The control of translation in Alzheimer’s disease

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Production of amyloid β-peptide (Aβ) deposits and formation of neurofibrillary tangles are key mechanisms involved in Alzheimer’s disease (AD) pathogenesis. Recent evidence suggests that inflammatory mechanisms may significantly also contribute to disease progression and chronicity. These mechanisms modulate many pro- and anti-apoptotic signalling pathways which could be explain why neurons die in AD, leading to the impairment of memory and behaviour. For the last five years, some studies have focused on the control of translation in AD. Results showed in different cellular and animal models of AD and in brain and lymphocytes of AD patients that the translation is downregulated not only by the PKR (double-stranded RNA-dependent Protein Kinase) activation but also the inhibition of mTOR/p70S6K signalling pathway which regulate negatively and positively the control of protein initiation, respectively. Recently, we showed that active PKR is closely associated with the apoptotic process in a new transgenic mouse model of AD. Taken together, these findings underline the possible role of PKR and p70S6K in the pathogenesis of AD. However, the molecular crosslink between the upregulation of PKR and the downregulation of mTOR by Aβ neurotoxicity remains unknown. Promoting new neuronal protective strategies in Alzheimer’s disease by PKR inhibition and/or p70S6K activation represents also an important objective in our laboratory.

Abbreviations
AD: Alzheimer’s disease; Akt/PKB: protein kinase B; APP: amyloid precursor protein; Aβ: amyloid-β peptide; CT: control of translation; eIF2: eukaryotic initiation factor 2; eIF4E: eukaryotic initiation factor 4E; 4E-BPs: eIF4E binding proteins; GSK3β: glycogen synthase kinase-3β; HD: Huntington’s disease; KI: knock-in; MPP+: 1-methyl-4-phenyltetrahydropyridinium; mTOR: mammalian Target Of Rapamycin; NFκB: nuclear transcription factor Kappa; NFTs: neurofibrillary tangles; 6-OHDA: 6-hydroxydopamine; 70/85-kDa S6 kinase; PD: Parkinson’s disease; PHFs: paired helical filaments; PI3K: phosphoinositide 3-kinase; PKR: double stranded (ds) RNA-activated protein kinase; PS1/2: presenilin 1/2; RS6K: ribosomal S6 kinase; SORL1: sortilin-related receptor 1; TOP mRNA: 5’ terminal oligopyrimidine tract-containing mRNA; TNFα: Tumor necrosis factor alpha; TSC: Tuberous sclerosis complex; TSC1/TSC2: tuberin/hamartin.

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INTRODUCTION

Alzheimer's disease (AD) is a progressive, degenerative disease of the brain, which causes serious impairment of thinking and memory. It is the most common form of dementia. AD represents 50 to 70% of dementia in the European Union. In France, over 850,000 cases of AD and related disorders have been estimated and this number is expected to increase to 1.3 million in 2020. Ageing is the most important risk factor, with a prevalence rate around 25% between 65-84 years of age. Unfortunately, no definitive therapy exists for resolution.

The disease was first identified by Dr. Alois Alzheimer in 1906. He described the two hallmarks of the disease: "plaques" - numerous tiny dense deposits scattered throughout the brain which become toxic to brain cells at excessive levels and "tangles" which interfere with vital processes eventually "choking" off the living cells. As well, when brain cells degenerate and die, the brain markedly shrinks in some regions. As AD progresses and affects different areas of the brain, various abilities and/or behaviour become impaired: difficulty in finding objects and remembering where they were placed; inability to retain memory of recent past; inability to recognize familiar faces, objects or places; inability to express thoughts clearly in writing; person may appear apathetic, uninterested; the ability to control mood and emotion may be lost; the ability to find the right words and follow a conversation is affected; the ability to independently perform day-to-day tasks is reduced.

