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Involvement of ‘stress-response’ kinase pathways in Alzheimer’s disease progression

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Abstract

Alzheimer’s disease (AD) is the most prevalent cause of dementia, affecting more than 25 million people worldwide. Current models of the pathophysiological mechanisms of AD suggest that the accumulation of soluble oligomeric forms of amyloid- β (A β) peptides causes early loss of excitatory synapses and impairs synaptic plasticity. The signaling pathways mediating A β oligomer-induced impairment of synaptic plasticity and loss of excitatory synapses are only beginning to be unraveled. Here, we review recent evidence supporting the critical contribution of conserved ‘stress-response’ kinase pathways in AD progression.

Introduction

There is currently no effective treatment for Alzheimer’s disease (AD) and the steady increase in human life span adds to the considerable burden that this devastating neurodegenerative disease imposes on our society. AD is characterized by the deposition in the brain of extracellular amyloid plaques composed of aggregated amyloid- β (A β) peptide, and of intracellular neurofibrillary tangles composed of aggregates of hyperphosphorylated protein Tau.

Loss of synapses in the hippocampus and neocortex is a cardinal feature that occurs at an early clinical stage of the disease, and strongly correlates with the degree of cognitive impairment [1]. In mouse models of AD, severe changes in synaptic function and maintenance can occur well before the appearance of amyloid plaques, supporting the amyloid hypothesis which suggests that soluble oligomers of A β are causal to synaptic toxicity [2], trigger synaptic dysfunction, synapse loss and impaired long-term potentiation (LTP) [3]. The exact nature of the A β species (dimers, trimers, A β *56, protofibrils) responsible for this early synaptotoxicity is still under investigation [4]. Most experimental

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designs use a mixture of various forms of synthetic A β oligomers, or natural A β oligomers isolated from the brain of AD subjects, to induce rapid loss of excitatory synapses in hippocampal and cortical neurons *in vitro* [5–10]. Transgenic mouse models of AD engineered to overexpress human mutant forms of Amyloid Precursor Protein (hAPP), with or without overexpression of mutant presenilins (PS), produce high levels of A β 1–40 and A β 1–42 peptides and oligomers, recapitulate the reduction of excitatory synapses, exhibit neuronal network dysfunction and cognitive deficits in spatial learning [6,11].

Current evidence suggests that the excitatory post-synaptic compartment represents the main target of A β toxicity [8], and several candidate receptors for A β oligomers have recently emerged, such as cellular prion protein PrP^C [12**] and EphB2 [13**]. Other cell surface receptors display altered function or expression in various AD mouse models, such as α 7-nicotinic acetylcholine receptor [14], the receptor for advanced glycation endproducts (RAGE) [15], metabotropic glutamate receptor mGluR5 [16], ionotropic glutamate NMDA and AMPA receptors [5,7,14,17]. A β oligomer binding to the post-synaptic compartment impairs the expression and function of these receptors, leading to altered synaptic plasticity and maintenance, and ultimately spine loss. The identification of the downstream signaling pathways mediating A β oligomer-dependent synaptotoxicity has considerable relevance for our understanding of the pathophysiology of AD and for the development of new therapeutic strategies. Here we review recently identified molecular pathways that contribute to A β oligomer-induced synaptotoxicity. Glycogen synthase kinase-3 (GSK-3) and CDK5 are not discussed in the present review because of space limitation and several recent reviews have addressed their potential contribution in AD [18–20].

