INTRODUCTION

Alzheimer’s disease (AD) is the most common form of dementia in the ageing population and affects millions of people worldwide [1]. At the neuropathological level, AD is characterized by neuronal cell loss and the combined presence of two lesions in the brain - extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles (NFTs) [2]. The extracellular deposits contain aggregated Aβ peptides [3], while intraneuronal tangles are aggregates of hyper-phosphorylated forms of the neurofilament-associated protein tau [4]. Evidence suggests that the pathogenesis of AD involves deleterious neurotoxic effects of both types of aggregates [5,6]. However, numerous studies strongly support a critical role of Aβ aggregates in the initiation phase of AD pathogenesis, while tau might mediate toxicity and impairment of neuronal function [5-9].

Aβ is a proteolytically processed fragment of the amyloid precursor protein (APP) [10,11]. It occurs in different length variants with peptides of 40 amino acid residues (Aβ40) and 42 amino acid residues (Aβ42) being the most prevalent. The longer Aβ42 variant has a much higher propensity to form aggregates. Genetic studies identified mutations in three genes that cause familial forms of AD (FAD): APP, presenilin-1 (PS1), and presenilin-2 (PS2) [12]. Mutations in each of these genes result in elevated levels of Aβ production and/or promote its aggregation. This genetic correlation strongly favours the key role of Aβ in AD. However,
mutations in APP and PS are very rare, and the causes of the much more common late onset forms of AD (LOAD) are largely unidentified. In line with a significant role of Aβ in pathogenesis, recent data show that various post-translational modifications of Aβ promote its aggregation and therefore could play important roles in the initiation of LOAD.

**Generation of Aβ by proteolytic processing of APP and effects of AD associated mutations**

APP is a type I membrane protein and ubiquitously expressed in most cell types. Alternative mRNA splicing leads to several cell type and development-specific isoforms [2]. In addition, two homologous APP-like proteins (APLPs) have also been identified,
that together form a small protein family with important physiological functions in perinatal and postnatal development and cell communication [13]. However, APLPs do not contain the Aβ sequence and thus APP is the sole source of Aβ peptides in the brain [14].

Aβ is produced during normal cellular metabolism and secreted to the extracellular milieu of the human brain and also found in cerebrospinal fluid (CSF) [15;16]. The presence of Aβ in the CSF of nondemented individuals and in the media from neuronal cell cultures during normal metabolism could indicate a physiological role of Aβ in the central nervous system [17]. Suggested physiological function of Aβ includes ion channel modulation [18], kinase activation [19], regulation of cholesterol transport [20], protection against metal-induced oxidative damage [21], learning and memory [22] and transcriptional regulation of AD-associated genes [23].

The generation of Aβ is initially starts with a cleavage of APP by β-secretase at the N-terminus of the Aβ domain (Figure 1A). This cleavage results in the shedding of the APP ectodomain and the generation of a membrane bound carboxyl (C)-terminal fragment (CTF-β). Subsequently, γ-secretase mediates the apparently intramembranous cleavage of CTF-β resulting in the liberation of Aβ into conditioned media of cultured cells or extracellular fluids of the brain or the periphery [2;11]. Alternatively, APP can also be cleaved in a non-amyloidogenic pathway that involves initial cleavage by α-secretase within the Aβ domain thereby precluding the subsequent generation of Aβ peptides (Figure 1A) [24].

The mutations within APP that causes early onset AD (EOAD), are all located within or close to the Aβ domain. Notably, a double mutation in APP at the cleavage site for β-secretase that cause EOAD increases the β-secretory cleavage resulting in an overall higher production of Aβ peptides (see Swedish mutation, Figure 1B) [25]. Additional EOAD-associated mutations located close to the cleavage site for γ-secretase at c-terminal of Aβ also alter the proteolytic processing of APP (Figure 1B). These mutations increase the ratio of Aβ42/40 peptides thereby promoting the relative production of Aβ variants with higher propensity to aggregate [26]. Mutations found in the middle of the Aβ domain might exert different effects (Figure 1B), (1) they might decrease the α-secretory cleavage of APP thereby facilitating amyloidogenic processing of APP [27], (2) these mutations could also increase the aggregation [28], (3) and/or alter the degradation by different proteases [29].

Beside the APP gene, two additional genes have been identified to contain mutations that lead to EOAD [30]. Both genes encode highly homologous PS proteins that are critical components of the γ-secretase complex, which includes three additional proteins such as nicastrin, APH-1 (anterior pharynx-defective 1), and PEN-2 (presenilin enhancer 2) to exert γ-secretase activity in cells [31]. The mutations in PS1 or PS2 also alter γ-secretase activity and/or cleavage specificity, resulting in higher ratios of Aβ42/40 [31]. Together, all mutations in the three genes known to be associated with EOAD affect the generation and/or aggregation of Aβ [25;27]. However, as mentioned before such mutations are very rare and mechanisms that increase the aggregation and accumulation of Aβ and cause the much more common sporadic forms of AD (>95% of all cases), are largely unknown. According to the 'amyloid hypothesis', accumulation of Aβ in the brain is the primary influence driving AD pathogenesis. The rest of the pathogenic events, including impaired synaptic function and cell communication [7;32;33], activation of microglia and astrocytes [34;35], neuronal ionic dysfunction [37], altered kinase/phosphatases activities leading to formation of neurofibrillary tangles containing tau protein, is proposed to result from an imbalance between Aβ production and Aβ clearance [38].

