ -cleavage alteration might initially be detected as a change in Alc  $\gamma$ -cleavage, and appear subsequently in A $\beta$  as brain aging progresses. Therefore, the use of a substrate panel would represent an effective means for detecting  $\gamma$ -secretase dysfunction and alterations in membrane environment that induce altered  $\gamma$ -cleavage of substrates in the pre-pathogenic state of AD.

## 18.5 Summary and Conclusion

The majority of AD patients have the sporadic type of disease, which is distinct from the much less common familial disease associated with mutations in APP and PSs. Although the brain pathologies of SAD patients are similar to those of FAD

patients, as can be observed from the formation of senile plaques and neurofibrillary tangles, SAD may have various primary causes. In some cases,  $\beta$ - or  $\gamma$ -secretase activity may be altered, resulting in elevated levels of pathogenic A $\beta$ , whereas in other cases, the ability to clear A $\beta$  may be reduced. Currently, we do not know which events in the aged brain are the major causes of AD. Because the critical causes may differ among individual patients, we should identify alterations that might contribute to AD pathogenesis to advance the development of personalized medicine.

In this chapter, we introduced three potential mechanisms that might alter the level of pathogenic A $\beta$ , as follows. (1) *Phosphorylation of APP at Thr668*. Phosphorylated APP CTFs, substrates of  $\gamma$ -secretase, are clearly capable of escaping from membrane microdomains in which  $\gamma$ -secretase is active. Aging generally decreases the phosphorylation level of CTF $\beta$  in monkey brain, and this reduction is significant in the brains of AD patients relative to age-matched non-demented subjects. (2) *The axonal transport system*. Dysfunction in axonal transport can induce many types of neurodegenerative diseases; thus, it is a general cause of brain disorders rather than a specific cause of AD. In this context, however, we should focus our attention on the function of APP as a cargo receptor. Although previous studies have described multiple APP functions, it is clear that APP's primary function in neurons is to serve as the cargo receptor for kinesin-1, the major anterograde motor in this cell type, suggesting that APP plays an important role in a transport of proteins. Defective transport of as-yet-unidentified proteins by APP cargo may promote neurotoxicity in the aged brain and trigger AD. (3) *Dysfunction of  $\gamma$ -secretase or  $\gamma$ -cleavage of APP, which is common in FAD patients carrying PS gene mutations*. Recent studies showed that alteration of  $\gamma$ -cleavage also occurs in SAD patients, even if they do not carry any mutation in PS genes. A $\beta$ , the pathogenic product of APP  $\gamma$ -cleavage, shows a strong tendency to aggregate; thus, because the majority of A $\beta$  is aggregated or precipitated in body fluids, it is difficult to determine the extent of qualitative and quantitative alteration of  $\gamma$ -cleavage of A $\beta$  peptides in quality and/or quantity. Almost 100 type I membrane proteins are candidate substrates of the  $\gamma$ -secretase. However, the detailed mechanism of  $\gamma$ -cleavages has been revealed for only a few substrates, including APP, APP family proteins, Notch, and the Alcadeins. We have proposed using a substrate panel to detect  $\gamma$ -secretase dysfunction and alteration of  $\gamma$ -cleavage, and thereby identify subjects in preclinical or very early stages of AD. Classification of AD patients based on major pathogenetic events and early detection of preclinical subjects will be essential for the effective development of personalized therapies for elderly people.

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