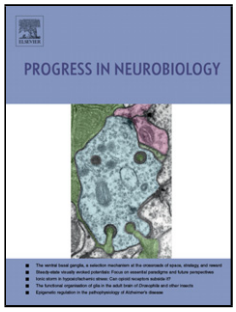

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Authors: Joao P. Lopes, Paula Agostinho

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CDK5: MULTITASKING BETWEEN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

Joao P. Lopes[(1)(2)] and Paula Agostinho[(1)]

[(1)] Center for Neuroscience and Cell Biology,
Faculty of Medicine, Biochemistry Institute
University of Coimbra, 3004 Coimbra
Portugal
Tel. 351 239 820190
Fax. 351 239 822776

[(2)] Istituto Italiano di Tecnologia
D3- Drug Discovery and Development
Via Morego, 30
16163 Genova
Italy
Tel. +39 01071781920
Fax. +39 010 7170187

Correspondence address:

João Pedro Lopes, joao.lopes@iit.it
Istituto Italiano di Tecnologia
D3- Drug Discovery and Development
Via Morego, 30
16163 Genova
Italy
Tel. +39 01071781920
Fax. +39 010 7170187

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ABSTRACT

Cyclin-dependent kinase 5 (Cdk5) is a peculiar proline-directed serine/threonine kinase. Unlike the other members of the Cdk family, Cdk5 is not directly involved in cell cycle regulation, being normally associated to neuronal processes such as migration, cortical layering and synaptic plasticity. This kinase is present mainly in post-mitotic neurons and its activity is tightly regulated by the interaction with the specific activators, p35 and p39.

Despite its pivotal role in CNS development, Cdk5 dysregulation has been implicated in different pathologies, such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and, most recently, prion-related encephalopathies (PRE). In these neurodegenerative conditions, Cdk5 overactivation and relocalization occurs upon association with p25, a truncated form of the normal activator p35. This activator switching will cause a shift in the phosphorylative pattern of Cdk5, with an alteration both in targets and activity, ultimately leading to neuronal demise.

In AD and PRE, two disorders that share clinical and neuropathological features, Cdk5 dysregulation is a linking event between the major neuropathological markers: amyloid plaques, tau hyperphosphorylation and synaptic and neuronal loss. Moreover, this kinase was shown to be involved in abortive cell cycle re-entry, a feature recently proposed as a possible step in the neuronal apoptosis mechanism of several neurological diseases.

This review focuses on the role of Cdk5 in neurons, namely in the regulation of cytoskeletal dynamics, synaptic function and cell survival, both in physiological and in pathological conditions, highlighting the relevance of Cdk5 in the main mechanisms of neurodegeneration in Alzheimer's disease and other brain pathologies.

1. INTRODUCTION

1.1. The Cdk family

The cyclin-dependent kinase (Cdk) family is composed of 9 small (30-35 kDa) serine/threonine kinases, numbered according to their discovery, from Cdk1 to Cdk9 (Morgan, 1997). The biological functions of Cdks are vast, going from mitosis to the regulation of cellular processes such as differentiation, senescence and apoptosis, through modification of gene transcription (Tannoch *et al.*, 2000). In proliferating cells, Cdk dysregulation is associated with tumour formation, whereas their disappearance/inhibition in neuronal precursors coincides with terminal differentiation (Okano *et al.*, 1993). Generally, in order to be activated, Cdks need to associate with regulatory subunits named cyclins. Although specific Cdks are linked to different phases of the cell cycle, their activities can sometimes overlap, depending on the association with different cyclins (Morgan, 1997; Nguyen *et al.*, 2002; Nigg, 2001).

Cdk activity can also be regulated by two other distinct mechanisms. A set of phosphorylation and dephosphorylation events primes Cdks for activation by regulatory subunits, as can be seen in the case of the Cdk4/cyclin D1 complex, which only becomes active after phosphorylation by the Cdk-activating kinase (CAK) (Diehl and Sherr, 1997; Kato *et al.*, 1994). Furthermore, a family of Cdk-inhibitory subunits (CKIs) can bind to and inactivate the Cdk-cyclin complex (Golias *et al.*, 2004; Pavletich, 1999; Peter, 1997).

1.2. Cdk5: different between equals

Cdk5 is an unusual member of the Cdk family (Dhariwala and Rajadhyaksha, 2008). Unlike the other Cdks, this serine-threonine kinase does not exert a direct control over the cell cycle (Dhavan and Tsai, 2001), although it has been shown that Cdk5 can phosphorylate the retinoblastoma protein (Rb), a major intervenient in cell cycle progression (Hamdane *et al.*, 2005). Similarly to the other members of its group, Cdk5 needs to associate with a regulatory subunit in order to be activated. However, this kinase does not associate with cyclins, but with the neuron-specific activators p35 and p39 (Tang *et al.*, 1995; Tsai *et al.*, 1994), which are structurally similar to cyclins, yet share no

homology at the amino acid level. Furthermore, Cdk5 does not require any additional phosphorylation in order to become active, although the phosphorylation at Tyr15 by Src-related tyrosine kinases can increase the activity of this protein (Zukerberg *et al.*, 2000).

Despite the widespread expression throughout the organism, enzymatic activity of Cdk5 is more prominent in the CNS, since the expression of this kinase and its activators is highest in post-mitotic neurons (Hisanaga and Endo, 2010; Nguyen *et al.*, 2002; Zheng *et al.*, 1998). The spacial and temporal expression of p35 and p39 appear to be complementary, with studies indicating that in growth cones, synapses and in detergent-insoluble cytoskeleton and membrane fractions they localize in distinct subcellular compartments that can be overlapped (Asada *et al.*, 2008; Humbert *et al.*, 2000a; Humbert *et al.*, 2000b; Paglini *et al.*, 1998), However, it remains unclear whether the two activators can confer substrate specificity to Cdk5. Interestingly, although p39 can compensate for some functions of p35, the absence of p39 can be masked by p35, as confirmed by the lack of obvious detectable abnormalities in p39-null mice (Ko *et al.*, 2001).

Cdk5 activators p35 and p39 are relatively unstable proteins. Studies in neuronal cultures show that the half-life of p35 is approximately 20-30 minutes (Patrick *et al.*, 1998). The levels of these proteins are regulated by their synthesis and degradation, and the expression of p35 was shown to be induced by extracellular stimuli. *In vitro* studies revealed that the extracellular matrix glycoprotein laminin can trigger an increase, not only in p35 mRNA, but also in protein levels, leading to an augment of Cdk5 activity (Paglini *et al.*, 1998). Neurotrophic factors, like nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), also cause an upregulation of p35 expression (Harada *et al.*, 2001; Tokuoka *et al.*, 2000). In order to be degraded, p35 is multi-ubiquitylated and processed via the ubiquitin-proteasome pathway. Interestingly, when Cdk5 activity is blocked, the stability of p35 is largely maintained and the half-life of this activator is increased (Kerokoski *et al.*, 2002), as demonstrated in Cdk5 dominant negative mutants that lack p35 phosphorylation (Patrick *et al.*, 1998), which can suggest a negative feedback mechanism for the regulation of Cdk5. Furthermore, the phosphorylation status of p35 also influences the membrane association of the Cdk5/p35 complex (Sato *et al.*, 2007). The interaction of this complex with the

membrane is a possible regulatory mechanism of Cdk5, since it has been shown that membrane-bound Cdk5/p35 is inactive, whereas the cytoplasmic complex is the active form (Zhu *et al.*, 2005). Moreover, a recent study showed that membrane association facilitates degradation of p35 and p39 (Minegishi *et al.*, 2010)

1.3. The physiological functions of Cdk5

Although it has been demonstrated that Cdk5 has a functional role in different organs and cell types (Daval *et al.*, 2011; Feldmann *et al.*, 2010; Lin *et al.*, 2009; Pallari *et al.*, 2011; Shimomura *et al.*, 2011), the vast majority of known actions for this kinase is associated with its activity at the CNS level (Hisanaga and Endo, 2010; Jessberger *et al.*, 2009; Lalioti *et al.*, 2010; Tsai *et al.*, 1993)}.

Gene-targeting experiments have demonstrated that Cdk5 plays a pivotal role in the cytoarchitecture of CNS. Indeed, Cdk5-deficient mice die just before or after birth, displaying widespread disruptions in neuronal layering of many brain structures, such as the cerebral cortex, hippocampus, cerebellum and olfactory bulb, indicating that neuronal migration is affected (Adle-Biassette *et al.*, 2006; Gilmore *et al.*, 1998; Ohshima *et al.*, 1999; Ohshima *et al.*, 1996). In a recent report, Jessberger and colleagues demonstrated that the knockdown of Cdk5 leads to the aberrant growth of dendritic processes, which is associated with an altered migration pattern of newborn cells in the hippocampus (Jessberger *et al.*, 2008). In the cerebral cortex, Cdk5 deficiency causes an inversion of the neuronal laminar organization (Tanaka *et al.*, 2001). Similarly, p35 null mice (p35 -/-) show a similar inverted cortical layering, although the hippocampus only suffers minor disruption and the cerebellum is unaffected (Chae *et al.*, 1997; Kwon and Tsai, 1998; Kwon *et al.*, 1999). In contrast to Cdk5 -/- mice, p35 -/- animals are viable and fertile, although they have increased susceptibility to seizures. The apparent discrepancy in the phenotypes of the p35 -/- and Cdk5 -/- mice can be explained by the compensatory role of p39. While p39-deficient mice (p39 -/-) do not show any noticeable defects, the phenotype of the p35/p39 double-mutant mice (p35 -/- and p39 -/-) is indistinguishable from that of Cdk5 -/- mice (Ko *et al.*, 2001).