PrP^C-mGluR5-Fyn versus EphB2

PrP^C has been identified through an unbiased genome-wide screen as a potential highaffinity receptor for A β oligomers [12**] (Figure 1A), and the binding of A β species to PrP^C has been confirmed in human AD brains [4,21*]. Experiments using genetic deletion or immunodepletion of PrP^C in hAPP mouse models support PrP^C as an essential mediator of A β oligomer-induced impairment of synaptic plasticity and memory, and synapse loss (reviewed in [22]), although this does not appear to be the case in every genetic backgrounds such as the J20 model [23]. The binding of synthetic or natural A β oligomers to PrP^C at the post-synaptic density activates the Src kinase Fyn [4,21*], which in turn phosphorylates NR2B subunit, leading to increased surface NMDA receptor and excitotoxicity, followed by spine and receptor loss [21*]. Interestingly, Tau targets Fyn to the post-synapse to induce A β toxicity, and Tau deletion in hAPP mice, which causes disruption of post-synaptic targeting of Fyn, prevents A β -induced NMDA receptor-mediated excitotoxicity [24*]. A β -dependent PrP^C-Fyn signaling induces Tau missorting and hyperphosphorylation at Y18 [4]. However, the requirement and functional role of this Fyn-dependent phosphorylation site on Tau for mediating A β toxicity at the synapse has not been investigated yet. Furthermore, mGluR5 is required for coupling A β oligomers/PrP^C complex with Fyn to induce dendritic spine loss and memory impairment [22]. mGluR5 antagonist 3-((2-Methyl-4-thiazolyl)ethynyl)pyridine (MTEP) blocks these deficits in APP/PS1dE9 and 3 \times Tg mouse models, suggesting that preventing mGluR5 activation by A β oligomers/PrP^C complex may be therapeutically relevant for treating AD [22].

EphB2 tyrosine kinase receptor has been recently identified as another high-affinity receptor for A β oligomers (Figure 1B) and EphB2 is known to play an important role in regulating the synaptic localization and function of NMDA receptors [25]. AD patients and hAPP transgenic mouse models of AD have reduced level of EphB2 in the hippocampus [26]. Recent studies revealed that A β oligomers can bind EphB2 to trigger EphB2 degradation in the proteasome, and knockdown of EphB2 impairs NMDA receptor signaling, leading to defective LTP [13**]. Remarkably, virus-mediated expression of EphB2 in the dentate gyrus of hAPP J20 mouse model blocks synaptic and memory deficits, suggesting that restoring EphB2 expression locally in the dentate gyrus may be an effective therapeutic strategy [13**].

Calcineurin-NFAT / GSK3-NFAT

A β oligomers dysregulate calcium homeostasis through a mechanism involving NMDA receptors (reviewed in [27]). A β -induced calcium elevation activates the calcium-dependent phosphatase B calcineurin, which in turn promotes nuclear translocation and activation of the transcriptional nuclear factor of activated T cells (NFAT) [28,29]. Both calcineurin and NFAT activity are increased in AD brains [28], and activation of the calcineurin-NFAT cascade in rodents results in dystrophic neurites, dendritic simplification and dendritic spine loss *in vitro* and *in vivo* [28,29]. Strategies aimed at blocking calcineurin or NFAT activation prevent spine loss and dendritic dystrophy in the Tg2576 transgenic mouse model of AD, supporting calcineurin- NFAT contribution to A β synaptotoxicity [28,29]. Furthermore, NFAT-dependent apoptotic pathway is activated by inhibition of GSK-3 [30], potentially explaining the conflicting outcomes in preclinical and clinical trials using GSK-3 inhibitors for treating AD and questioning the single therapeutic strategy of inhibiting GSK-3 [18].

Centaurin- α 1-Ras-Elk-1

Centaurin- α 1 (also named p42/IP4) is an ADP ribosylation factor (Arf) GTPase-activating protein (GAP) that is required for normal dendritic development [31]. It interacts with Ras and activates Ras-E26-like kinase 1 (Elk-1). Both centaurin- α 1 and Elk-1 can associate with the mitochondrial permeability transition pore complex to regulate its function [32]. Centaurin- α 1 protein level is increased in the brain of AD patients and hAPP J20 mouse model [33], and increased association of Elk-1 with mitochondria is also observed in the J20 mice [34]. Recent studies demonstrated that A β 1–42 oligomers increase the expression level of centaurin- α 1 in cultured neurons, which induces a Ras-dependent association of Elk-1 with mitochondria, leading to mitochondrial and synaptic dysfunction in organotypic hippocampal slices [34]. Blockade of the centaurin- α 1-Ras-Elk-1 pathway rescues A β -induced dendritic spine loss, spine structural plasticity, and mEPSC (miniature Excitatory Post-synaptic Currents) amplitude and frequency, suggesting the contribution of this pathway in neuronal dysfunction in early stage of AD. Further studies are required to determine the relevance of this pathway *in vivo*. Interestingly, extensive experimental evidence from the cancer field has demonstrated that the Ras-MAPK pathway is tightly connected to the AMPK (AMP-activated Kinase) and mTOR (mammalian target of

rapamycin) pathways [35,36] which have been recently shown to play a critical role in the early synaptotoxic effects of A β oligomers (see below).