We know that the majority of cases of AD in people over the age of 65 are the sporadic (or "late onset") form, suggesting that the disease has no family link. However, about ten per cent of the Alzheimer population have "Familial" (FAD) or "early onset" AD via autosomal dominant inheritance. FAD is identical to the sporadic form but it occurs because of the inheritance of certain genes which at some point in the family's history "mutated" from having normal to abnormal characteristics. In FAD, when a parent is affected, each child has a 50 per cent risk of inheriting the disease gene and will develop AD in adulthood. Most cases of FAD result from mutations in one of the three genes: the amyloid precursor protein (APP), the presenilin 1 and 2 (PS1, PS2). All mutations result in elevated levels of amyloid-β (Aβ) peptide [1].

The most important genetic risk factor for both the familial and sporadic forms of AD is the apoE4 gene [2,3]. ApoE4 is one variant of the apoE gene, the others being the benign apoE2 and apoE3 genes. If a person's pair of apoE genes includes one apoE4, the risk to develop AD is three times higher, but if one carries two apoE4 genes the risk increases to ten times. However, people with no apoE4 genes can still get AD and people with two apoE4 genes will not necessarily get the disease. ApoE4 enhances the rate of amyloid fibril formation, interacts with the microtubule-associated proteins [4,5] and directly affects cholinergic activity in the brain of AD subjects [6].

A recent study confirms the association between genetic variants in sortilin-related receptor (SORL1) and AD [7,8]. SORL1 binds APP and acts as a sorting receptor for APP. Absence of SORL1 switches APP away from the recycling endosomes pathway and instead directs APP into the β- and γ-secretase cleavage pathways to generate Aβ [7]. However, it is unclear whether these changes are causal or simply reactive to AD.

In addition to the risk factors described above, all of the following have been documented as risk factors for AD: type 2 (adult) diabetes, chronic inflammatory conditions (such as certain forms of arthritis), a history of episodes of clinical depression, strokes or "ministrokes", high cholesterol, high blood pressure, stress, inadequate exercising of the brain and obesity. Risk factors that are less firmly established include smoking, excessive alcohol consumption and abuse drugs.

AD is characterized by three major pathologic hallmarks that consist of extracellular plaques of the 39-43 amino acid Aβ aggregated, intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein and the neuronal death. Aβ is derived by proteolytic cleavage of APP. The action of β-secretase liberates a C-terminal fragment which is subsequently processed by γ-secretase to release Aβ. Cleavage by γ-secretase releases a number of Aβ species, which vary in length. Presenilin proteins are an integral part of γ-secretase complex involved in the processing of APP. The major form of Aβ contains 40 amino-acid residues (Aβ40), although minor C-terminally extended forms (Aβ42 and Aβ43) are also produced. Aβ42 has a higher
Increased production of Aβ42 is closely associated with the development of AD [9-11]. Besides amyloid plaques, NFTs are composed of arrays of paired helical filaments (PHFs) as described for the first time by Kidd [12]. NFTs are mainly present in the hippocampus, entorhinal cortex and amygdala. PHFs are anomalous structures generated by self-aggregation of hyperphosphorylated forms of tau protein that form a compact filamentous network [1].

Furthermore, increasing evidence suggests that inflammation significantly contributes to the pathogenesis of AD. Research performed in the last twenty years supports the hypothesis of Fisher [13]. Senile plaques might be the result of an inflammation mediated regenerative response of surrounding nerve fibres against extracellular deposits of a foreign substance. In the following years, it was shown that the “foreign” substance, now identified as Aβ fibrils, could indeed induce a local inflammatory response [14,15]. However, the chronic inflammatory response in AD brains is described in the plaques containing fibrillar Aβ deposits but not in the diffuse plaque with the non-congophilic low-fibrillar Aβ depositions [16,17]. A spectrum of inflammatory mediators upregulated in AD has been demonstrated [15, 18-21] and indicates that such inflammation is an important part of pathology and suggests many routes for future therapeutic intervention.