A crucial role for Cdk5 in corticogenesis is supported by observations showing that this kinase promotes migration by acting positively on pro-migratory signals, and possibly by antagonizing anti-migratory signals. In fact, Cdk5 has been identified as a regulator of neuroblast migration in the postnatal subventricular zone (Hirasawa *et al.*, 2004; Hirota *et al.*, 2007). In cultured primary neurons, the reduction of Cdk5 activity by expressing dominant-negative Cdk5 mutants, or by using antisense oligonucleotides of Cdk5, p35 or p39, inhibits neurite outgrowth (Nikolic *et al.*, 1996). Defects observed in fasciculation of several prominent axon tracts of p35- mutant mice also suggest a role for Cdk5 in axonal guidance and targeting (Kwon *et al.*, 1999). The regulation of microtubule and intermediate-filament cytoskeletal components by Cdk5 also implicates this kinase in the modulation of cell adhesion and of intracellular signalling and transport (Dhavan and Tsai, 2001). Indeed, amongst the substrates of Cdk5 are several proteins involved in axonal transport, such as the microtubule and neurofilament-associated proteins tau and microtubule-associated protein 1B (MAP1B) (Ahlijanian *et al.*, 2000; Grant *et al.*, 2001; Paglini *et al.*, 1998), as well as NUDEL, a protein proposed to bind to neurofilaments and facilitate their assembly (Holzbaur, 2004; Niethammer *et al.*, 2000).

The regulatory role of Cdk5 in the CNS also extends to several synaptic functions, such as synapse formation, synaptic plasticity, learning and memory. This kinase, as well as p35 and p39, is present in both pre- and postsynaptic compartments (Humbert *et al.*, 2000b; Niethammer *et al.*, 2000). Studies have shown that synapsin 1 and MUNC18, two presynaptic proteins involved in the regulation of exocytosis, are substrates of Cdk5 (Fletcher *et al.*, 1999; Matsubara *et al.*, 1996; Shuang *et al.*, 1998). This kinase is also supposed to be involved in the modulation of synaptic plasticity through the regulation of dendritic spine formation (Cheung and Ip, 2007; Tada and Sheng, 2006). Dendritic spines are small membranous protrusions from a neuron's dendrite that usually receive excitatory input from axons and can be modified by synaptic activity (Bourne and Harris, 2008). Since spines need to rapidly change their volume or shape in response to stimuli, alterations in dendritic spine morphology will depend on the dynamic regulation of the actin cytoskeleton (Schubert and Dotti, 2007; Tada and Sheng, 2006). As a large number of cytoskeleton-binding proteins are Cdk5 substrates

in neurons, it is valid to hypothesize that this kinase plays a role in dendritic spine formation. Indeed, phosphorylation of the Cdk-related protein kinase Pctaire 1 by Cdk5 was shown to modulate the development of dendrites in differentiating neurons (Fu *et al.*, 2011). Furthermore, both spinophilin, which is enriched at dendritic spines and negatively regulates their development (Feng *et al.*, 2000), and its related protein neurabin I, are also phosphorylating targets for Cdk5 (Causeret *et al.*, 2007; Futter *et al.*, 2005), although the functional consequences of this mechanism on spine formation remains to be determined.

An increasing number of reports also points out Cdk5 as an important regulator of the activity of two major neurotransmitter systems, the cholinergic and the glutamatergic (Fu *et al.*, 2001; Fu *et al.*, 2005; Hawasli *et al.*, 2007; Li *et al.*, 2001). In fact, Cdk5 and its activator p35 were shown to co-localize with the acetylcholine receptor on the postsynaptic muscle membrane, where they regulate the trafficking of these receptors (Fu *et al.*, 2001). Interestingly, acetylcholine has been shown to negatively regulate the formation of synapses at the neuromuscular junction through a mechanism involving Cdk5 (Lin *et al.*, 2005). The regulation of synaptic function by Cdk5 is also linked to its action on the glutamatergic neurotransmitter system, through the modulation of N-methyl-D-aspartate (NMDA) receptor activity. In fact, the conditional Cdk5 knock-out will cause enhanced synaptic plasticity through an increase in the amount of NMDA receptors containing NR2B subunits and in the related excitatory postsynaptic currents (Hawasli *et al.*, 2007). Likewise, NMDA receptor activity can be increased through the phosphorylation of its NR2A subunit by Cdk5 (Li *et al.*, 2001). Dopaminergic signalling is also controlled by Cdk5 through the phosphorylation of dopamine cAMP-regulated phosphoprotein of 32 kDa, DARPP32 (Bibb *et al.*, 1999).

2. CDK5 IN NEURODEGENERATION

2.1. Cdk5 dysregulation: when things go wrong

Although Cdk5 activity is vital for a correct CNS development, as well as several other important physiological nervous system functions, the dysregulation of this kinase has been shown to be involved in the neurodegenerative processes of several diseases, including AD, prion-related

encephalopathies (PRE), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) or acute neuronal injury caused by ischemia/stroke (Alvira *et al.*, 2008; Lopes *et al.*, 2007, 2010; Nguyen and Julien, 2003; Slevin and Krupinski, 2009; Tsai *et al.*, 2004).

A main step in Cdk5 dysregulation is the cleavage of the activator proteins p35 and p39 by calpains, a group of Ca^{2+}-activated cytosolic proteases (Adle-Biassette *et al.*, 2006; Camins *et al.*, 2006; Kusakawa *et al.*, 2000; Lee *et al.*, 2000; Patrick *et al.*, 1999) (Fig. 1). Calpains participate in various important physiological processes and are crucial in some neuronal functions like learning and memory. Their activation during sustained synaptic activity is vital for Ca^{2+}-dependent neuronal functions, such as neurotransmitter release, synaptic plasticity, vesicular trafficking and structural stabilization (Liu *et al.*, 2008a; Wu and Lynch, 2006; Zadran *et al.*, 2010). Several studies suggest the involvement of calpains in different neurodegenerative conditions, including some where Cdk5 dysregulation also occurs, like stroke, PD or AD (Alvira *et al.*, 2006; Araujo *et al.*, 2010; Bano and Nicotera, 2007; Camins *et al.*, 2006; Grammer *et al.*, 2008; Langou *et al.*, 2010; Raynaud and Marcilhac, 2006; Yadavalli *et al.*, 2004). Indeed, Cdk5 dysregulation in AD and PRE is intimately related to calpains overactivation (Liang *et al.*, 2010; Lopes *et al.*, 2007). The dependence of Ca^{2+} for activation makes calpains vulnerable to changes in the homeostasis of this ion and turns calpains into important elements of neurodegeneration (Araujo *et al.*, 2010; Ferreiro *et al.*, 2006; Green *et al.*, 2007; Lopes *et al.*, 2007; Raynaud and Marcilhac, 2006; Resende *et al.*, 2007). In fact, the imbalance in intracellular Ca^{2+} levels occurs both through the entry from the extracellular space via NMDA and AMPA receptors (Alberdi *et al.*, 2010), and by the release of this ion from the intracellular compartments, namely the endoplasmic reticulum (Ferreiro *et al.*, 2006; Resende *et al.*, 2008) (Fig. 1).

Calpain cleavage of p35 and p39 generates, respectively, p25 and p29. These truncated Cdk5 activators show distinct properties from their original precursors. The half-life times of p25 and p29 are significantly longer than p35 and p39 (around 3-fold increase) (Patrick *et al.*, 1998) and the binding of these cleaved activators to the kinase is stronger (Amin *et al.*, 2002), resulting in increased Cdk5 activity when compared to Cdk5/p35 (or p39). Furthermore, p25 and p29 lack an amino-terminal myristoilation site, which will cause the Cdk5/p25 (or p29) complex to exhibit a different

subcellular localization, passing from the cellular periphery (mainly in the synapses) to a more perinuclear region (in the cell body), thus altering the substrates phosphorylated by this kinase (Asada *et al.*, 2008; Kusakawa *et al.*, 2000; Patrick *et al.*, 1999). Interestingly, when p35 is phosphorylated by Cdk5, the calpain-mediated cleavage of this activator is reduced, suggesting that that the formation Cdk5/p25 complex can be autoregulated (Saito *et al.*, 2003).