Aβ aggregation - routes to neurotoxic assemblies

Amyloid formation in AD is conceptualized as a complex process of protein aggregation, involving the misfolding of Aβ into soluble and insoluble assemblies [39]. Monomeric Aβ is mainly composed of α-helical and/or unordered structure, whereas the misfolded polymers are rich in β-sheet conformation. The conformational changes leading to the formation of extended β-sheets promotes homophilic interactions and eventually leads to Aβ oligomer formation. Kinetic studies have suggested that misfolding of monomeric Aβ precedes formation of oligomers, which then serve as seeds/nuclei for accelerated fibril growth (Figure 2) [40].

A widely accepted concept for the formation of amyloid fibrils is the nucleation-dependent polymerization model [41-43], which separates the fibrilization process into a nucleation phase and an elongation phase. Nucleation requires the self-association of soluble
monomers, which is thermodynamically unfavourable and so occurs slowly. In the nucleation phase, monomers undergo conformational change/misfolding and associate to form oligomeric nuclei, and (ii) a elongation phase/growth phase, in which the nuclei rapidly grow by further addition of monomers and form larger polymers/fibrils until saturation. The ‘nucleation phase’, is thermodynamically unfavourable and occurs gradually, whereas ‘elongation phase’, is much more favourable process and proceeds quickly. Thus, kinetics of amyloid formation is well represented by a sigmoidal curve with a lag phase followed by rapid growth phase (green curve). The rate limiting step in the process is the formation of nuclei/seeds to promote aggregation. Thus, amyloid formation can be substantially speedup by the addition of preformed seeds (nuclei). The addition of seeds reduces the lag time and induces faster aggregate formation (red curve).

In a landmark discovery, Pike et al., [44], established that innocuous monomers of Aβ become neurotoxic upon aggregation. It was further shown that toxicity of Aβ involved self-association of monomers into oligomers and higher aggregated forms [45]. This is further supported by in vitro [46-48], and in vivo studies showing that oligomeric and pre-fibrillar Aβ assemblies are potent neurotoxins [5;49;50]. A correlation between soluble oligomeric Aβ levels and the extent of synaptic loss and severity of cognitive impairment further corroborate the findings [7;32]. Thus, neurotoxicity appears to require toxic oligomeric assemblies of Aβ. The formation of such neurotoxic assemblies in the brain generated due to higher production and/or decreased clearance of Aβ [51;52].

Effect of post-translational modification on aggregates formation, toxicity and clearance

Amyloid plaques in the human AD brain are known to contain a heterogeneous mixture of Aβ peptides [53]. In addition to main Aβ species (Aβ40 and Aβ42), a variety of post-translationally modified variants have been identified [54], including truncation [55-58], racemization [59;60], isomerization [61;62], pyroglutamination [63;64], metal induced oxidation [65] and phosphorylation [66-68].
The N-terminal truncated variants of Aβ beginning at amino acid 3, 11 and 25 are present in senile plaques and vascular amyloid deposits [56;57;58;69-71]. The truncated Aβ25-35 is shown to favour aggregation in vitro [72]. Due to potential toxic effects of truncated Aβ25–35, it has been frequently used for aggregation or toxicity studies [73]. Racemization of Aβ at Asp7, Asp23 and Ser26 was reported in the human brain and aggregation properties of Aβ were influenced by the position of the racemized residue [59;60]. Isomerization of aspartate residues at position 1, 7 and 23 of Aβ results in structural transition of Aβ and also shown to occur in vivo [62]. Isomerization of Aβ promotes fibril formation in vitro and resistance to proteolytic degradation [61]. In addition Aβ can undergo pyroglutammatin also resulting in faster aggregation [74;75].

Thus, post-translational modifications of Aβ could promote oligomer and aggregate formation, thereby also reducing the degradation by a variety of proteases [76-79]. Modified Aβ peptides show enhanced cytotoxicity as compared to non-modified peptides [73], and serve as seeding species for Aβ aggregate formation in vivo [66;74;78]. These post-translationally modified Aβ variants appear to be present at an early stages of the disease [58;66;71;74].

