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Review article:

Cutting Edge Science

The Emphatic Role of Mitogen-Activated Protein Kinases (MAPKs) in the Cellular Mechanisms Mediating Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is a neurogenetic condition that affects the processes via which the brain functions. Major observable hallmarks of AD are accumulated clusters of proteins in the brain. These clusters, termed neurofibrillary tangles (NFT), resemble pairs of threads wound around each other in a helix fashion accumulating within neurons. These tangles consist of a protein called *Tau*, which binds to tubulin, thus forming microtubules. Unlike NFTs, deposits of amyloid precursor protein (β-APP) gather in the spaces between nerve cells. The nearby neurons often look swollen and deformed, and the clusters of protein are usually accompanied by reactive inflammatory cells, microglia, which are part of the brain's immune system responsible for degrading and removing damaged neurons or plaques. Since phosphorylation/dephosphorylation mechanisms are crucial in the regulation of *Tau* and β-APP, a superfamily of mitogen-activated protein kinases (MAPKs) has recently emerged as key regulators of the formation of plagues, eventually leading to dementia and AD. The complex molecular interactions between MAPKs and proteins (plagues) associated with the evolution of AD form a cornerstone in the knowledge of a still burgeoning field of neurodegenerative diseases and ageing. This review overviews current understanding of the molecular pathways related to MAPKs and their role in the development of AD and, possibly, dementia. MAPKs, therefore, may constitute a neurogenetic, therapeutic target for the diagnosis and evolution of a preventative medical strategy for early detection, and likely treatment, of Alzheimer's.

***Keywords*:** Alzheimer's; β-Amyloid precursor protein; Dementia; MAPK; Neurodegeneration; Neurofibrillary tangles; Tau.

Contents

1. Introduction

The syndrome of dementia, which represents a progressive deterioration in intellectual abilities, can be so severe to an extent it may interfere with the person's usual social and occupational functioning (Burnham, 1988; Floyd, 1999; Harper and LoGrasso, 2001; Peel, 2004; Samanta et al., 2006; Wurtman, 1985). An estimated 5-10 % of the US adult population (ages 65 and older) is affected by a dementing disorder, and the incidence doubles almost every 5 years among people falling within this age group (Drachman, 2006; Erickson, 1991; Sellcoe, 1991; Runden et al., 1998).

Alzheimer's disease (AD) is the most common form of dementia, especially in the US; it and related dementias affect at least 2 million, and possibly as many as 4 million, residents. Despite its prevalence, dementia often goes unrecognized or is misdiagnosed in its early stages (Beardsley, 1993; Beardsley, 1995; Jellinger, 2006; Zhu et al., 2001a). Some disorders that result in dementia are 'reversible' or 'potentially reversible,' which means that they can be treated effectively to restore normal or nearly normal intellectual function (Giannakopoulos, et al., 2007; St George-Hyslop, 2000).

Among the most frequent reversible causes of dementia are depressions, alcohol abuse and drug toxicity (Suram et al., 2006). In elderly persons, drug use — particularly drug interactions caused by 'poly-pharmacy' (simultaneous use of multiple drugs) — is a common cause of cognitive decline (Uhlhaas and Singer, 2006). Depression also is an under-diagnosed condition in this population (Erickson, 1991). However, the majority of dementias, including AD, are considered non-reversible. Even for these conditions, correct diagnosis of the problem in its early stages can be beneficial (Martindale, 2002). In addition, many of the non-reversible dementias include symptoms such as incontinence, wandering and depression that can be treated effectively (Dickey and Petrucelli, 2006; Wurtman, 1985).

The underlying factors contributing to the evolution and perpetuation of AD remain largely obscure (Martindale, 2002; St. George-Hyslop, 2000). Biomedical researchers have devised psychological screening tests to try to identify people in the early stages of AD. Apart from memory loss, there are nine other warning signs — among them difficulty performing familiar tasks, problems with language, such as remembering words, disorientation in time and space, and changes in personality. Susceptible people appear to have a greater sensitivity to the drug tropicamide, which is ordinarily used to dilate the pupils in a routine eye exam (Martindale, 2002; Wurtman, 1985). But for the most part, diagnosis has been a process of elimination. Microscopic views, for instance, have revealed a loss of nerve cells in certain regions of the brain, such as the hippocampus, a center for memory, and the cerebral cortex, which is involved in reasoning, memory, language and other important thought processes (Morgan, 2006; St George-Hyslop, 2000).