When dysregulated, Cdk5 hyperphosphorylates the cytoskeleton protein tau (Ahlijanian *et al.*, 2000; Lopes *et al.*, 2010; Plattner *et al.*, 2006), causing its release from the microtubules and accumulation in the form of cytoplasmic filaments and tangles (Alonso *et al.*, 2001; Grundke-Iqbal *et al.*, 1986a) (Fig. 1). This is caused by the change in substrate specificity of Cdk5, derived from its association with p25. Indeed, major evidences regarding the involvement of p25/Cdk5 in neurodegeneration were also obtained from p25-transgenic mouse models (Ahlijanian *et al.*, 2000; Cruz *et al.*, 2003). Cruz and colleagues demonstrated that in p25-overexpressing animals, endogenous tau is hyperphosphorylated at different epitopes and the accumulation of aggregated tau and neurofibrillary pathology progressively increased. Furthermore, while the phosphorylation of tau and other substrates was upregulated, the phosphorylation of known physiological Cdk5 substrates is not increased in p25-transgenic mice (Cruz *et al.*, 2003). Mice expressing p25 during long periods of time were shown to suffer from impaired hippocampal long term potentiation (LTP) and memory deficits, along with significant synaptic and neuronal loss. However, when p25 was expressed in a transient form, hippocampal LTP was improved, the number of dendritic spines and synapses was increased and no neurodegeneration was observed (Fischer *et al.*, 2005). Altogether, these results point out that it is the persistent increase in p25 expression that turns the physiological Cdk5 function into a pathological one.

(Insertion of Figure 1)

2.2. Cdk5 and AD: two old acquaintances

Alzheimer's disease (AD) is the most common type of dementia (Rodgers, 2008; Smith, 1998). Affecting an estimate of 35 million people worldwide (Shah and Reichman, 2006), AD has turned into an alarming health care problem with a vast socio-economic impact (Wimo and Prince, 2010).

Neuropathologically, the AD brain is characterized by 3 main markers: amyloid plaque deposition, neurofibrillary tangle formation and severe selective neuronal loss (Armstrong, 2006; Braak and Braak, 1991). Amyloid-β (Aβ), the main component of amyloid plaques, is considered a key molecule in AD pathogenesis (Alberdi *et al.*, 2010; Findeis, 2007; Lopes *et al.*, 2010; McLarnon and Ryu, 2008; Tseng *et al.*, 2007). Generated by a two-step proteolytic cleavage of the integral membrane glycoprotein APP (amyloid precursor protein) (Esler and Wolfe, 2001; Thinakaran and Koo, 2008) via the amyloidogenic pathway, Aβ can trigger a series of processes known as the amyloid cascade, a multi-step series of events that disrupts neuronal homeostasis, causing aberrant activation of kinases and ultimately resulting in neurofibrillary tangle formation and neuronal loss (Golde, 2003; Hardy and Selkoe, 2002; Newman *et al.*, 2007).

Cdk5 has been proposed as an attractive candidate to connect Aβ toxicity, tau pathology and neurodegeneration (Cruz *et al.*, 2006; Lee and Tsai, 2003; Lopes *et al.*, 2010; Piedrahita *et al.*, 2010). Indeed, in human AD brains, there is a significant augment in Cdk5 activity compared with age-matched control brains (Lee *et al.*, 1999). In accordance with this finding, the levels of p25 and activated calpain are increased in AD brains (Grynspan *et al.*, 1997; Tseng *et al.*, 2002) and different studies point out to a correlation between the presence of Cdk5/p25 and neurofibrillary tangle (NFT) formation (Liu *et al.*, 2004b; Pei *et al.*, 1998; Wang *et al.*, 2007). Also, p25 levels were shown to be increased both *in vitro* and *in vivo* through the administration of Aβ peptide (Lopes *et al.*, 2007, 2010; Patrick *et al.*, 1999). Aβ neurotoxicity was shown to be decreased by the direct inhibition of Cdk5 (Alvarez *et al.*, 1999; Chang *et al.*, 2011; Lopes *et al.*, 2007) or by acting upstream, on calpain activation, with the consequent blockage of p25 formation (Granic *et al.*, 2010; Lopes *et al.*, 2010).

2.2.1. Cdk5 and Aβ generation

All mutations currently known to cause AD are located either in the APP gene or in the genes encoding presenilins 1 (PS1) and 2 (PS2), two proteins that are part of the γ-secretase complex, one of the secretases responsible for APP cleavage to Aβ (Ahn *et al.*, 2008; Esler and Wolfe, 2001; Florean *et al.*, 2008; Hardy, 1997). Therefore, besides the evidence that Aβ can trigger Cdk5 dysregulation (Kusakawa *et al.*, 2000; Lopes *et al.*, 2007, 2010; Patrick *et al.*, 1999), the link between Cdk5 and AD was further reinforced by reports showing that dysregulated Cdk5 phosphorylates APP at Thr668 (Iijima *et al.*, 2000; Lee *et al.*, 2003), thus regulating its processing and increasing Aβ production. In fact, augmented generation and intraneuronal accumulation of Aβ has been described in inducible p25-transgenic mice (Cruz *et al.*, 2006). Furthermore, APP phosphorylation will induce the nuclear translocation of the APP intracellular domain, which will lead to neuronal demise (Chang *et al.*, 2006).

Cdk5 dysregulation can also be linked to alterations in the presenlin system. Actually, in the cerebral cortex of PS conditional knock-out mice increased levels of activator p25 activator are associated with tau hyperphosphorylation and neurodegeneration (Saura *et al.*, 2004). Moreover, and similarly to other kinases such as protein kinases A (PKA) and C (PKC) and glycogen-synthase kinase 3 beta (GSK3β) (Kirschenbaum *et al.*, 2001; Seeger *et al.*, 1997; Walter *et al.*, 1998), Cdk5 can phosphorylate PS1, altering its interaction with other molecules and regulating the stability of this phosphoprotein. Human PS1 contains two threonine (thr) residues preceding a proline, thr112 and thr354, which are prone to phosphorylation by Cdk5. Indeed, Cdk5/p35 has been show to directly phosphorylate PS1thr354 both *in vitro* and *in vivo*, increasing the levels of this presenilin (Lau *et al.*, 2002). As an indirect mode of action, the complex Cdk5/p35 can bind and phosphorylate β-catenin and modulate the interactions between this protein and PS1 (Kesavapany *et al.*, 2001). Moreover, Cdk5 is involved in the regulation of N-cadherin-mediated adhesion in cortical neurons, and N-cadherin itself is a γ-secretase substrate (Kwon *et al.*, 2000; Marambaud *et al.*, 2002). Remarkably, Dab1, a key regulator of reelin signaling that is downregulated in PS1 mutants, is also a substrate for Cdk5/p35, and is known to interact with APP (Howell *et al.*, 1999; Keshvara *et al.*, 2002). Therefore,

alterations in Cdk5 activity may lead to changes in PS1 metabolism, contributing to the pathogenesis of AD (Fig. 3).

2.2.2. Cdk5 and tau pathology

Besides Aβ accumulation, in brain regions affected by AD many neurons also display intracellular inclusions in the form of large nonmembrane-bound bundles of abnormal fibres, which occupy much of the perinuclear cytoplasm. These inclusions are designated neurofibrillary tangles (NFTs) and are composed primarily of hyperphosphorylated tau (Adle-Biassette *et al.*, 2006; Eckermann *et al.*, 2007; Grundke-Iqbal *et al.*, 1986a; Grundke-Iqbal *et al.*, 1986b; Kosik *et al.*, 1986). Tau is a member of the microtubule associated proteins (MAP) family, which have as their main function the binding and stabilization of the cellular microtubular network (Iqbal *et al.*, 2005). Therefore, tau is essential to vital processes such as axonal transport, cytoskeletal organization or mitotic division (Cuchillo-Ibanez *et al.*, 2008; Grundke-Iqbal *et al.*, 1986b). However, upon hyperphosphorylation, tau no longer associates with the microtubules and can aggregate in the form of filaments and tangles (Alonso *et al.*, 2001; Grundke-Iqbal *et al.*, 1986a) (Fig. 1), ultimately leading to synaptic loss and neuronal death (Buee *et al.*, 2010; Eckermann *et al.*, 2007; Iqbal *et al.*, 2005) (Steinhilb *et al.*, 2007) (Fig. 3).

The high number (about 42) of serine/threonine (Ser/Thr) residues available in tau (Shahani and Brandt, 2002) make this protein a good target for several kinases, such as Cdk5 (Li *et al.*, 2006; Lopes *et al.*, 2010; Piedrahita *et al.*, 2010; Wang *et al.*, 2007), Gsk3β (Li *et al.*, 2006; Mandelkow *et al.*, 1992; Resende *et al.*, 2008; Wang *et al.*, 2008) or PKA (Liu *et al.*, 2006; Liu *et al.*, 2004a; Liu *et al.*, 2008b). Indeed, both *in vitro* (Lopes *et al.*, 2007; Patrick *et al.*, 1999) and *in vivo* (Ahlijanian *et al.*, 2000; Cruz *et al.*, 2003; Lopes *et al.*, 2010) studies support a role for Cdk5 in tau hyperphosphorylation, mainly when associated with p25: i) Cdk5 can phosphorylate tau on sites that are found in paired helical filaments (a form of tau aggregation associated with different pathologies) (Sengupta *et al.*, 2006; Wang *et al.*, 2007), ii) transgenic animals and cell lines overexpressing p25 display tau hyperphosphorylation (Cruz *et al.*, 2003; Hamdane *et al.*, 2003), and iii) the inhibition of

Cdk5 activity, either by blocking directly the kinase or by avoiding p35 cleavage to p25, prevents tau hyperphosphorylation in neuronal cultures exposed to Aβ and in animals intracerebroventricularly injected with the peptide (Adle-Biassette *et al.*, 2006; Lopes *et al.*, 2007, 2010).