Yet, it is clear that a variety of cellular mechanisms can lead to this neurodegenerative disorder. Elucidation of the factors that trigger the sequence changes in the normal neuronal machinery leading to neurodegeneration and the mechanisms underlying signal transductions that determine neuronal death in AD brains is of utmost importance.

Some works reported the mitotic hypothesis in which vulnerable neurons in the AD brain not only show activation of cell cycle components, but at least in some cases, show evidence of DNA replication (S-phase) prior to neuronal death [22]. Studies of the lysosomal system in AD identified a robust mobilization of this system and its impairment in neurons which may actively promote disease pathogenesis not only by accelerating amyloidogenesis but also by triggering degeneration [23]. Another mechanism for substrate degradation in eucaryotic cells is the proteasome. In neuritic plaques and tangles in brain patients with AD, the inhibition of the proteasome may result in neuronal death [24]. In addition, several studies have provided evidence implication of the oxidative stress as a major pathogenic mechanism in AD [25].

For the last five years, works have begun to focus on pathways of the control of translation, in particular the control of initiation [26-29]. Our works are related to these molecular signalling mechanisms in order to explain the neuronal death observed in brains of AD patients. In this review, we firstly describe the control of translation (CT) in eukaryotic cells. Then, we explain the results known in CT and AD.

Control of translation

In eukaryotes, protein translation includes three consecutive phases: initiation, elongation, and termination. The initiation phase corresponds to processes associated with the connection between mRNA and ribosomes. The elongation phase includes the links between amino acids at the ribosomal level, and is followed by the termination phase. These three phases are highly regulated by proteins called translation factors, which can interact directly with mRNAs. In the initiation phase, two major factors are involved: the eukaryotic initiation factor 2 (eIF2) and the eukaryotic initiation factor 4E (eIF4E).

eIF2 can fix the GTP and interact with the tRNA carrying the methionin. The formed complex can bind to the small 40S sub-unit of the ribosome, a phase in which eIF4E plays a major role. The availability of these two initiation factors is necessary for the start of protein synthesis. The control of eIF2 is linked to the state of phosphorylation at serine 51 site on the α sub-unit. When phosphorylated, eIF2α is unable to bind the tRNA and the translation is stopped. There are four protein kinases able to phosphorylate eIF2α: PKR (double-stranded (ds) RNA-dependent protein kinase), PERK (PKR-like endoplasmic reticulum (ER) kinase), HRI (Heme regulated kinase) and GCN2 (amino-acid regulated kinase).

Several studies revealed an increased PKR expression with ageing [30] or in neurodegenerative diseases [31-33] or the activation of eIF2α in cerebral ischemia-reperfusion, hypoxia or zinc toxicity [34-36]. It
is demonstrated that these kinases play an important role in the process of cellular apoptosis by interacting with protein translation and apoptotic factors. In addition, phosphorylation of eIF2α by PKR converts it from a substrate to a competitive inhibitor of eIF2B resulting to a protein synthesis shutdown [37,38]. Besides, the β-isoform of the Glycogen Synthase Kinase-3 (GSK3β) can phosphorylate the ε subunit of eIF2B blocking the GDP/GTP exchange eIF2 and thereby leads to the inhibition of translation [39]. Furthermore, it is well known that GSK3β, the main tau kinase, can induce memory deficits in vivo and may be involved in neuronal death by inactivation of transcription factors involved in cellular defense systems [40].