**Extracellular phosphorylation**

Phosphorylation is an important reversible post-translational modification that regulates the structural and functional properties of proteins in health and disease [80]. Phosphorylation is a key step in the regulation of protein activity, cell cycle control, gene regulation, learning and memory [81]. In addition to intracellular protein kinases (PKs), extracellular PK activities have also been described [82]. These extracellular kinases phosphorylate cell-surface proteins and soluble extracellular substrates, and thus could affect many physiological processes involving cell-cell contacts, cellular differentiation and proliferation, ion transport [82]. Depending on the localization, these PKs are differentiatied as ecto-PKs and exo-PKs. Ecto-PKs are localized at the external surface of the plasma membrane (membrane bound) where they exert their catalytic activity [83-86]. Exo-PKs are secreted/shedded to the extracellular milieu [87;88]. Ecto- and Exo-PKs can phosphorylate extracellular membrane bound proteins and soluble proteins. Both Ecto- and Exo-PKs use extracellular ATP as co-substrate, which can be released by intact cells [89;90]. Extracellular ATP plays physiological roles in neurite outgrowth, neurotransmission and glial communication [91]. The release of extracellular ATP is mediated by metabotropic (P2Y) and ionotropic (P2X) receptors, both are widely expressed in the nervous system [92]. In the brain, extracellular ATP is present in low nanomolar concentrations. However, the local ATP concentration can increase upon certain stimuli, including synaptic activation [89;93], inflammation [94] and ischaemia in vivo [95]. Therefore, extracellular phosphorylation is likely to play a role in normal as well as pathological processes in the brain.

**Phosphorylation of Aβ**

A variety of AD associated proteins including APP [96-98], BACE [99;100], PS [101;102] and tau [103;104], are shown to be phosphorylated. Phosphorylation of these proteins affects subcellular trafficking, interaction with adapter proteins, signal transduction cascades, APP processing, Aβ generation and tangle formation. In AD, tau is shown to be abnormally hyperphosphorylated at several Ser/Thr residues. Hyperphosphorylation and subsequent accumulation of neurofilament subunits is a typical feature of the AD brain [105;106]. However, the pathophysiological relevance of tau phosphorylation is still under debate.

In silico analysis revealed that Aβ contain potential phosphorylation sites at serine residue at 8th and 26th position and tyrosine residue at 10th position. Aβ can undergo phosphorylation by protein kinase A and cdc2 in vitro [68], as well as by cultured cells and in human CSF (Kumar, 2009; URN: urn:nbn:de:hbz:5N-18193).

We recently showed that Aβ is phosphorylated at serine-8 by extracellular protein kinase A. Phosphorylation of Aβ promoted the formation of toxic aggregates [66]. The formation of small soluble oligomers is associated with the conformational transition of Aβ from α-helical and random coiled state to a β-sheet structure, as demonstrated by circular dichroism. Phosphorylation-state specific antibodies were used in western-blotting and immunohistochemistry to demonstrate the occurrence of phosphorylated Aβ in murine AD models and AD patient’s brain tissue. Notably, these antibodies further confirmed that phosphorylation occurs at free extracellular Aβ rather than at the full-length APP or β-CTF, the precursors of Aβ peptide. Phosphorylated Aβ co-localized with non-phosphorylated Aβ in extracellular plaques [66]. Interestingly, phosphorylated
Aβ appeared to be concentrated in the centre of individual plaques and was detected as early as at 2 months of age in APP transgenic mice, and then accumulated with aging. The detection of phosphorylated Aβ in oligomeric assemblies in mouse brain homogenates suggested that phosphorylation also increases aggregation of Aβ in vivo. Therefore, we hypothesize that phosphorylation of Aβ might act as a conformational switch, thereby promoting the formation of aggregates.

To test the effect of Aβ phosphorylation on toxicity in vivo, transgenic Drosophila models were employed. Since Drosophila allows the selective expression of Aβ independent of its precursor APP [107;108], transgenic Drosophila flies expressing either the wild type Aβ (AβWT) or pseudophosphorylated mutant (AβS8D) were generated. When expressing AβWT and AβS8D mutant in photoreceptor cells in Drosophila eyes, the pseudophosphorylated AβS8D variant showed significant cell degeneration compared to AβWT, demonstrating increased toxicity of pseudophosphorylated Aβ. Notably, pseudophosphorylated AβS8D also accumulated to much higher levels in aged flies than AβWT, strongly indicating increased aggregation. In addition, transgene expression in the fly brain showed stronger age-dependent accumulation of pseudophosphorylated Aβ peptides as compared to AβWT. The increased toxicity of pseudophosphorylated Aβ was revealed by altered climbing behaviour upon aging. This progressive age-dependent phenotype, correlates with Aβ peptide accumulation, indicating that pseudophosphorylated Aβ can mimic the effect of phosphorylation on Aβ aggregation in vivo [66].

The Aβ plaque formation could be induced by inoculation of amyloid containing brain homogenates from human or transgenic mouse into brains of monkeys or APP transgenic mice, suggesting the occurrence of nucleation-dependent fibrillization in vivo [109;110]. As phosphorylation of Aβ promotes oligomer formation, phosphorylated Aβ oligomers could serve as seeds or nuclei that increase the rate of aggregation. In agreement with this hypothesis, the nuclei of phosphorylated Aβ were capable to promote aggregation of non-phosphorylated Aβ in vitro [66].