Although Aβ peptides cause a significant increase in tau phosphorylation (Adle-Biassette *et al.*, 2006; Lopes *et al.*, 2007, 2010; Resende *et al.*, 2008), therefore sustaining a link between amyloid and tau pathologies in AD, the overexpression of p25 in transgenic mice can lead to neurofibrillary tangle formation and neuronal loss even in the absence of changes in Aβ levels (Cruz *et al.*, 2003), thus confirming neuropathological studies showing that tau pathology alone can contribute for memory loss and behavior changes associated with AD (Arriagada *et al.*, 1992; Quon *et al.*, 1991) and that Cdk5 dysregulation is a major part of this process.

Hyperphosphorylation of tau also reduces its degradation rate, further promoting tau accumulation in the neurons (Khatoon *et al.*, 1992). Indeed, whereas proteasomal activity has been demonstrated as a major responsible for tau degradation (David *et al.*, 2002; Oddo, 2008), tau aggregates isolated from AD brain can have inhibitory action on the activity of proteasome (Keck *et al.*, 2003), similarly to what has been observed with Aβ oligomers (Tseng *et al.*, 2007), culminating in the pathological accumulation of Aβ and tau. Interestingly, in order to be degraded, p35 is multi-ubiquitylated and processed via the ubiquitin-proteasome pathway (Patrick *et al.*, 1998). This may ultimately lead to a feedback-loop mechanism, where Aβ and/or hyperphosphorylated tau impair proteasomal activity, leading to a decrease in the rate of p35 degradation. More of this activator will therefore be available for cleavage onto p25, generating Cdk5 dysregulation, a phenomenon known to cause the hyperphosphorylation of tau and increments on Aβ levels (Fig. 1).

2.3. Cdk5 dysregulation in other neurodegenerative conditions: causes and consequences

Although the deleterious effect of Cdk5 have been mostly studied in the context of AD, its dysregulation has also been shown to be involved in other neuropathologies, such as prion encephalopathies, PD, ALS and stroke.

2.3.1 Cdk5 in prion diseases

Prion-related encephalopathies (PRE) are a lethal type of neurodegenerative diseases, some of which with a vast socio-economic impact, like bovine spongiform encephalopathy (BSE) or the new variant Creutfeldt-Jakob disease (nvCJD) (Belay and Schonberger, 2005; Feraudet-Tarisse *et al.*; Johnson, 2005). Although they can have genetic or sporadic etiology, PRE are mainly of infectious origin (Blennow *et al.*, 2006; Johnson, 2005) and, therefore, in most prion diseases the onset age will depend on when the contact with the pathogenic agent occurs.

The central molecule of PRE pathogenesis is the prion, an exclusively proteic infectious particle that amplifies in a self-catalytic misfolding process without requiring any nucleic acid contribution (Aguzzi *et al.*, 2008). However, the normal product of the prion gene is a protein designated as cellular isoform of the prion protein or PrP^C. Although the exact function of this ubiquitously expressed glycoprotein remains unknown, diverse lines of evidence point to a potential role of PrP^C in cell adhesion, oxidative stress and cell signalling (Encalada *et al.*, 2008; Mouillet-Richard *et al.*, 2000; Roucou *et al.*, 2004; Vassallo and Herms, 2003). PrP^C has also been largely described as a copper (Cu^{2+})-dependent antioxidant (Adle-Biassette *et al.*, 2006; Brown, 2001; Millhauser, 2007; Zomosa-Signoret *et al.*, 2008). Studies suggest that PrP^C may be a major Cu^{2+}-binding protein in brain membrane fractions, even controlling the activity of other membrane-associated Cu^{2+}-binding proteins. Furthermore, prion protein expression was shown to alter cellular Cu^{2+} uptake and enhance the incorporation of this ion into the antioxidant enzyme superoxide dismutase (SOD), or may also act as a SOD by itself, scavenging reactive oxygen species (Brown *et al.*, 1999). Remarkably, in a transgenic mouse model of AD, Cu^{2+} exposure not only increased Aβ generation, (particularly Aβ40) but also triggered pathological tau phosphorylation and tangle formation in the brain, in a mechanism correlated with the increased formation of p25 and subsequent aberrant activation of Cdk5/p25 (Kitazawa *et al.*, 2009). It is, however, not well understood how Cu^{2+} in the brain triggers these pathological changes and if the alterations in the homeostasis of this ion can somehow be related to the recent discovery that cellular prion protein is required for memory impairment in transgenic AD mice (Gimbel *et al.*, 2010).

Cdk5 dysregulation is a major contributor to PrP-induced neurodegeneration (Lopes *et al.*, 2007, 2009b). Indeed, a recent work showed that hamsters infected with prion strains had a significant increase in the levels of tau hyperphosphorylated on Ser 202/Thr 205, a fact that was correlated with a marked increase of Cdk5 levels (Wang *et al.*, 2010). Also, the exposure of cortical neurons to the toxic peptide $PrP_{106-126}$ can lead to tau hyperphosphorylation and cell cycle re-entry, a cascade that will culminate in apoptotic neuronal death (Lopes *et al.*, 2007, 2009b). This effect of PrP, similarly to what happens with the Aβ peptide, is mediated by the imbalance of calcium homeostasis (Agostinho and Oliveira, 2003). However, whereas Aβ causes Ca^{2+} influx from the extracellular space via NMDA receptors and voltage-sensitive Ca^{2+} channels (Alberdi *et al.*, 2010), PrP peptides will block Ca^{2+} entry through this type of channels (Florio *et al.*, 1998; Sandberg *et al.*, 2004). In alternative, the synthetic prion fragment $PrP_{106–126}$ has been demonstrated to form nonselective ionic channels in planar lipid bilayers (Kourie and Shorthouse, 2000; Lin *et al.*, 1997), which could permit Ca^{2+} entry into cells and consequent calpain activation, leading to the cleavage of p35 to p25 and the overactivation of Cdk5 without altering the levels of this kinase (Lopes *et al.*, 2007) (Fig. 1). The infectious isoform of the prion protein is also known as scrapie prion (PrP^{Sc}), due to the fact that this protein-only pathogenic agent is the cause of sheep transmissible spongiform encephalopathy, scrapie. This 33-35 kDa protein has a protease-resistant core known as PrP_{27-30} (McKinley *et al.*, 1991). PrP^{Sc} is resistant to physical and chemical proteolysis, heat and radiation (Aguzzi and Heppner, 2000; Harris, 1999; Prusiner, 1998). Interestingly, a recent study has shown that the phosphorylation of the recombinant PrP fragment PrP_{23-231} by Cdk5 can lead to the generation of a proteinase K resistant species (which forms Congo Red-positive fibrils) and aggregates that can be immunostained with anti-PrP antibodies (Giannopoulos *et al.*, 2009). These new results raise the possibility that phosphorylation by Cdk5 can be part of a physiological mechanism of PrP conformational alteration and its putative conversion into a pathological infectious form.

2.3.2 Cdk5 in Parkinson's disease

Parkinson's disease (PD) is a debilitating neurodegenerative disorder that affects both cognitive and motor skills. Neuropathologically, PD is characterized by a progressive and selective degeneration of dopaminergic neurons, in particular those from substantia nigra pars compacta (Aguzzi and Weissmann, 1996; Przedborski, 2005; Przedborski and Vila, 2001). Neuronal loss and the formation of intracellular aggregates of α-synuclein named Lewy bodies are the two main hallmark features of this pathology (Meredith *et al.*, 2009; Pollanen *et al.*, 1993; Venda *et al.*, 2010).

Although the exact mechanism of dopaminergic neuronal death remains unknown, recent studies have demonstrated that Cdk5 is involved in the process. The activity and levels of this kinase were found to be altered in the brain of PD patients (Alvira *et al.*, 2008), as well as in pharmacological animal models of the disease, in particular those corresponding to the administration of MPTP(a neurotoxin that causes parkinsonian symptoms), or in cell cultures exposed to the MPTP metabolite, MPP+ (Alvira *et al.*, 2006; Smith *et al.*, 2003), with increased Cdk5 activation correlating to the augment in the levels of the pathogenic activator p25 due to calpain cleavage (Alvira *et al.*, 2008; Alvira *et al.*, 2006). This mechanism probably involves glutamate excitotoxicity, since MPTP exposure substantially increases glutamate levels (Meredith *et al.*, 2009), leading to the imbalance of calcium and consequent calpain activation. Inhibition of Cdk5, either directly, by the cdk inhibitor flavopiridol, or indirectly, by blocking the cleavage of p35 to p25, was shown to provide neuroprotection against both compounds, with effects at the cellular level (reduction in the number of degenerating dopaminergic neurons), as well as in the behavioural aspect, with the animals displaying a significant locomotor improvement (Smith *et al.*, 2003).