mTOR

The serine/threonine kinase mTOR plays a central role in various cellular processes, including cell size, cell proliferation through regulation of protein synthesis, and also negatively regulates autophagy (reviewed in [37]). In neurons, mTOR plays an important role in long-term synaptic plasticity, axon pathfinding and regeneration, dendrite arborization and spine morphology (reviewed in [38]). mTOR signaling has been shown to be up-regulated in mouse models and human cases of AD, although data appears to be conflicting, and increased mTOR signaling seems to be caused by A β (reviewed in [39]). Therefore, A β -mediated hyperactivation of mTOR may contribute to early cognitive defects in Alzheimer's disease. Accordingly, inhibition of mTOR by rapamycin treatment improves cognitive deficits and rescues both A β and Tau pathologies in AD mouse models [40,41]. However, given the central role of mTOR in learning and memory, further studies may be required to clarify its potential relevance to treat AD.

MARK kinases

The serine/threonine microtubule-affinity regulating kinases (MARK) belong to the AMPK-like family of kinases, and consist of four members (MARK1 to MARK4) in mammals. MARKs are the closest orthologs of the fruit fly partition defective-1 (PAR-1) which plays an important role in regulating cell polarity [42]. MARK1 and MARK2 were originally identified as regulators of microtubule stability [43]. Through their ability to phosphorylate Tau on KXGS motifs in the microtubule binding domain, MARKs cause Tau detachment from microtubules and microtubule destabilization (reviewed in [44,45]). Genome-wide association studies suggest a potential link of MARK4 to late onset AD [46], and recent functional studies suggest that MARK kinases participate to the synaptotoxic effects of A β *in vitro* [47,48]. Overexpression of MARK4 in hippocampal neurons leads to Tau hyperphosphorylation (Figure 2), reduced expression of synaptic markers, loss of dendritic spines and synapses, while an inhibitor of all MARK family members abrogated the toxic effects of A β oligomers on dendritic spines and synapses, assayed at the morphological and electrophysiological levels [47]. However, conflicting studies reported that overexpression of MARK2 prevents A β oligomer-induced synaptotoxicity, whereas pharmacological inhibition of the kinase results in spine loss in presence or absence of A β [48]. Future studies will have to define A β -dependent signaling pathway leading to activation of MARKs, and use loss- and gain-of-function approaches in hAPP mouse models to better understand the role of these group of kinases in mediating the toxic effects of A β .

Role of the CAMKK2-AMPK pathway in neurodegenerative diseases

Calcium/Calmodulin-dependent Kinase Kinase 2 (CAMKK2) is a serine/threonine protein kinase whose activity is increased upon Ca²⁺ influx through Ca²⁺/calmodulin binding. It is the upstream activator of calcium/calmodulin kinases CAMKI and CAMKIV, and of the metabolic sensor AMPK [49]. CAMKK2 is mainly expressed in the central nervous system,

where it plays important roles, presumably through via CAMKI, in axon and synaptic development, synaptic plasticity and memory (reviewed in [50]).