Several proteases or peptidases have been reported that are able to cleave Aβ and thereby contribute to efficient removal of Aβ in the brain [52;111]. It will therefore also be interesting to assess the effect of phosphorylation on protease dependent degradation of Aβ.

CONCLUSION

Increasing evidence suggests that phosphorylation of proteins involved in several neurodegenerative diseases and plays a serious role during the pathogenesis [67;112;113]. The role of phosphorylation in modulating the aggregation and fibrillogenesis of tau in AD and α-synuclein in Parkinson’s disease (PD) is currently a subject of intense investigation [103;114;115]. Our studies provide evidence that Aβ can undergo phosphorylation. Phosphorylation promotes conformational transition and formation of toxic aggregates. Further, phosphorylated Aβ aggregates could serve as endogenous seeds triggering further aggregation of soluble, extracellular Aβ into plaques in the brain. Phosphorylation stabilizes the Aβ against degradation by various proteases in vitro and in cell cultures (Kumar et al., Unpublished data). The stabilization of Aβ by phosphorylation might play a crucial role in AD pathogenesis, because it would eventually result in increased concentrations of this peptide in the brain. Therefore, inhibition of extracellular kinases or stimulation of Aβ dephosphorylation could be pursued as valuable targets to prevent or slow down the progression of AD. Further, the detection of phosphorylated Aβ in biological fluids could also be explored for evaluation as biomarkers. Together, phosphorylation of Aβ might have very important implications for AD pathogenesis and offer novel therapeutic avenues.

ACKNOWLEDGEMENTS

We thank Dr. Peter Breuer and Dr. Patrick Wunderlich for critically reading the manuscript. Although we have made a thorough and extensive search of the literature, we apologize to our colleagues if we mistakenly excluded their studies in our reference list. Work in the laboratory was supported by Deutsche Forschungsgemeinschaft (DFG) grant (WA1477/6, SFB645, KFo177).

CONFLICT OF INTERESTS STATEMENT

The authors of this manuscript have no conflict of interest to declare.
REFERENCES