Similarly to what happens in AD with the cytoskeleton protein tau (Ahlijanian *et al.*, 2000; Piedrahita *et al.*, 2010), Cdk5 has been implicated in the phosphorylation of two proteins, α-synuclein and parkin, with significant importance in the pathogenesis of PD (Avraham *et al.*, 2007; Muntane *et al.*, 2008; Rubio de la Torre *et al.*, 2009). α-synuclein is the major component of Lewy bodies, and it is primarily found in nervous tissue (Iwatsubo, 2003; Spillantini *et al.*, 1998), where it is known to play a role in neurotransmission (Dev *et al.*, 2003), although its main function remains unknown,

whereas parkin acts as an E3 ubiquitin ligase, a member of the ubiquitin-proteasome system (Shimura *et al.*, 2000). Mutations in the gene coding for parkin are known to cause early-age familial PD (Kitada *et al.*, 1998; Lucking *et al.*, 2000). The phosphorylation of parkin by Cdk5, both *in vitro* and *in vivo*, was shown to affect its ubiquitin-ligase activity, leading to a reduction in its auto-ubiquitylation (Avraham *et al.*, 2007). Furthermore, the conjugated action of Cdk5 with casein kinase I decreases the solubility of parkin, leading to its aggregation(Rubio de la Torre *et al.*, 2009). Cdk5 phosphorylation of α-synuclein also leads to its agglomeration (Muntane *et al.*, 2008). Indeed, in the brains of PD patients, Cdk5 and p35 were found to co-localize with Lewy bodies, which reinforces the idea that the Cdk5 is involved in the generation of the α-synuclein fibrils that form these PD hallmark structures (Nakamura *et al.*, 1997).

Alterations in Cdk5 activity can also have an impact on the antioxidant capacity of the dopaminergic neurons: the overactivation of Cdk5 by MPP+ was shown to downregulate the peroxidase activity of the peroxiredoxin Prx2, thus leading to a decrease to the capacity of the cells to eliminate reactive oxygen species (ROS), which will ultimately induce neuronal loss (Qu *et al.*, 2007).

2.4.3 Cdk5 in other neurological disorders

Cdk5 has also been linked to the onset of other neurodegenerative conditions, such as ALS (Nguyen and Julien, 2003) or stroke (Slevin and Krupinski, 2009) with the overactivation of this kinase resulting from a strong intracellular calcium imbalance, which in turn activates calpains and leads to the generation of p25 and ultimately contributing for neuronal loss (Patrick *et al.*, 1999).

In ALS, an adult-onset neurodegenerative disorder characterized by a selective demise of motor neurons from the brain and spinal cord (Cleveland, 1999), mutations of the copper/zinc superoxide dismutase (SOD1) gene trigger a series of toxic effects including glutamate excitotoxicity, which overstimulates the glutamatergic receptors and leads to excessive calcium entry into the motor neurons (Cudkowicz *et al.*, 1997; Rosen, 1993). The involvement of Cdk5 in ALS is mainly correlated with its phosphorylative action, since the dysregulation of this kinase seems to cause a hyperphosphorylation of neurofilaments, namely the heavy subunit (NF-H), as well as of tau (Nguyen

et al., 2001). In fact, besides the co-localization between phosphorylated NF-H and the Cdk5/p25 complex in motor neurons of SOD1 mutant mice (Nguyen *et al.*, 2001), a partial overlapping of NF-H aggregates and Cdk5 was observed in the brain of ALS patients (Bajaj *et al.*, 1999). Although NF-H hyperphosphorylation is associated with a disruption of the cytoskeleton (similarly to what is observed in the case of tau in AD) (Patzke and Tsai, 2002), perikaryal aggregates of NF-H can capture Cdk5/p25, thus preventing it, up to a certain point, from targeting other substrates (Nguyen *et al.*, 2001).

Cerebral ischemia (stroke) consists of a loss of brain functions due to a transient or permanent blocking of the blood flow to the brain (Lo *et al.*, 2003). The neuronal demise resulting from an ischemic event is caused by multiple overlapping mechanisms, such as the generation of reactive oxygen species, inflammatory reactions and excitotoxicity caused by and excessive activation of ionotropic glutamate receptors, in particular NMDA (Mehta *et al.*, 2007; Nakka *et al.*, 2008). Therefore, and similarly to other neurodegenerative conditions, the calcium imbalance generated by NMDA receptor overactivation (Mehta *et al.*, 2007) is probably the main generator of Cdk5 dysregulation in stroke. In *in vivo* models of ischemia, Cdk5 overactivation was shown to cause tau hyperphosphorylation (Wen *et al.*, 2007) and will also phosphorylate the NMDA receptors, thus amplifying the calcium influx and potentiating neuronal death (Wang *et al.*, 2003). Furthermore, ischemic stroke leads to increases in Cdk5 expression (Mitsios *et al.*, 2007) and affects the antioxidant capability of the neurons, since similarly to what occurs in PD, Cdk5 phosphorylates the enzyme Prx2, therefore inactivating it and abolishing its peroxidase activity (Rashidian *et al.*, 2009).

3. CDK5 AND THE CELL CYCLE

3.1. The cell cycle: Cdks in control

Cell cycle activity/progression is dependent on the activity of Cdks. The formation of different Cdk/cyclin complexes is the base for a precise control of cell cycle progression (Golias *et al.*, 2004; Nguyen *et al.*, 2002; Nigg, 2001). The phosphorylation of the retinoblastoma protein (Rb), first by Cdk4/cyclin D1 and Cdk6/cyclin D1-3 and further by Cdk2/cyclin E, is considered to be the initiating

point of cell cycle (Tannoch *et al.*, 2000; Weinberg, 1995). At the G1/S checkpoint, a complex formed by Rb, E2F-1, histone deacetylases (HDAC) amongst other proteins, blocks protein transcription and arrests the cell cycle (Nguyen *et al.*, 2002; Panteleeva *et al.*, 2004). Upon phosphorylation, Rb is released from this transcription-blocking complex and occurs the transcription of S phase-associated proteins (Park *et al.*, 2000) and consequent progression in the cell cycle.

Since cell cycle-associated Cdks do not play a significant role in differentiated neurons, their activity in the CNS is considerably reduced (Nguyen *et al.*, 2002). Despite the tight regulation of the cell cycle, this process can sometimes be disrupted by powerful stimuli, such as excitotocitity, oxidative stress, DNA damage or ischemia, forcing mature neurons to leave a steady G0 state and re-enter the cell cycle (Katchanov *et al.*, 2001; Kruman *et al.*, 2004; Kuan *et al.*, 2004; Nguyen *et al.*, 2003).

Cell cycle re-entry has been observed in different neurodegenerative conditions, such as AD, PD, ALS or stroke (Ahn *et al.*, 2008; Andorfer *et al.*, 2005; Hoglinger *et al.*, 2007; Lopes *et al.*, 2009c; Neve and McPhie, 2006; Nguyen *et al.*, 2003; Rashidian *et al.*, 2005; Wen *et al.*, 2005). Although in these neurons the passage into the G1 phase is closely related to the re-expression of cell cycle Cdks, namely Cdk2, 4 and 6 (Copani *et al.*, 1999; Kuan *et al.*, 2004; Lopes *et al.*, 2009a; Nguyen *et al.*, 2003), a very important part in the abortive cell cycle re-entry is played by Rb. Under pathological conditions, Rb phosphorylation/inactivation causes re-cycling neurons to overcome the G1/S checkpoint and DNA synthesis will occur (as confirmed by BrdU incorporation) (Hoglinger *et al.*, 2007; Wen *et al.*, 2005). Nevertheless, these neurons never reach the M phase and, somewhere between the S and the G2 phases, degenerate by apoptosis (Hernandez-Ortega *et al.*, 2007).

3.2. Cdk5 and cell cycle re-entry: a new pathway to degeneration

Abnormal cell cycle reactivation can in fact be considered as an important neuropathological feature of AD (Ahn *et al.*, 2008; Hernandez-Ortega *et al.*, 2007; Lopes *et al.*, 2009a; Majd *et al.*, 2008; Yang *et al.*, 2006). Cell cycle events have been described in the brains of patients (Busser *et al.*, 1998) and in animal models of AD (Ahn *et al.*, 2008; Lopes *et al.*, 2010; McShea *et al.*, 2007), as well

as in cultured neurons exposed to Aβ (Lopes *et al.*, 2009b; Wu *et al.*, 2000). Furthermore, in AD brains, ectopic expression of cell cycle molecules was shown to occur in the same regions as disease-associated neurodegeneration (Busser *et al.*, 1998). Recently, abnormal cell cycle re-entry was also observed in neuronal cultures exposed to prion peptides (Lopes *et al.*, 2009b), which may indicate that the ectopic reactivation of the cell cycle is also part of the neurodegenerative mechanism of PRE.