PKR is an interferon-inducible eIF2α kinase that binds to dsRNA and then undergoes homodimer formation and autophosphorylation at several sites, resulting in its activation. Considerable evidence confirmed that PKR, a ubiquitous protein, plays an important role in host defense against virus infection. However, in response to stress signals, an additional dsRNA-independent mechanism of activation of this enzyme is mediated by the human protein PACT or murine RAX in vitro and in vivo [41,42]. Important roles in the regulation of protein synthesis and the control of cell growth and survival were demonstrated for PKR [37,43,44]. Several studies established that activation of PKR can either induce apoptotic cell death or at least enhance this process when apoptosis is initiated by other agents [45,46]. Conversely, in cells deficient for the kinase or containing a dominant-negative form, there is substantial resistance to apoptosis [29,45,47]. It is not clear whether the phosphorylation of eIF2α is sufficient to mediate the proapoptotic effects of PKR. The cell expression of an inhibitor of eIF2α phosphorylation or the nonphosphorylatable eIF2α mutant does partially protect cells from apoptosis [47].

The availability of the eIF4E factor is linked to the binding of specific proteins called 4E-BPs. When these proteins are non-phosphorylated, they have a great affinity for eIF4E, which is unable to bind to mRNA leading to the reduction of translation. These proteins are mainly phosphorylated by a kinase called mTOR (mammalian Target Of Rapamycin) or FRAP [48,49]. mTOR can also phosphorylate the ribosomal S6 kinases (RS6K) such as p70S6K that stimulate protein synthesis [50]. The regulation of mTOR activity is important for the availability of eIF4E. mTOR is activated by the phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt/PKB) pathway. The mTOR signalling is physiologically active and allows protein and ribosome synthesis. Many studies have shown that cellular stresses can increase the binding of 4E-BPs to the eIF4E factor and can reduce protein translation [43]. Recently, it has been demonstrated that two tumor suppressor genes, the tuberous sclerosis complex (TSC) gene products TSC1 (or Hamartin) and TSC2 (or Tuberin) were negative regulators of mTOR phosphorylation [51] by blocking the small GTPase Rheb activity which is a positive regulator of mTOR [52,53].

In addition, the reduction of eIF4E level is not enough to induce cellular apoptosis [54] and PKR can dephosphorylate the proteins 4E-BPs in activating the phosphatase 2A, leading to cell apoptosis [55,56]. Electrophysiological results have also demonstrated that mTOR could play a role in neuronal plasticity and in the process of learning and memory. In brain slices treated with the mTOR inhibitor rapamycin, the authors have observed a decrease of the late phase of LTP induced by synaptic stimulation or BDNF exposure [57,58].

According to these results, it appears worth studying these two pathways: PKR (pro-apoptotic) and mTOR (anti-apoptotic), involved in the control of translation initiation, in different models of neurodegenerative disorders and in patients. These both molecular signalling pathways were largely described in AD. Recently, studies have reported that brain tissues from patients with Parkinson's (PD) and Huntington's (HD) diseases displayed also strong induction of phosphorylated PKR in hippocampal neurons compared with age-matched disease controls [33].
AD and control of translation

It has been recognized for several years that the mRNA translation was disturbed in the brain of AD patients [59]. The inhibition of the ribosomal translation could be responsible for the modifications of gene expression detected in affected brain regions. An increased of the elongation factor 2 was also noted in the AD brains [60].

For the last four years, our studies have focused on the control of translation, in particular the control of initiation. We have recently demonstrated that Aβ exposure induces a sustained reduction of mTOR/p70S6K signalling in neuroblastoma cell cultures marked by a progressive decrease of phosphorylated mTOR and phosphorylated p70S6K [26,61]. In addition, the mTOR signalling is reduced in the cortex but not in the cerebellum of APPsl/PS1 mutant transgenic mice and in lymphocytes of AD patients as compared with results observed in control individuals [26]. These modifications were positively correlated with cognitive and memory test scores performed in AD patients: the higher expression of mTOR, the higher cognitive tests score [62,63]. Furthermore, previous reports revealed that phosphorylated and activated PKR was associated with degenerating neurons in AD brains [32,64,65]. Some data also showed that phosphorylated PKR and eIF2α co-localized with phosphorylated tau protein in affected neurons in AD brains [32]. Another report demonstrated in vitro that Aβ neurotoxicity was associated with an increased PKR and eIF2α phosphorylation linked to increased intracellular calcium and caspase 8 activation following Aβ exposure [27,29]. All these results could suggest that PKR could be involved in the pathogenesis of AD.