1. Thies W, Bleiler L: 2011 Alzheimer's disease facts and figures. Alzheimers Dement 2011;7:208-244.
2. Selkoe DJ: Alzheimer’s disease: genes, proteins, and therapy. Physiol Rev 2001;81:741-766.
3. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K: Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci U S A 1985;82:4245-4249.
4. Querfurth HW, LaFerla FM: Alzheimer’s disease. N Engl J Med 2010;362:329-344.
5. Selkoe DJ: Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior. Behav Brain Res 2008;192:106-113.
6. LaFerla FM: Pathways linking Abeta and tau pathologies. Biochem Soc Trans 2010;38:993-995.
7. Haass C, Selkoe DJ: Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer’s amyloid beta-peptide. Nat Rev Mol Cell Biol 2007;8:101-112.
8. Trojanowski JQ, Shin RW, Schmidt ML, Lee VM: Relationship between plaques, tangles, and dystrophic processes in Alzheimer’s disease. Neurobiol Aging 1995;16:335-340.
9. Vossel KA, Zhang K, Brodbeck J, Daub AC, Sharma P, Finkbeiner S, Cui B, Mucke L: Tau reduction prevents Abeta-induced defects in axonal transport. Science 2010;330:198.
10. Haass C, De Strooper B: The presenilins in Alzheimer’s disease—proteolysis holds the key. Science 1999;286:916-919.
11. Walter J, Kaether C, Steiner H, Haass C: The cell biology of Alzheimer’s disease: uncovering the secrets of secretases. Curr Opin Neuromol Biol 2001;11:585-590.
12. Goate AM: Molecular genetics of Alzheimer’s disease. Geriatrics 1997;52 Suppl 2:59-12.
13. Anliker B, Muller U: The functions of mammalian amyloid precursor protein and related amyloid precursor-like proteins. Neurogeodegener Dis 2006;3:239-246.
14. Walsh DM, Minogue AM, Sala FC, Fadeeva JV, Wasco W, Selkoe DJ: The APP family of proteins: similarities and differences. Biochem Soc Trans 2007;35:416-420.
15. Seubert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, Davis D, Sinha S, Schlossmacher M, Whaley J, Swindlehurst C, ..: Isolation and quantification of soluble Alzheimer’s beta-peptide from biological fluids. Nature 1992;359:325-327.
16. Haass C, Schlossmacher MG, Hung AY, Vigo-Pelfrey C, Mellon A, Ostaszewski BL, Lieberburg I, Koo EH, Schenk D, Teplow DB, ..: Amyloid beta-peptide is produced by cultured cells during normal metabolism. Nature 1992;359:325-327.
17. Pearson HA, Peers C: Physiological roles for amyloid beta peptides. J Physiol 2006;575:5-10.
18. Kerrigan TL, Atkinson L, Peers C, Pearson HA: Modulation of ‘A'-type K+ current by rodent and human forms of amyloid beta protein. Neuroreport 2008;19:839-843.
19. Tabaton M, Zhu X, Perry G, Smith MA, Giliberto L: Signaling effect of amyloid-beta(42) on the processing of AbetaPP. Exp Neurol 2010;221:18-25.
20. Yao XZ, Papadopoulos V: Function of beta-amyloid in cholesterol transport: a lead to neurotoxicity. FASEB J 2002;16:1677-1679.
21. Zou K, Gong JS, Yanagisawa K, Michikawa M: A novel function of monomeric amyloid beta-protein serving as an antioxidant molecule against metal-induced oxidative damage. J Neurosci 2002;22:4833-4841.
22. Morley JE, Farr SA, Banks WA, Johnson SN, Yamada KA, Xu L: A physiological role for amyloid-beta protein: enhancement of learning and memory. J Alzheimers Dis 2010;19:441-449.
23. Bailey JA, Maloney B, Ge YW, Lahiri DK: Functional activity of the novel Alzheimer’s amyloid beta-peptide interacting domain (AbetaID) in the APP and BACE1 promoter sequences and implications in activating apoptotic genes and in amyloidogenesis. Gene 2011.
24. Kojo E, Fahrenholz F: The non-amyloidogenic pathway: structure and function of alpha-secretases. Subcell Biochem 2005;38:105-127.
25. Citron M, Oltersdorf T, Haass C, McConlogue L, Hung AY, Seubert P, Vigo-Pelfrey C, Lieberburg I, Selkoe DJ: Mutation of the beta-amyloid precursor protein in familial Alzheimer’s disease increases beta-protein production. Nature 1992;360:672-674.
26. Herl L, Thomas AV, Lill CM, Banks M, Deng A, Jones PB, Speolgen R, Hyman BT, Berezovska O: Mutations in amyloid precursor protein affect its interactions with presenilin/gamma-secretase. Mol Cell Neurosci 2009;41:166-174.
27. Haass C, Hung AY, Selkoe DJ, Teplow DB: Mutations associated with a locus for familial Alzheimer’s disease result in alternative processing of amyloid beta-protein precursor. J Biol Chem 1994;269:17741-17748.
28. Chiti F, Stefani M, Taddei N, Ramponi G, Dobson CM: Rationalization of the effects of mutations on peptide and protein aggregation rates. Nature 2003;424:805-808.
29. Betts V, Leissing MA, Dolios G, Wang R, Selkoe DJ, Walsh DM: Aggregation and catabolism of disease-associated intra-Abeta mutations: reduced proteolysis of AbetaA21G by nephrilysin. Neurobiol Dis 2008;31:442-450.
30. Donoviel DB, Hadjantonakis AK, Ikeda M, Zheng H, Hyslop PS, Bernstein A: Mice lacking both presenilin genes exhibit early embryonic patterning defects. Genes Dev 1999;13:2801-2810.
31. De Strooper B: Loss-of-function presenilin mutations in Alzheimer disease. Talking Point on the role of presenilin mutations in Alzheimer disease. EMBO Rep 2007;8:141-146.
32. Lacer PN, Buniel MC, Furlow PW, Clemente AS, Velasco PT, Wood M, Viola KL, Klein WL: Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer’s disease. J Neurosci 2007;27:796-807.
33. Lacer PN, Buniel MC, Chang L, Fernandez SJ, Gong Y, Viola KL, Lambert MP, Velasco PT, Bigio EH, Finch CE, Kraft G, Klein WL: Synaptic targeting by Alzheimer’s-related amyloid beta oligomers. J Neurosci 2004;24:10191-10200.
34. Choi SH, Bosetti F: Cyclooxygenase-1 null mice show reduced neuroinflammation in response to beta-amyloid. Aging (Albany NY) 2009;1:234-244.
35. Candelario-Jail E: A role for cyclooxygenase-1 in beta-amyloid-induced neuroinflammation. Aging (Albany NY) 2009;1:350-353.
36. Pratico D, Delanty N: Oxidative injury in diseases of the central nervous system: focus on Alzheimer’s disease. Am J Med 2000;109:577-585.
37. Massaad CA, Pautier RG, Klann E: Mitochondrial superoxide: a key player in Alzheimer’s disease. Aging (Albany NY) 2009;1:758-761.
38. Hardy J, Selkoe DJ: The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002;297:353-356.

39. Yoshiike Y, Minai R, Matsuo Y, Chen YR, Kimura T, Takashima A: Amyloid oligomer conformation in a group of natively folded proteins. PLoS One 2008;3:e3235.

40. Ni CL, Shi HP, Yu HM, Chang YC, Chen YR: Folding stability of amyloid-beta 40 monomer is an important determinant of the nucleation kinetics in fibrillization. FASEB J 2011;25:1390-1401.