(Insertion of Figure 2)

Interestingly, Cdk5 overactivation has been described in several neurodegenerative conditions in which ectopic cell cycle events were also reported (Hoglinger *et al.*, 2007; Lopes *et al.*, 2009b; Nguyen *et al.*, 2003; Wen *et al.*, 2005; Zhang *et al.*, 2010). Moreover, Cdk5 dysregulation due to increased levels of oxidative stress, like in the mutant SOD1 mouse model of ALS, can cause neurodegeneration via changes in Cdk4 expression/activity (Nguyen *et al.*, 2003). In a recent report, it was demonstrated that Cdk5 can also act as a mediator of neuronal cell cycle reactivation induced by Aβ and PrP, since both peptides increased the levels of Cdk4, pRb and PCNA; however, these changes in the levels of cell cycle markers were prevented when Cdk5 activity was blocked. The levels of the M phase marker, phospho-histone H3 (phH3), were identical before and after Cdk5 dysregulation, confirming that although neurons challenged with Aβ or PrP manage to reach the S or the G2 phases, they do not pass the G2/M checkpoint and proceed in the apoptotic pathway (Fig. 2). Curiously, the presence of a marker for the S phase was observed in neurons already at an advanced apoptotic stage, what seems to imply cell cycle reactivation, not as a cause for, but rather as a component of the cell death pathway (Lopes *et al.*, 2009b).

Besides the "normal" alterations in Cdk5 localization, with a passage from a more peripheral position to the cell body, recent studies have shown the toxic effects of this kinase are also associated with a translocation of nuclear Cdk5 to the cytoplasm (Fig. 2), an event which appears to be intimately linked with the ectopic cell cycle re-entry observed in both AD animal models and cultured neurons exposed to Aβ (Lopes *et al.*, 2010; Zhang *et al.*, 2008a). Indeed, nuclear Cdk5 appears to be

responsible for the suppression of cell cycle activity in post-mitotic neurons, possibly through the stabilization of the cell cycle inhibitor p27 and by preventing the association of E2F-1 with its co-activator DP1. Furthermore, the blockage of cell cycle progression by Cdk5 requires that this kinase is associated with its normal activator p35. Thus, the cleavage of p35 to p25, which will lead to Cdk5 dysregulation, is probably a major step in the abortive cell cycle re-entry triggered by Aβ exposure (Zhang *et al.*, 2008a; Zhang *et al.*, 2010), an hypothesis supported by the recent discovery that upon p25 generation, either via Aβ exposure *in vitro* or in p25-inducible transgenic mice, Cdk5 will cause the dispersion of the nuclear envelope, an event directly associated with apoptotic neuronal death (Chang *et al.*, 2011). Since the nature of Cdk5 dysregulation is mainly based on the imbalance of intracellular calcium homeostasis (Camins *et al.*, 2006; Lee *et al.*, 2000) (Fig. 3), a common feature of different neurodegenerative pathologies (Araujo *et al.*, 2010; Bano and Nicotera, 2007; Green *et al.*, 2007; Liu *et al.*, 2008a; Melo *et al.*, 2007), it is likely that the exposure to pathological stimuli other than Aβ (such as prion peptides, oxidative stress or ischemia) can trigger common mechanisms that lead to ectopic cell cycle re-entry (Fig.2).

4. CDK5 AT THE SYNAPSES: FUNCTIONS AND DYSFUNCTION

There are increasing evidences that point out a role for Cdk5 in the mechanisms of synaptic plasticity (Angelo *et al.*, 2006; Cheung *et al.*, 2006; Hawasli *et al.*, 2007; Hawasli *et al.*, 2009; Lai and Ip, 2009). Both Cdk5 and its normal activator p35 have been found in neuronal synapses as well as in the neuromuscular junction (Fu *et al.*, 2011; Humbert *et al.*, 2000b), where they colocalize with acetylcholine receptors (Fu *et al.*, 2001). Furthermore, several of the synaptic proteins isolated from adult mouse brain synaptosomes have been identified as substrates for Cdk5 (Collins *et al.*, 2005). Cdk5 also plays a relevant role neurotransmitter trafficking since it has the ability to modulate neurotransmitter release through the phosphorylation of P/Q-type voltage-dependent calcium channels (Tomizawa *et al.*, 2002). Moreover, Cdk5 inhibition allows the access to a pool of synaptic vesicles which is not normally available (Kim and Ryan, 2010). Besides regulating the exocytosis of

neurotransmitters, Cdk5 also regulates clathrin-mediated endocytosis, by phosphorylating dynamin 1 and amphiphysin 1(Floyd *et al.*, 2001; Tomizawa *et al.*, 2003).

Recent studies show that Cdk5 controls hippocampus-dependent learning and synaptic plasticity (Adle-Biassette *et al.*, 2006; Hawasli *et al.*, 2007; Hawasli *et al.*, 2009; Li *et al.*, 2001). Indeed, the conditional knock-out of Cdk5 improved performance in several hippocampal learning tasks and reduced the threshold for LTP induction (Hawasli *et al.*, 2009). However, although the knock-out of Cdk5 improves learning and synaptic plasticity, after some time Cdk5 knock-out mice displayed an increase in seizure susceptibility, suggesting a progressive increase in excitability (Hawasli *et al.*, 2009). Interestingly, the transient expression of p25 was also shown to enhance LTP and spatial learning (Angelo *et al.*, 2003; Fischer *et al.*, 2005), whereas in p35 null mice (p35 -/-) the long-term depression (LTD) and spatial learning were impaired (Ohshima *et al.*, 2005).

The modulation of learning and synaptic plasticity by Cdk5 occurs mainly through the regulation of NMDA receptor (NMDAR) trafficking and degradation. Indeed, in hippocampal neurons, Cdk5 can complex with calpains to promote the proteolysis of the NMDAR subunit NR2B (Hawasli *et al.*, 2007). On the other hand, the regulation of NMDAR endocytosis by Cdk5 is made through the phosphorylation of PSD-95 (Zhang *et al.*, 2008b), a postsynaptic scaffolding protein with a major role in the organization, function, and plasticity of excitatory synapses (Ehrlich and Malinow, 2004; Kim and Sheng, 2004). Studies show that Cdk5 inhibition increases the binding of PSD-95 to the tyrosine kinase Src, which in turn induces phosphorylation of NR2B and attenuates activity-induced endocytosis of this NMDAR subunit (Zhang *et al.*, 2008b). Interestingly, Cdk5 can also act on another type of NMDAR subunits, the NR2A. In fact, the action of Cdk5 over this subunit type is different from NR2B, since NR2A can be directly phosphorylated by Cdk5, a mechanism that will increase the activity of the receptor. Thus, unlike in NR2B, the inhibition of Cdk5 will block LTP induction in hippocampal neurons (Li *et al.*, 2001). This dual mechanism through which Cdk5 regulates NR2A and NR2B receptors may also be associated to their different nature, since NR2A is eminently synaptic, whereas NR2B can occur both synaptically and extrasynaptically (Hardingham and Bading, 2010).

Additionally, Cdk5 was shown to regulate the expression of acetylcholine neurotransmitter receptors (Fu *et al.*, 2001; Xie *et al.*, 2004) and affect acetylcholine and NMDA receptors clustering through PSD-95 phosphorylation (Fu *et al.*, 2005; Morabito *et al.*, 2004).The interaction between Cdk5 and PSD-95 is also considered relevant for the synaptic impairment that occurs in AD. Excitatory synapses are considered to be early targets for soluble Aβ, a view supported by the evidence that oligomerized Aβ can bind to synaptic sites, namely postsynaptic ones containing PSD-95 (Lacor *et al.*, 2004), Remarkably, inhibition of Cdk5 by roscovitine was shown to block the effect of Aβ on PSD-95 protein levels, an observation confirmed by the fact that the levels of PSD-95 do not decline after Aβ treatment of cultured cells expressing the triple alanine mutant form of PSD-95, which lacks phosphorylation sites (Roselli *et al.*, 2005).

5. STRATEGIES TO CONTROL CDK5 AND CONCLUSIONS

The studies comprehended in this review demonstrate the vital role of Cdk5 in the brain development through the participation in processes as important as neuronal migration or synaptic plasticity. It was our objective to unravel the physiological part that Cdk5 phosphorylation takes on the modulation of NMDA receptor activity and expression, neurotransmitter release, degradation of synaptic proteins, or even in gene expression modulation.

On the other hand, this review addressed the consequences of Cdk5 dysregulation , with a particular focus on Alzheimer's disease, but also outlining the role of this kinase in other neurodegenerative pathologies, such as prion encephalopathies or Parkinson's disease. Indeed, the overactivation and myslocalization of Cdk5 through a mechanism involving Ca^{2+}- induced calpain activation was shown to mediate tau hyperphosphorylation and apoptotic neuronal death, amongst other noxious effects. (Fig. 3).