The other more directly observable hallmarks of AD are clusters of proteins that accumulate in the brain. These accumulations usually occur in two forms: those found inside nerve cells and those found between cells. The clusters in the interior are called neurofibrillary tangles (NFTs) and they resemble pairs of threads wound around each other in a form of a helix. Biochemical analyses showed that these tangles consist of a protein called *Tau* (Anderton et al., 2001; Dickey and Petrucelli, 2006). *Tau* is significant because it binds to a protein named tubulin, which in turn forms structures known as microtubules (microtubules are crucially important in the structural framework of living cells). Like the girders and pillars of buildings, they run through cells, thereby imparting support and shape. Microtubules also provide routes along which nutrients, other molecules and cellular components such as vesicles and mitochondria move through cells (Johnson, 2006; Martindale, 2002; St George-Hyslop, 2000).

Unlike NFTs, deposits of amyloid protein gather in the spaces between nerve cells. The nearby neurons often look swollen and deformed, and the clusters of protein — sometimes called senile or amyloid plaques — are usually accompanied by reactive inflammatory cells called microglia, which are part of the brain's natural immune system that help degrade and remove damaged neurons or perhaps the plaques themselves (Haddad 2002e; Martindale, 2002). It is unclear, however, whether the neurons in or near these plaques function normally, because the density of plaques is only weakly correlated with the severity of dementia.

Further, such plaques are present in most elderly people. Nevertheless, their extensive presence in the hippocampus and the cerebral cortex is specific to AD and they appear long before NFTs do (Higuchi et al., 2005).

Intensive efforts to isolate the ingredients of these plaques culminated with the discovery that a principal component was a peptide made up of 40-42 amino acids (St George-Hyslop, 2000). The sequencing of the gene for the longer protein from which this peptide originates quickly followed this identification of what is now termed the β-amyloid peptide: the β-amyloid precursor protein (β-APP). Although the precise biological role of normal β-APP molecules remains obscure, it is currently known that many kinds of cells and tissues produce β-APP and that it can be between 695 and 770 amino acids long (Martindale, 2002). The protein runs through the outer cell membrane, with a short piece jutting into the cell and a longer piece sticking into the extracellular space. The β-amyloid peptide, for its part, is snipped out of the section of β-APP that spans the cell membrane. β-APP is cut in one of two ways: in one process, the protein is first cleaved by an enzyme called α-secretase; it is then cut by another putative enzyme, γ-secretase. Together these cuts produce a harmless peptide fragment called p3 (St George-Hyslop, 2000). The second way in which β-APP is cleaved is another two-step process, one that is not always so harmless. First, an enzyme called β-secretase clips the protein; one of the resulting pieces, called C99-β-APP fragment, is then snipped by γ-secretase and the β-amyloid peptide is born (Dickey and Petrucelli, 2006).

At the molecular level, the biochemical, molecular, genetic, epidemiological and clinical discoveries have significantly advanced our understanding of the mechanisms underlying the evolution of AD and made it increasingly likely that, in the years to come, useful therapeutic treatments will be proposed. Some of these will probably come from the recent insights into the mis-processing or phosphorylation of *Tau*, a key mechanism in the regulation of Alzheimer's (Blennow et al., 2006; St George-Hyslop, 2000). Indeed, the insights into β-APP and β-amyloid peptide are already fueling treatment research. For instance, some investigators are designing compounds that will block the ability of either β- or γ-secretase enzymes to cut β-APP, thus preventing the creation of the damaging β-amyloid peptide. Others are seeking to alleviate the peptide's effects once it has been created. Clinical trials are under way to investigate whether antioxidants, such as vitamin E, or non-steroidal anti-inflammatory drugs (NSAIDS), such as ibuprofen, could alleviate some of the toxic effects of β-amyloid (Kanaan et al., 1997; Blennow et al., 2006; St George-Hyslop, 2000).