Interestingly, our results showed that activated PKR and eIF2α were significantly increased in lymphocytes of AD patients. These modifications were negatively correlated with cognitive and memory test scores performed in AD patients: the higher expression of PKR, the lower cognitive tests score [66].

In 2005, we proposed a working hypothesis to explain the crosslink between the upregulation of PKR and the downregulation of mTOR by Aβ neurotoxicity and in lymphocytes of AD patients. We received a LECMA grant for this...
research project entitled: “Role of p53, PKR and mTOR molecular pathways in Aβ neurotoxicity”. Three molecular factors p53, RTP801/Redd1 and TSC1/TSC2 complex seem to be involved in this crosslink and promote the role of PKR in the neuronal death. Definitive results will be published at the end of this year. Recently, authors identified RTP801 as a gene whose transcripts were highly induced in a cellular model of PD in which death of neuronal catecholaminergic PC12 cells was triggered by the PD mimetic 6-hydroxydopamine (6-OHDA) and in PD brains. RTP801 induce neuron death appears to involve repression of p70S6K activity in cells treated with 6-OHDA, and such death is inhibited by shRNAs targeting TSC2, a protein with which RTP801 interacts to block mTOR signalling pathway [67]. We also studied the control of translation in the newly engineered APPSL/PS1 knock in transgenic mice (APPSL/PS1 KI) which display, in addition to extra-cellular Aβ deposits, more than 50% CA1 neuron loss at 10 months of age, starting approximately at 6 months combined with intraneuronal Aβ accumulations [68]. Western blot results revealed an increase of activated PKR and eIF2α in the brains of these transgenic mice. A progressive increase of apoptotic nuclei is observed at 3, 6 and 12 months of age in APPSL/PS1 KI mice and PKR is closely co-localized with DNA strand breaks in apoptotic nuclei of hippocampal neurons [69]. Furthermore, we showed in APPSL, APPPS/PS1 and APPSL/PS1 KI that phospho-PKR was expressed at the level of amyloid deposits (data not published) as Peel and Bredesen [28] in another APP transgenic mouse model. Phospho-PKR was not colocalized with Aβ, but surrounded these amyloid deposits as the terminal membrane attack complex (MAC) attacking dystrophic neurites in brains of AD patients [16,20]. In addition, our results showed a great reactive microglia and activated astrocytes by Fluorojade® B staining around amyloid deposits in hippocampus and cortex of APPSL (10 month-old), APPPS/PS1 (12 month-old) and APPSL/PS1 KI (starting at 6 month-old) [70]. In the last mouse model, phospho-PKR was expressed in nuclei of neurons at 3 months of age before the presence of amyloid deposits and astrogliosis. In brain of AD patients, phospho-PKR was also expressed in nuclei and around amyloid deposits but the Braak score was not indicated [32,64]. However, the Braak score IV to V is characterized by the increased presence of neuroinflammation and activated microglia cells associated with fibrillar Aβ deposits [71,72].

Besides the factor eIF2α, PKR has other potential targets such as the tumor suppressor protein p53, death adaptors [73,74]. PKR is also required for the cytotoxic response to the inflammatory cytokine, the tumor necrosis factor (TNFα) by phosphorylation of I-κBα, leading to release and activation of the nuclear transcription factor NFκB [75,76]. Furthermore, the PKR gene is transcribed from a promoter containing a large number of potential regulatory elements, including the IL-6-sensitive elements as well as NFκB elements [77]. The death receptor signalling could be a critical step in PKR-induced apoptosis because activities of NFκB can also partially depend on the activation of this pathway. Until now, no work has reported the role of PKR in death receptor signalling in AD. In 2005, we received a AIRMA grant for a research project entitled: Role of PKR in death neuronal signalling in Alzheimer’s disease. The aim of this project is to determine physical and functional interactions of PKR with Death Receptor signalling in Aβ neurotoxicity and in APPSL/PS1 KI transgenic mice. The levels of TNFα and the ratio phospho-FADD(S191)/FADD were significantly increased in brain of APPSL/PS1 KI mice at 3 and 6 months of age compared to PS1 KI and control littermates and phospho-PKR may be involved in the degradation of a protein leading to the first step nuclear condensation of apoptosis in Aβ neurotoxicity (data will be published at the end of the year).