(Insertion of Figure 3)

Due to its importance in these various neurodegenerative pathways of several brain pathologies, it is logical to assume that Cdk5 can be a good pharmacological target to prevent or even halt these pathologies. Indeed, diverse *in vitro* and *in vivo* studies have demonstrated that the blockage of Cdk5 activity can have a beneficial effect and provide neuroprotection. Two main strategies have been used for these purposes: direct inhibition, with the use of Cdk5 inhibitors, and indirect action, by preventing the excessive generation of the pathogenesis-associated activator p25 through the use of calpain inhibitors. Although the efficacy of these compounds has been demonstrated in various disease models, several concerns remain regarding the possible effects on the several physiological mechanisms controlled by Cdk5, since not only this kinase is vital for different neuronal processes, but even p25, when generated in a transient form, can play a positive role in cognitive and memory functions. Furthermore, although both roscovitine (one of the best know Cdk5 inhibitors), and calpain inhibitors (such as MDL28170), have the capability to cross the blood-brain barrier, they are not completely specific, which implies that their administration can affect several other pathways, both pathological and physiological.

In conclusion, the understanding of how such a multifaceted kinase executes its role in both normal and pathological conditions is of vital importance, since it establishes a basis for the development of novel therapeutic approaches designed to block Cdk5 dysregulation in diverse pathologies.

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7. FIGURE LEGENDS

Figure 1. Schematic representation of Cdk5 dysregulation by Aβ or PrP peptides

Neurons exposed to Aβ or PrP suffer a dysregulation in Ca^{2+} homeostasis, caused mainly by an influx through NMDA receptors and voltage-sensitive Ca^{2+} channels (in the case of Aβ) and non-selective ion channels (for PrP peptides), and potentiated by a calcium-induced calcium release from intracellular compartments, in particular the endoplasmic reticulum. This increase of the intracellular Ca^{2+} level will trigger the overactivation of calpains, which in turn will cleave the normal Cdk5 activator p35 to the pathogenic form p25. The augment in p25 levels promotes the formation of a hyperactive p25/Cdk5 complex, which, amongst other toxic effects, is responsible for the

hyperphosphorylation of the cytoskeleton protein tau, which will accumulate in the cytoplasm as paired helical filaments and neurofibrillary tangles.

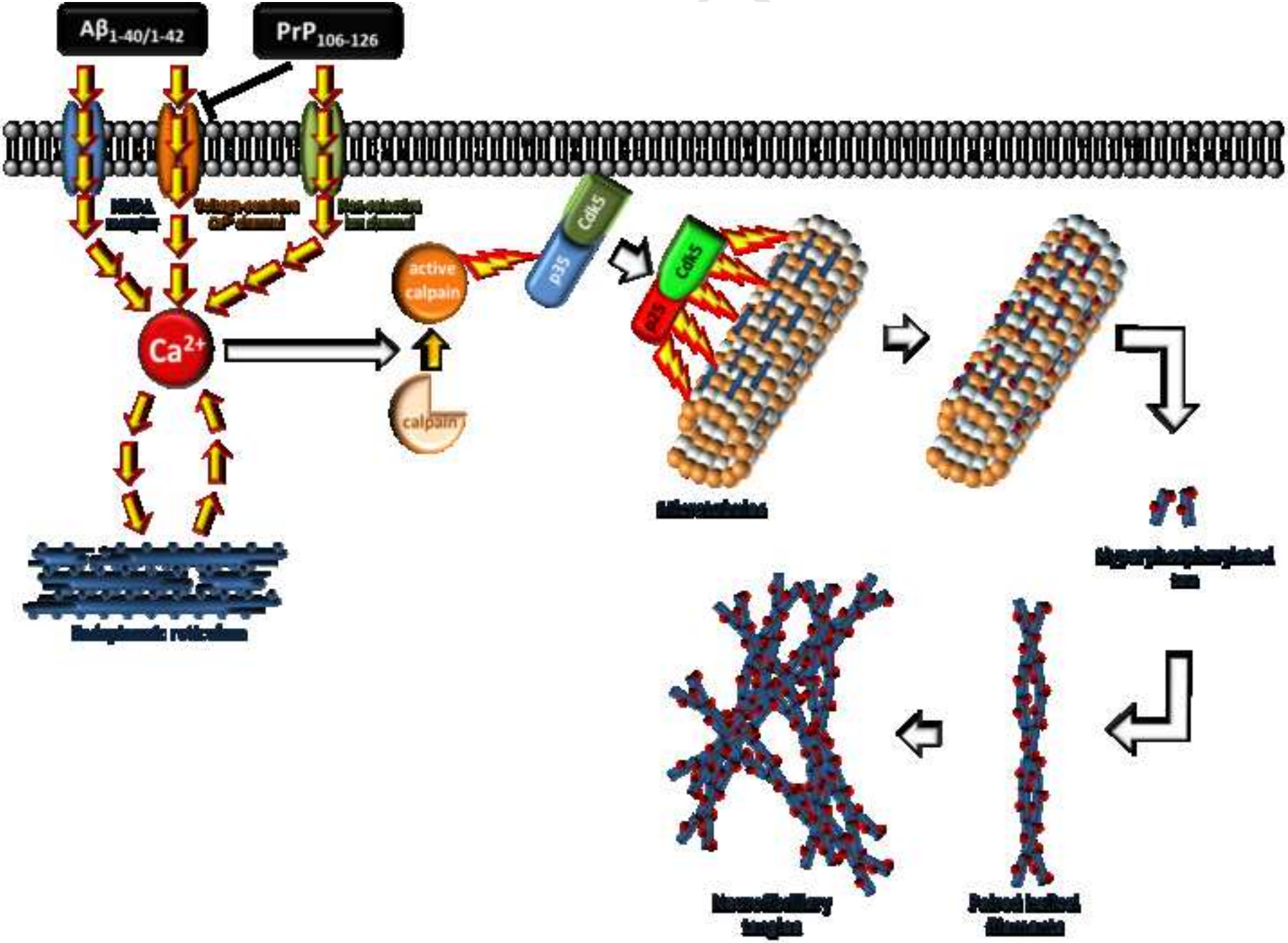
Figure 2. Cdk5 is a key molecule in the cell cycle re-entry triggered by Aβ

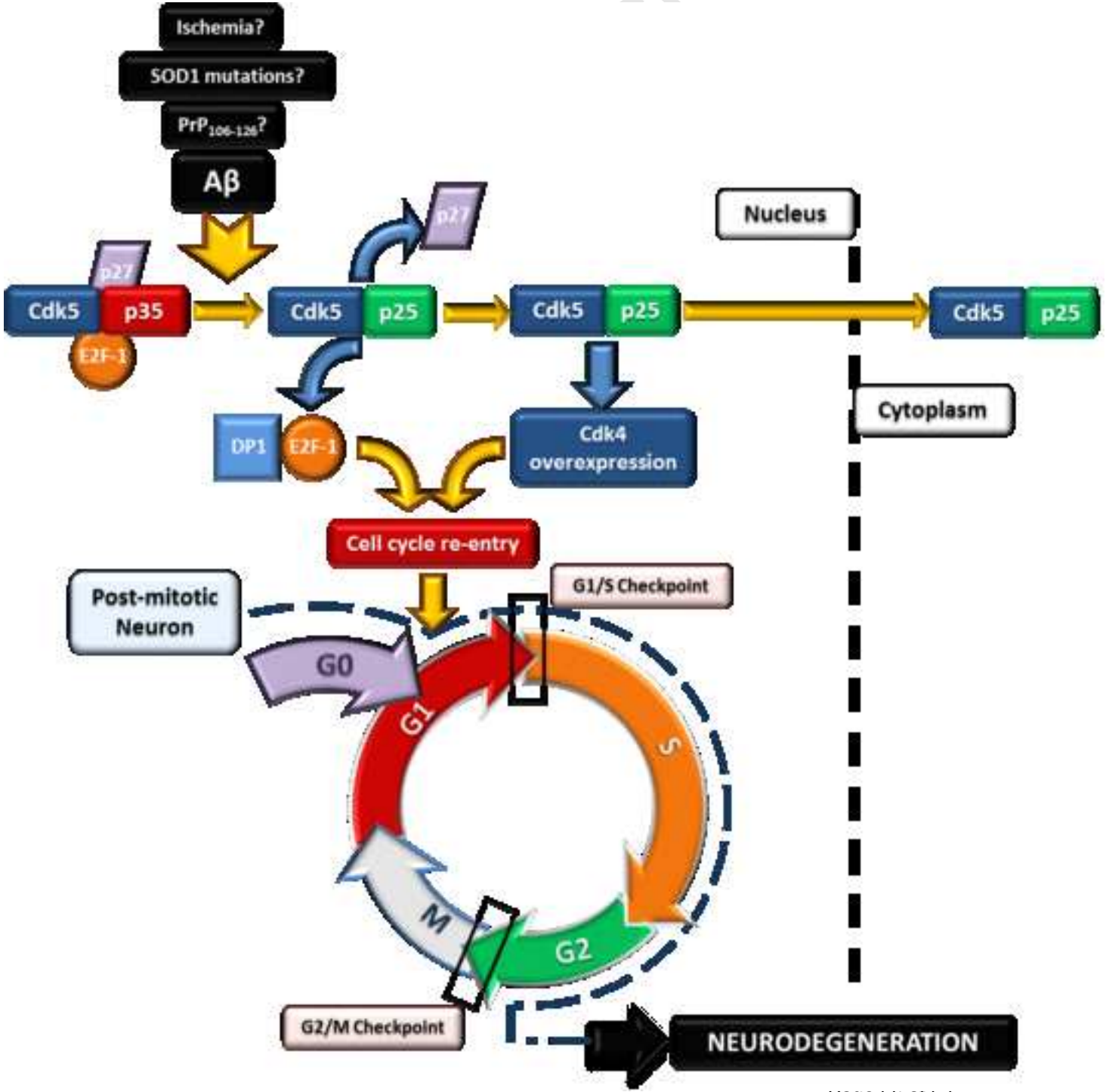
Besides the cytoplasm, Cdk5 is also known to be located in the nucleus, where the Cdk5/p35 complex will stabilize p27, a protein known to affect multiple processes in neurons, including cell cycle suppression. Furthermore, Cdk5/p35, by associating with E2F-1, blocks the binding of this nuclear factor with its activator DP1, thus halting the cell cycle in post-mitotic neurons. However, the cleavage of p35 to p25 triggered by Aβ (and possibly by other stimuli associated with different neurodegenerative pathologies, such as prion peptides, SOD1 mutations or ischemia) causes the disruption of this complex. Cdk5 is shuttled to the cytoplasm and, in the nucleus, the association of E2F-1 with DP1, as well as anupregulation of different proteins, such as the Cdks 2, 4 and 6, will induce the progression from the G1 to the S phase, where DNA replication will occur. However, these cell cycle active neurons do not overcome the G2/M checkpoint, probably exiting the cell cycle somewhere along the S or the G2 phases and advancing through the apoptotic pathway.