Since phosphorylation/dephosphorylation mechanisms are crucial in the regulation of *Tau* and β-APP (Hwang et al., 2004; Iqbal and Grundke-Iqbal, 2006), a superfamily of mitogen-activated protein kinases (MAPKs) has recently emerged as key regulators of the formation of plagues, eventually leading to dementia and AD (Wang et al., 2004) (Fig. 1). To this end, this review will focus on the mechanisms mediated by MAPKs, which may modulate AD-related proteins and their functions and thereby can potentially determine the development of NFTs and, perhaps, the evolution of AD.

2. MAPK signaling modules and pathways: A network overview

2.1. MAPK signaling pathways as viewed through their identification and bifurcations

Signal transduction at the cellular level refers to the movement of signals (messages) from outside (extracellular) the cell to inside (intracellular) (Chattopadhyay and Brown, 2000; Dhanasekaran and Dermott, 1996; Guha and Mackman, 2001; Haddad, 2001; Haddad, 2002a; Haddad et al., 2002; Haddad et al., 2003; Holmes-McNary, 2002; Inui et al., 2001; Kim et al., 1999; Levin, 2002; Lowes et al., 2002; Meriin et al., 1998; Nakamura et al., 1996; 1998; 2002; Plyte et al., 2000; Sontag, 2001). The movement of signals can be simple, like that associated with receptor molecules of the acetylcholine class receptors that constitute channels which, upon ligand interaction, allow signals to be passed in the form of small ion movement, either into or out of the cell (Chattopadhyay and Brown, 2000; Dhanasekaran and Dermott, 1996; Guha and Mackman, 2001; Haddad, 2002b; Haddad et al., 2003; Pursiheimo et al., 2002; Sontag, 2001).

These ion movements result in changes in the electrical potential of the cells that, in turn, propagates the signal spatially along the cell. More complex

signal transduction, furthermore, involves the coupling of ligand-receptor interactions to many intracellular events. These events include phosphorylations by tyrosine kinases and/or serine/threonine kinases (Haddad, 2001; Haddad, 2002c; Haddad et al., 2003; Holmes-McNary, 2002; Levin, 2002; Lowes et al., 2002). The phosphorylation of proteins may change enzyme activities and protein conformations. The eventual outcome is an alteration in cellular activity and changes in the programming of genes expressed within the responding cells (Haddad, 2005). Phosphorylation and dephosphorylation mechanisms schematically involved in the regulation of gene transcription are shown in Fig. 1.