AMPK is a serine/threonine protein kinase composed of three subunits, including a catalytic subunit ($\alpha 1$ or $\alpha 2$ in mammals), a β adaptor subunit ($\beta 1$ or $\beta 2$), and a γ subunit ($\gamma 1$, $\gamma 2$ or $\gamma 3$) that is allosterically activated upon binding to AMP or ADP (reviewed in [35,36,51]). Phosphorylation of the threonine residue T172 within the activation loop of the α subunit is required for catalytic activation. Several kinases can act as direct upstream activators of AMPK through phosphorylation of T172, including CAMKK2 and LKB1. AMPK is instrumental at restoring proper ATP level through a myriad of effectors, converging on repression of the mTOR pathway and promotion of autophagy [35,36,51], which are both deregulated in AD mouse models and human patients (reviewed in [39,52]). In neurons, AMPK can be activated by increased intracellular Ca^{2+} (through depolarization and opening of voltage-gated calcium channels or through activation of ionotropic glutamate receptors such as NMDA receptors) and therefore is not only a metabolic sensor but also reports changes in neuronal activity [53**,54*].

In the brain, AMPK activity is increased by metabolic stresses associated with ischemia, hypoxia or glucose-deprivation [55,56], and is abnormally elevated in several human neurodegenerative disorders including AD and other tauopathies, amyotrophic lateral sclerosis, and Huntington's disease [57–59]. However, whether over-activation of AMPK in these different pathological contexts plays a role in the disease progression remained controversial (see [63] for example). Activated AMPK seems strongly enriched in tangle- and pre-tangle bearing neurons in AD patients, suggesting that AMPK might participate to AD progression by modulating Tau phosphorylation [59]. Recent reports indicated that AMPK over-activation might have deleterious outcomes by increasing A β generation through β -secretase transcriptional up-regulation [64] and inhibition of mTOR-dependent protein synthesis [65**], and by aberrantly promoting autophagosome formation through RAGE-CAMKK2 pathway [66].

The CAMKK2-AMPK pathway was recently shown to mediate the early synaptotoxic effects of A β oligomers, in part through Tau phosphorylation [53**] (Figure 2). We found that the CAMKK2-AMPK pathway is robustly activated by acute application of A β 42 oligomers *in vitro* confirming a previous report [54*], and that AMPK activation is elevated in the cortex and hippocampus of the J20 hAPP-overexpressing mouse model at 4 and 12 month-old [53**]. More importantly, we found that abolishing CAMKK2 or AMPK catalytic activity or expression protects hippocampal and cortical neurons from the synaptotoxic effects of A β 42 oligomers *in vitro* as well as the loss of dendritic spines observed in the J20 mouse model *in vivo* [53**].

AMPK, BRSK1/2 and MARK1-4 can robustly phosphorylate Tau on S262 in the KXGS motif of the microtubule-binding domain, and preventing phosphorylation on this site blocks the synaptotoxic effects induced by A β oligomers or by overexpression of these kinases [47,48,53**,54*,67]. Tau aggregation and clearance seems to be regulated by a balance between phosphorylation and acetylation at the KXGS motifs [68]. The relevance of AMPK

and MARK kinases over-activation and Tau phosphorylation in AD pathogenesis requires further *in vivo* evidence.

AMPK-mTOR-JNK3

A recent report suggests that c-Jun N-terminal kinase 3 (JNK3) activation, which is abnormally increased in the brain of human AD cases and hAPP mouse models, is central to the development of AD pathology by maintaining a positive feedback loop that leads to continued production of A β [65**] (Figure 2). Accordingly, genetic deletion of JNK3 in a hAPP mouse model (5XFAD) results in dramatic reduction in A β 42 levels and A β plaque loads, and improved cognition. Interestingly and in accordance with the recent report discussed above [53**], *in vitro* studies suggest that A β 42 oligomer-dependent activation of AMPK inhibits mTOR, resulting in a translational block responsible for endoplasmic reticulum (ER) stress which activates JNK3. In turn, JNK3 phosphorylates APP at T688, facilitating its endocytosis and processing, thereby increasing A β production *in vitro*. This study contrasts with other studies who proposed to inhibit mTOR as a therapeutic approach for AD [40,41].