In brain of APPSL/PS1 KI mice at 3 and 6 months of age, we also analysed upstream and downstream factors of mTOR. While the mTOR activation was not modified, we found a great activation of Akt/PKB with a robust immunoreactivity of Phospho-Akt(T308) in the cytoplasm of hippocampal and cortical neurons at 6 months of age. At the opposite, a significant decrease of the ratio Phospho-p70S6K(T389)/p70S6K was observed in brain of PS1 KI and APPSL/PS1 KI mice. Confocal immunostainings showed no nuclear Phospho-p85S6K(T389) in neurons of APPPS/PS1 KI compared to PS1 KI and control littermates (data submitted). No active nucleocytoplasmic
RS6K1 was present in apoptotic neurons. These findings underline the role of the nuclear isoform of RS6K1 in survival and cell proliferation. Further experiments are needed to determine the role of nuclear substrates of RS6K1, such as cAMP-response element modulator [78]. The decrease and absence of active p85S6K in PS1 KI and APP3′/PS1 KI neurons, respectively, could be due to the activation of the type 2A phosphatases known to dephosphorylate RS6K1 [79].

CONCLUSION

Activation of PKR and inhibition of RS6K1 (probably mTOR-independent) are the main alterations of the control of translation found in cellular and transgenic mouse models of AD and in the brain and lymphocytes of AD patients. In PD, the decrease of p70S6K activity was only demonstrated in cellular model treated with 6-OHDA [67] or with 1-methyl-4-phenyl tetrahydropyridinium (MPP+) [80] and this alteration was mainly due to the mitochondrial stress. The kinase p70S6K phosphorylates the 40S ribosomal protein S6, an event resulting in enhanced translation of 5′ terminal oligopyrimidine tract-containing (5′TOP) mRNAs [50,81]. In AD, we think that RS6K1 may be involved in the control of memory gene expression and could be novel potential therapeutic target for the disease.

 Activation of PKR could lead to the inhibition of p70S6K and induce the neuronal death by a mechanism which is still unknown. Previous findings underline the fact that PKR could play a functional role in the triggering of neuronal apoptosis. Chang et al. [32] demonstrated that, using neurons from PKR knock out transgenic mice, the down regulation of this kinase was able to dramatically attenuate extra-cellular Aβ toxicity in primary neuronal cell cultures. Recently, we have used two PKR inhibitors, C16 [82] and the PRI peptide [83], and observed that Aβ-induced apoptosis in SH-SY5Y cells is blocked by both compounds and that C16 also induced a nearly complete extinction of phosphorylated PKR. In the future, the use of new inhibitors of PKR, able to cross the blood brain barrier but which are not presently available, will determine if, in vivo, the reduction of PKR activation, can attenuate the neuronal degeneration detected in the disease.

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REFERENCES


67. Malagelada C, Ryu EJ, Biswas SC, Jackson-Lewis V, Greene LA. RTP801 is elevated in Parkinson brain substantia nigral neurons and mediates death in cellular models of Parkinson's disease by a


79. Peterson RT, Desai BN, Hardwick JS, Schreiber SL. Protein phosphatase 2A interacts with the 70-kDa S6 kinase and is activated by inhibition of PKB/P12-rapamycin-associated protein. Proc Natl Acad Sci USA. 1996; 93:4438-4442.