41. Jarrett JT, Lansbury PT, Jr.: Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? Cell 1993;73:1055-1058.

42. Harper JD, Lansbury PT, Jr.: Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. Annu Rev Biochem 1997;66:385-407.

43. Naiki H, Gejyo F: Kinetic analysis of amyloid fibril formation. Methods Enzymol 1999;309:305-318.

44. Pike CJ, Walenczewicz AJ, Glabe CG, Cotman CW: In vitro aging of beta-amyloid protein causes peptide aggregation and neurotoxicity. Brain Res 1991;563:311-314.

45. Lorenzo A, Yankner BA: Beta-amyloid neurotoxicity requires fibril formation and is inhibited by congen. Proc Natl Acad Sci U S A 1994;91:12243-12247.

46. Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trotter B, Viola KL, Wals P, Zhang C, Finch CE, Kratf GA, Klein WL: Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. Proc Natl Acad Sci U S A 1998;95:6448-6453.

47. Hartley DM, Walsh DM, Ye CP, Diehl T, Vasquez S, Vassilev PM, Teplow DB, Selkoe DJ: Prototibrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. J Neurosci 1999;19:8876-8884.

48. Townsend M, Shankar GM, Mehta T, Walsh DM, Selkoe DJ: Effects of secreted oligomers of amyloid beta-protein on hippocampal synaptic plasticity: a potent role for trimers. J Physiol 2006;572:477-492.

49. Walsh DM, Selkoe DJ: A beta oligomers - a decade of discovery. J Neurochem 2007;101:1172-1184.

50. Klein WL, Krafft GA, Finch CE: Targeting small Abeta oligomers: the solution to an Alzheimer's disease conundrum? Trends Neurosci 2001;24:219-224.

51. Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, Yarasheski KE, Bateman RJ: Decreased clearance of CNS beta-amyloid in Alzheimer's disease. Science 2010;330:1774.

52. Iwata N, Higuchi M, Saito TC: Metabolism of amyloid-beta peptide and Alzheimer's disease. Pharmacol Ther 2005;108:129-148.

53. Walker LC, Rosen RF, LeVine H, III: Diversity of Abeta deposits in the aged brain: a window on molecular heterogeneity? Rom J Morphol Embryol 2008;49:5-11.

54. Kuo YM, Kokjohn TA, Beach TG, Sue L, Brune D, Lopez JC, Kalback WM, Abramowski D, Sturchler-Pierrat C, Staufenbiel M, Roher AE: Comparative analysis of amyloid-beta chemical structure and amyloid plaque morphology of transgenic mouse and Alzheimer's disease brains. J Biol Chem 2001;276:12991-12998.

55. Saito TC, Yamao-Harigaya W, Iwatsubo T, Kawashima S: Amino- and carboxyl-terminal heterogeneity of beta-amyloid peptides deposited in human brain. Neurosci Lett 1996;215:173-176.

56. Tekirian TL, Saito TC, Markesbery WR, Russell MJ, Wekstein DR, Patel E, Geddes JW: N-terminal heterogeneity of parenchymal and cerebrovascular Abeta deposits. J Neuropathol Exp Neurol 1998;57:76-94.

57. Miravalle L, Calero M, Takao M, Roher AE, Gheetti B, Vidal R: Amino-terminally truncated Abeta peptide species are the main component of cotton wool plaques. Biochemistry 2005;44:10810-10821.

58. Hartig W, Goldhammer S, Bauer U, Wegner F, Wirths O, Bayer TA, Grosche J: Concomitant detection of beta-amyloid peptides with N-terminal truncation and different C-terminal endings in cortical plaques from cases with Alzheimer's disease, senile monkeys and triple transgenic mice. J Chem Neuroanat 2010;40:82-92.

59. Mori H, Ishii K, Tomiyama T, Furiya Y, Sahara N, Asano S, Endo N, Shirasawa T, Takio K: Racemization: its biological significance on neuropathogenesis of Alzheimer's disease. Tohoku J Exp Med 1994;174:251-262.

60. Tomiyama T, Asano S, Furiya Y, Shirasawa T, Endo N, Mori H: Racemization of Asp23 residue affects the aggregation properties of Alzheimer amyloid beta protein analogues. J Biol Chem 1994;269:10205-10208.

61. Murakami K, Uno M, Masuda Y, Shimizu T, Shirasawa T, Irie K: Isomerization and/or racemization at Asp23 of Abeta42 do not increase its aggregative ability, neurotoxicity, and radical productivity in vitro. Biochem Biophys Res Commun 2008;366:745-751.

62. Shimizu T, Watanabe A, Ogawara M, Mori H, Shirasawa T: Isoaspartate formation and neurodegeneration in Alzheimer's disease. Arch Biochem Biophys 2000;381:225-234.

63. Saito TC, Iwatsubo T, Mann DM, Shimada H, Ihara Y, Kawashima S: Dominant and differential deposition of distinct beta-amyloid peptide species, A beta N3(e), in senile plaques. Neuron 1995;14:457-466.