The microtubule-binding protein Tau as a critical effector of A β -induced synaptotoxicity

Remarkably, the adverse effects of A β on neuronal degeneration and cognitive dysfunction appear to depend in large part on Tau (reviewed in [69–71]). For example, synthetic A β oligomers or A β dimers isolated from the brain of AD subjects induce hyperphosphorylation of Tau at AD-relevant epitopes in hippocampal neurons, Tau mislocalization into the somatodendritic compartment, disrupt the microtubule cytoskeleton and cause neuritic degeneration [9,72]. Knocking down endogenous Tau does not appear to be phenotypic in neurons but instead fully prevents neuritic changes, whereas overexpressing human Tau accelerated them [9,48]. *In vivo*, genetic deletion of Tau in the hAPP J20 mouse model prevents behavioral deficits without altering A β burden, and protects both transgenic and non-transgenic mice against excitotoxicity [73,74**].

Conclusion

Many kinase pathways have been implicated in AD progression. However, it is presently unclear which pathways are mediating the synaptotoxic effects of A β oligomers and which are activated in response to impairment of synaptic homeostasis and/or neuronal viability. Furthermore, there is increasing evidence that A β oligomers can trigger the over-activation of ‘stress-response’ pathways including the CAMKK2-AMPK pathway [53**,54*] and the downstream mTOR-ER stress-JNK3 pathway [65**]. These pathways seem to play important roles in the early steps of AD progression, inducing loss of excitatory synapses and reduction of synaptic plasticity in hippocampal and cortical circuits. Future investigations need to determine how these ‘stress-response’ kinase pathways interact functionally during AD progression, and whether preventing their over-activation could represent a new therapeutic avenue to prevent the cognitive decline characterizing AD

progression. This study provides the first *in vivo* evidence that genetic deletion of tau prevents cognitive deficits in a hAPP transgenic mouse model without altering A β burden.