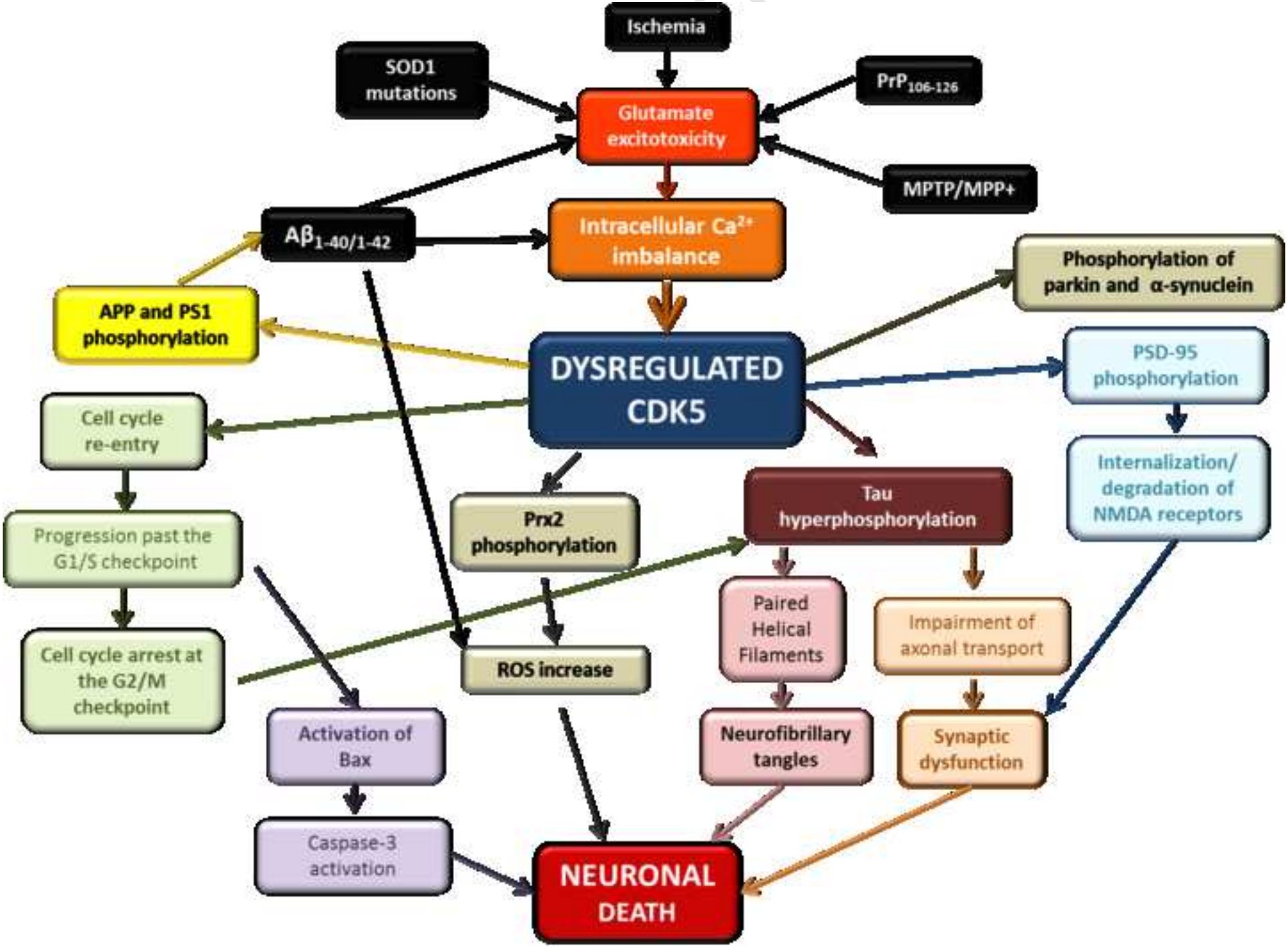
Figure 3. Cdk5 in different pathways of neurodegeneration

Although the triggering stimuli may be diverse, Cdk5 dysregulation appears to depend on the disruption of intracellular calcium homeostasis, generally do to an excessive activation of ionotropic glutamate receptors. One of the main consequences from Cdk5 overactivation is the excessive phosphorylation of the cytoskeleton protein tau, which correlates with the synaptic loss and formation of neurofibrillary tangles in AD and in some PRE, conducing also to neuronal death. Similarly, Cdk5 phosphorylates α-synuclein and parkin, two proteins involved in the pathogenesis of PD. Cdk5 is also a trigger for synaptic dysfunction via the phosphorylation of PSD-95 leading to the internalization and degradation of NMDA receptors. Cell cycle reactivation has also been recently linked to changes in the activity and localization of Cdk5. However, this cell cycle is not fully completed and the neurons exhibit activation of pro-apoptotic proteins of the Bcl-2 family and consequent caspase-3 activation.

Recent studies have further shown that cell cycle re-entry leads to AD-like changes such as increased Aβ and APP levels. Cdk5 can also influence Aβ production both by altering APP processing via the phosphorylation of this transmembrane protein and by modulating the activity of presenilins. The capacity of the cells to handle oxidative stress is also impaired by Cdk5 dysregulation, as is demonstrated by the inactivation of the peroxidase Prx2, via Cdk5 phosphorylation, in PD and ALS. Altogether, these evidences clearly point out Cdk5 dysregulation as a major step in the neurodegeneration pathways of diverse neurological disorders.


Aβ1-40/1-42
PrP106-126
Ca2+
active calpain
calpain
Cdk5
p35
Cdk5
p35


Ischemia?
SOD1 mutations?
PrP106-126?
Aβ
p27
Cdk5
p35
E2F-1
Cdk5
p25
p27
Cdk5
p25
Nucleus
Cdk5
p25
Cytoplasm
DP1
E2F-1
Cdk4 overexpression
Cell cycle re-entry
Post-mitotic Neuron
G1/S Checkpoint
G0
G1
S
M
G2
G2/M Checkpoint
NEURODEGENERATION


Ischemia
SOD1 mutations
PrP106-126
Glutamate excitotoxicity
MPTP/MPP+
Aβ1-40/1-42
Intracellular Ca2+ imbalance
Phosphorylation of parkin and α-synuclein
APP and PS1 phosphorylation
DYSREGULATED CDK5
PSD-95 phosphorylation
Cell cycle re-entry
Internalization/ degradation of NMDA receptors
Tau hyperphosphorylation
Prx2 phosphorylation
Progression past the G1/S checkpoint
Paired Helical Filaments
Impairment of axonal transport
Cell cycle arrest at the G2/M checkpoint
ROS increase
Activation of Bax
Neurofibrillary tangles
Synaptic dysfunction
Caspase-3 activation
NEURONAL DEATH