64. Kuo YM, Emmerling MR, Woods AS, Cotter RJ, Roher AE: Isolation, chemical characterization, and quantitation of A beta 3-pyroglutamyl peptide from neuritic plaques and vascular amyloid deposits. Biochem Biophys Res Commun 1997;237:188-191.

65. Dong J, Atwood CS, Anderson VE, Siedlak SL, Smith MA, Perry G, Carey PR: Metal binding and oxidation of amyloid-beta within isolated senile plaque cores: Raman microscopic evidence. Biochemistry 2003;42:2768-2773.

66. Kumar S, Rezaei-Ghaileh N, Terwel D, Thal DR, Richard M, Hoch M, Mc Donald JM, Wullner U, Glebov K, Heneka MT, Walsh DM, Zweckstetter M, Walter J: Extracellular phosphorylation of the amyloid beta-peptide promotes formation of toxic aggregates during the pathogenesis of Alzheimer's disease. EMBO J 2011;30:2255-2265.

67. Milton NG: Phosphorylated amyloid-beta: the toxic intermediate in alzheimer's disease neurodegeneration. Subcell Biochem 2005;38:381-402.

68. Milton NG: Phosphorylation of amyloid-beta at the serine 26 residue by human cdc2 kinase. Neuroreport 2001;12:3839-3844.

69. Gunter A, Dobeli H, Bohrmann B: High sensitivity analysis of amyloid-beta peptide composition in amyloid deposits from
human and PS2APP mouse brain. Neuroscience 2006;143:461-475.

70. Miller DL, Papayannopoulos IA, Styles J, Bobin SA, Lin YY, Biemann K, Igla K: Peptide compositions of the cerebrovascular and serine plaque core amyloid deposits of Alzheimer’s disease. Arch Biochem Biophys 1993;310:41-52.

71. Sergeant N, Bombois S, Ghestem A, Drobecq H, Kostanjvecki V, Missaen C, Wattez A, David JP, Vanmechelen E, Sergheraert C, Delacourte A: Truncated beta-amyloid peptide species in pre-clinical Alzheimer’s disease as new targets for the vaccination approach. J Neurochem 2003;85:1581-1591.

72. Pike CJ, Overman MJ, Cotman CW: Amino-terminal deletions enhance aggregation of beta-amyloid peptides in vitro. J Biol Chem 1995;270:23895-23898.

73. Millucci L, Ghezzi L, Bernardini G, Santucci A: Conformations and biological activities of amyloid beta peptide 25-35. Curr Protein Pept Sci 2010;11:54-67.

74. Schilling S, Zeitschel U, Hoffmann T, Heiser U, Francke M, Kehlen A, Holzer M, Hutter PA, Prokesch M, Windisch M, Jagla W, Schlenzig D, Lindner C, Rudolph T, Reuter G, Cynis H, Montag D, Demuth HU, Rossner S: Glutaminyl cyclase inhibition alters structure and function of proteins. Prog Neurobiol 2010;89:236-272.

75. Kubler D, Barneck A: Ecto-protein kinase activity that phosphorylates Kemptide in a cyclic AMP-dependent mode. J Biol Chem 1989;264:14549-14555.

76. Rodríguez P, Mitton B, Kranias EG: Phosphorylation of glutathione-S-transferase by protein kinase C-alpha implications for affinity-tag purification. Biotechnol Lett 2005;27:1869-1873.

77. Walter J, Scholzer M, Pyerin W, Kinzel V, Kubler D: Induced release of cell surface protein kinase yields CK1- and CK2-like enzymes in tandem. J Biol Chem 1996;271:111-119.

78. el-Moattassim C, Dornand J, Mani JC: Extracellular ATP and cell signalling. Biochim Biophys Acta 1992;1134:31-45.

79. Dubyak GR, el-Moattassim C: Signal transduction via P2-purinergic receptors for extracellular ATP and other nucleotides. Am J Physiol 1993;265:C577-C606.

80. Burnstock G: Purinergic signalling and disorders of the central nervous system. Nat Rev Drug Discov 2008;7:575-590.

81. Abbracchio MP, Burnstock G, Verkhratsky A, Zimmermann H: Purinergic signalling in the nervous system: an overview. Trends Neurosci 2009;32:19-29.

82. Fuji S: ATP- and adenosine-mediated signalling in the central nervous system: the role of extracellular ATP in hippocampal long-term potentiation. J Pharmacol Sci 2004;94:103-106.

83. Gourine AV, Dale N, Llaudet E, Poputnikov DM, Spyer KM, Gourine VN: Release of ATP in the central nervous system during systemic inflammation: real-time measurement in the hypothalamus of conscious rabbits. J Physiol 2007;585:305-316.

84. Melani A, Turchi D, Vannucchi MG, Cipriani S, Gianfriddo M, Pedata F: ATP extracellular concentrations are increased in the rat striatum during in vivo ischemia. Neurochem Int 2005;47:442-448.