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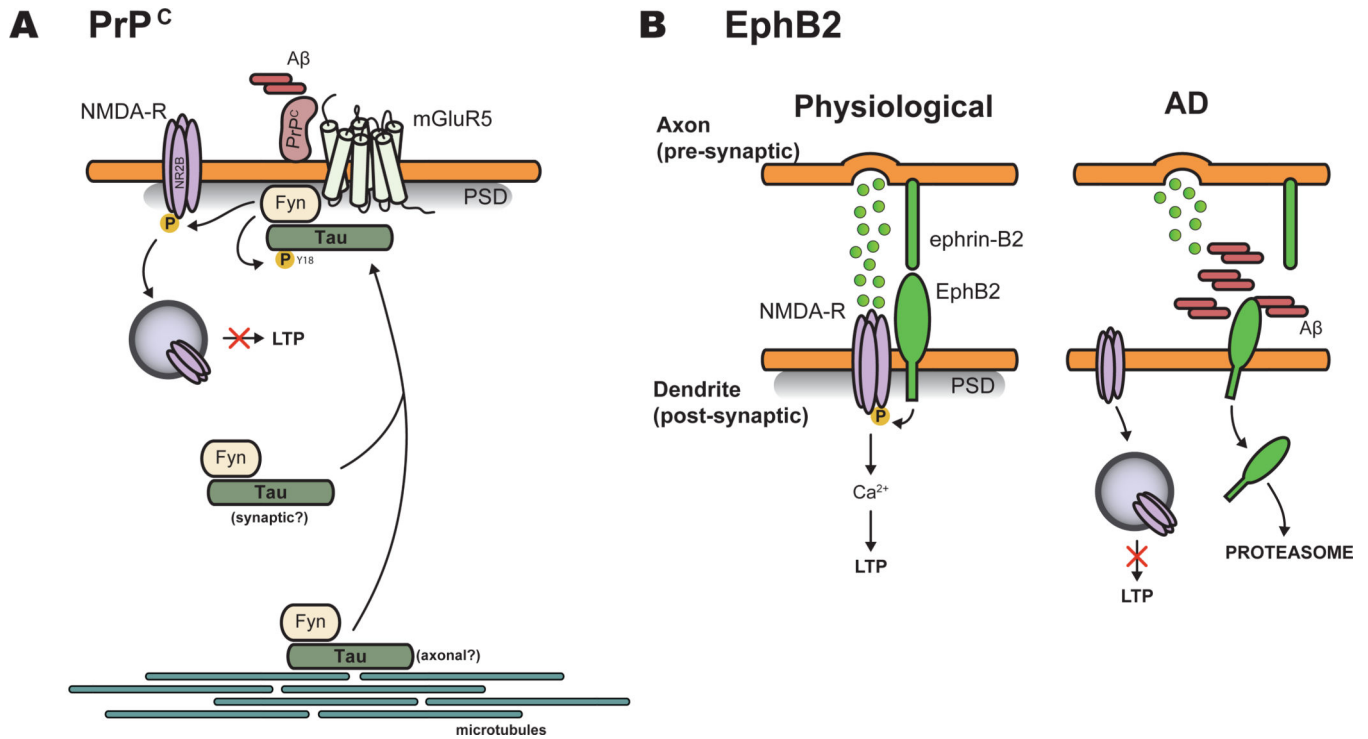
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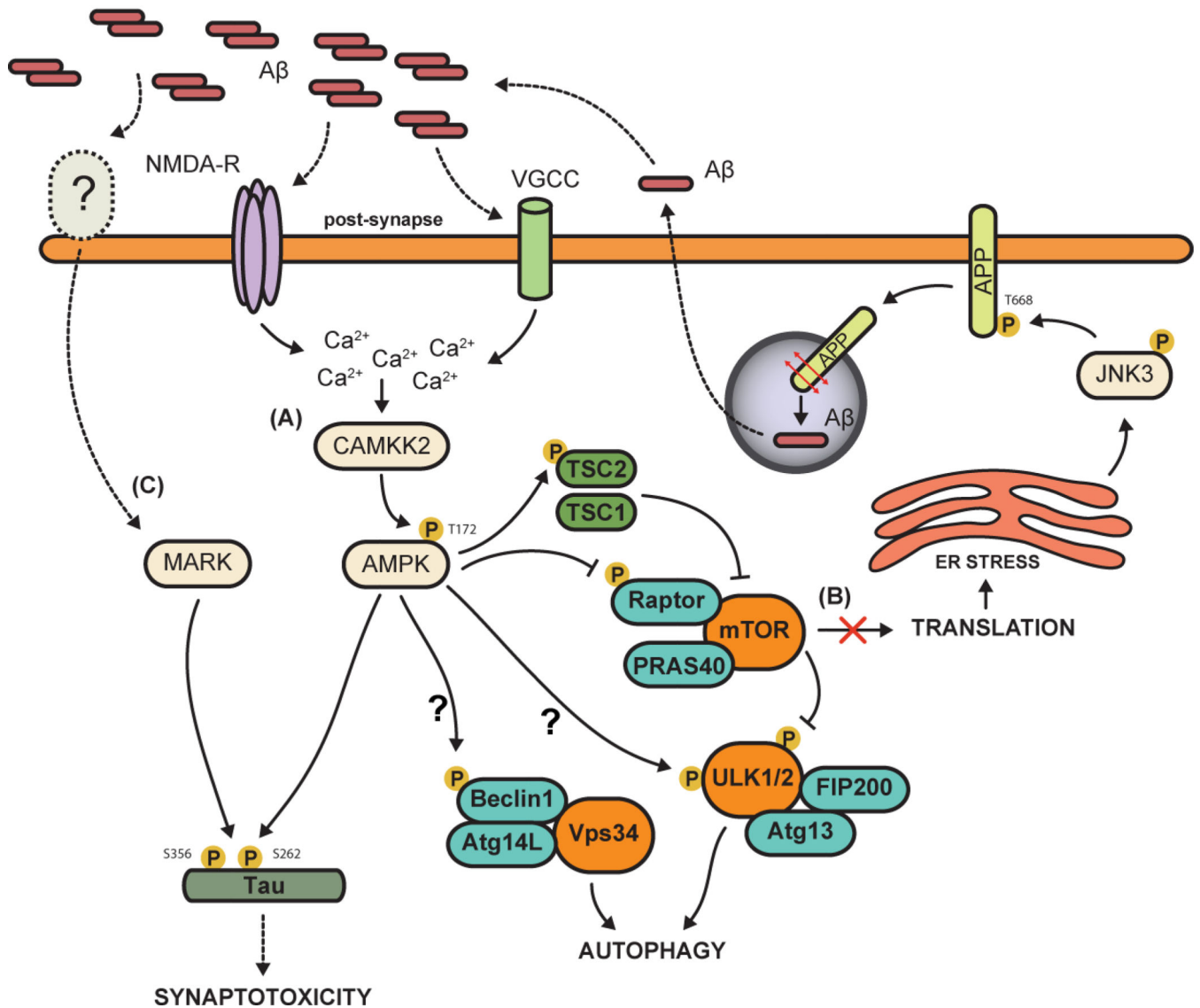
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- Several 'stress-response' kinase pathways are over-activated in Alzheimer's disease
- A β oligomers activate the CAMKK2-AMPK pathway which phosphorylates Tau on Serine 262
- Recent results involve mTOR inhibition, protein synthesis block and ER stress in AD progression
- Blocking these 'stress-response' pathways prevents the synaptotoxic effects of A β oligomers

**Figure 1.**

Involvement of the PrP^C-mGluR5-Fyn and EphB2-NMDA pathways in Aβ oligomer-induced synaptic pathology. **(A)** PrP^C is a functional receptor of Aβ oligomers. Upon binding, Aβ/PrP^C complexes activate Fyn. mGluR5 receptor seems required for coupling Aβ oligomers/PrP^C complexes to Fyn. Tau plays an important role in signal transduction by tethering Fyn post-synaptically where it phosphorylates NR2B subunit and Tau at Y18. The origin of Tau/Fyn complexes, either axonal and/or postsynaptic, is currently unknown. Phosphorylation of NMDA receptors by Fyn leads to increased surface NMDA receptor (NMDA-R) and excitotoxicity, followed by spine (and receptor) loss. **(B)** EphB2 is another functional receptor for Aβ oligomers. In physiological conditions, EphB2/ephrin-B2 complexes regulate NMDA receptor function via NR1 and/or NR2B subunit phosphorylation. In AD, Aβ oligomer binding to EphB2 receptor induces EphB2 internalization and degradation in the proteasome. Decreased EphB2 receptor alters NMDA receptor function, resulting in LTP impairment. This figure is adapted from [13**]. PSD: postsynaptic density.

**Figure 2.**

Aβ oligomers activate several ‘stress-response’ kinase pathways in neurons. **(A)** Oligomeric Aβ as well as increased intracellular calcium flux, induced either by NMDA receptor activation or membrane depolarization through opening of voltage-gated calcium channels (VGCC), can activate the CAMKK2-AMPK pathway. AMPK over-activation leads to increased phosphorylation of Tau at S262 which results in dendritic spine loss. Furthermore, AMPK promotes autophagy, which is abnormally activated in AD, by acting on three main pathways: (1) indirectly, by inhibiting mTORC1 (mTOR complex 1, which inhibits autophagy) through phosphorylation of Raptor and/or TSC2 (Tuberous Sclerosis Complex 2); (2) directly, by activating ULK1/2 (Unc-51 Like Kinases), the mammalian orthologs of yeast ATG1, an upstream regulator of the autophagy pathway; and (3) via Beclin1 phosphorylation which promotes its assembly with class III PI3-kinase (PIK3c3; also named Vps34) and ATG14L. The two question marks represent ways whereby AMPK has been shown to regulate autophagy in non-neuronal cells but not in neurons yet. **(B)** mTOR

inhibition further results in a protein translation block which causes widespread ER stress, leading to JNK3 activation. In turn, JNK3 phosphorylates APP at T688, promoting its endocytosis and processing by secretases, consequently increasing A β 40/42 biogenesis. (C) The AMPK-related kinases MARKs can also phosphorylate Tau at ADrelevant epitopes (KXGS motifs) to mediate the synaptotoxic effects of A β oligomers, although the upstream pathway leading to their activation in mammalian neurons is currently unclear, and could be either LKB1 or CAMKK2 [53**].