85. Iijima K, Ando K, Takeda S, Satoh Y, Seki T, Itohara S, Greengard P, Kirino Y, Nairn AC, Suzuki T: Neuron-specific phosphorylation of Alzheimer’s beta-amyloid precursor protein by cyclin-dependent kinase 5. J Neurochem 2000;75:1085-1091.

86. Suzuki T, Nakaya T: Regulation of amyloid beta-protein precursor by phosphorylation and protein interactions. J Biol Chem 2008;283:29633-29637.

87. Walter J, Schindzielorz A, Hartung B, Haass C: Phosphorylation of the beta-amyloid precursor protein at the cell surface by ectoasein kinases 1 and 2. J Biol Chem 2000;275:23523-23529.

88. Walter J, Fluhler R, Hartung B, Willem M, Kaether C, Capell A, Lammich S, Multhaup G, Haass C: Phosphorylation regulates intracellular trafficking of beta-secretase. J Biol Chem 2001;276:14634-14641.

89. von Arnim CA, Tangredi MM, Peltan ID, Lee BM, Irizarry MC, Kinosita H, Hyman BT: Demonstration of BACE (beta-secretase) phosphorylation and its interaction with GGA1 in cells by fluorescence-lifetime imaging microscopy. J Cell Sci 2004;117:5437-5445.

90. Walter J, Capell A, Grunberg J, Puelson B, Schindzielorz A, Prior R, Podlisny MB, Fraser P, Hyslop PS, Selkoe DJ, Haass C: The Alzheimer’s disease-associated presenilins are differentially phosphorylated proteins located predominantly within the endoplasmic reticulum. Mol Med 1996;2:673-691.

91. De Strooper B, Barnekow A, Contreras B, Levesque L, Czerecki K, Cordell B, Moechars D, Bollen M, Fraser P, George-Hyslop PS, Van LF: Phosphorylation, subcellular
localization, and membrane orientation of the Alzheimer's disease-associated presenilins. J Biol Chem 1997;272:3590-3598.

103. Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI: Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proc Natl Acad Sci U S A 1986;83:4913-4917.

104. Delobel P, Flamant S, Hamdane M, Mailliot C, Sambo AV, Begard S, Sergeant N, Delacourte A, Vilain JP, Buee L: Abnormal Tau phosphorylation of the Alzheimer-type also occurs during mitosis. J Neurochem 2002;83:412-420.

105. Mi K, Johnson GV: The role of tau phosphorylation in the pathogenesis of Alzheimer's disease. Curr Alzheimer Res 2006;3:449-463.

106. Chun W, Johnson GV: The role of tau phosphorylation and cleavage in neuronal cell death. Front Biosci 2007;12:733-756.

107. Moloney A, Sattelle DB, Lomas DA, Crowther DC: Alzheimer's disease: insights from Drosophila melanogaster models. Trends Biochem Sci 2010;35:228-235.

108. Crowther DC, Page R, Rival T, Chandraratna DS, Lomas DA: Using a Drosophila model of Alzheimer's disease. SEB Exp Biol Ser 2008;60:57-77.

109. Walker LC, Bian F, Callahan MJ, Lipinski WI, Durham RA, LeVine H: Modeling Alzheimer's disease and other proteopathies in vivo: is seeding the key? Amino Acids 2002;23:87-93.

110. Meyer-Luehmann M, Coomaraswamy J, Bolmont T, Kaeser S, Schaefer C, Kilger E, Neuenschwander A, Abramowski D, Frey P, Jaton AL, Vigouret JM, Paganetti P, Walsh DM, Mathews PM, Ghiso J, Staufenbiel M, Walker LC, Jucker M: Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. Science 2006;313:1781-1784.

111. LeVine H, III: The Amyloid Hypothesis and the clearance and degradation of Alzheimer's beta-peptide. J Alzheimers Dis 2004;6:303-314.

112. Paleologou KE, Oueslati A, Shaked G, Rospiglioni CC, Kim HY, Lamberto GR, Fernandez CO, Schmid A, Chegini F, Gai WP, Chiappe D, Moniatte M, Schneider BL, Aebischer P, Eliezer D, Zweckstetter M, Masliah E, Lashuel HA: Phosphorylation at S87 is enhanced in synucleinopathies, inhibits alpha-synuclein oligomerization, and influences synuclein-membrane interactions. J Neurosci 2010;30:3184-3198.

113. Liang FC, Chen RP, Lin CC, Huang KT, Chan SI: Tuning the conformation properties of a peptide by glycosylation and phosphorylation. Biochem Biophys Res Commun 2006;342:482-488.

114. Mbefo MK, Paleologou KE, Boucharaba A, Oueslati A, Schell H, Fournier M, Olschewski D, Yin G, Zweckstetter M, Masliah E, Kahle PJ, Hirling H, Lashuel HA: Phosphorylation of synucleins by members of the Polo-like kinase family. J Biol Chem 2010;285:2807-2822.

115. Cavalarin N, Vicario M, Negro A: The role of phosphorylation in synucleinopathies: focus on Parkinson's disease. CNS Neurol Disord Drug Targets 2010;9:471-481.