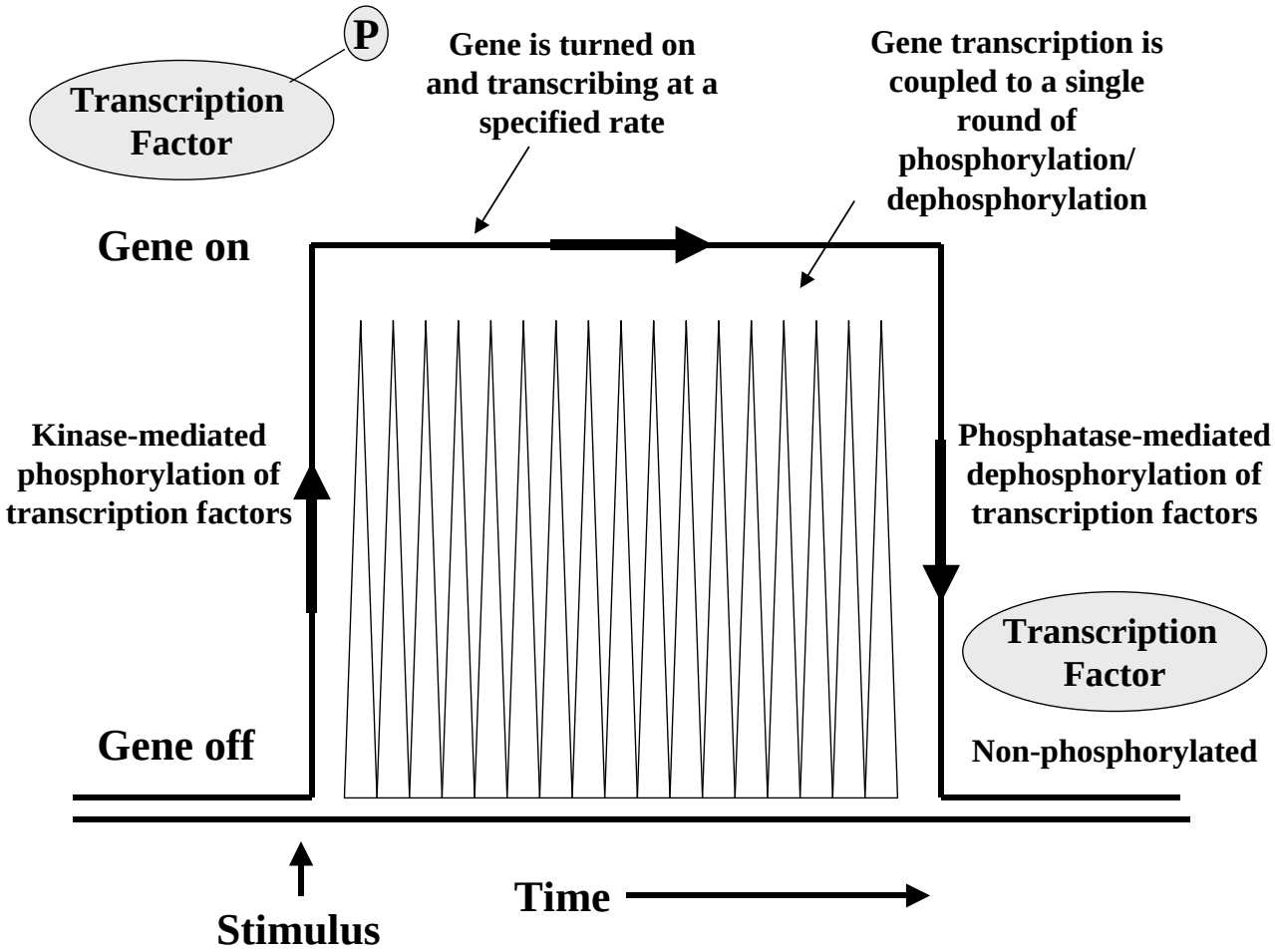

Figure 1: A hypothetical model of gene regulation where the switch on/off mediates a series of phosphorylation/dephosphorylation steps regulated by kinases and phosphatases, respectively. This sequential propagation of signals occurs in response to a stimulus over a pre-specified period of time.

MAPKs were identified by virtue of their activation in response to growth factor stimulation of cells in culture, hence the name mitogen-activated protein kinases (Fig. 2) (Avruch et al., 2001; Cano and Mahadevan, 1995; Chakraborty, 2001; Haddad, 2002a; Haddad et al., 2003; Kennedy et al., 1999; Lee et al., 2000; Mordret, 1993; Pierce et al., 2001). MAP kinases are also called extracellular receptor kinases (ERKs) for extracellular-signal regulated kinases. On the basis of *in vitro* substrates, the MAP kinases have been variously called microtubule associated protein-2 kinase (MAP-2 kinase), myelin basic protein kinase (MBP kinase), ribosomal S6 protein kinase (RSK-kinase; i.e., a kinase that phosphorylates a kinase) and epidermal growth factor (EGF) receptor threonine kinase (ERT kinase) (Avruch et al., 2001; Bardwell, 2006; Cano and Mahadevan, 1995; Chakraborty, 2001; Haddad, 2001; Haddad et al., 2003; Kennedy et al., 1999; Lee et al., 2000; Pierce et al., 2001). All of these proteins have similar biochemical properties,

immuno-cross-reactivities, amino acid sequence and ability to *in vitro* phosphorylate similar substrates.

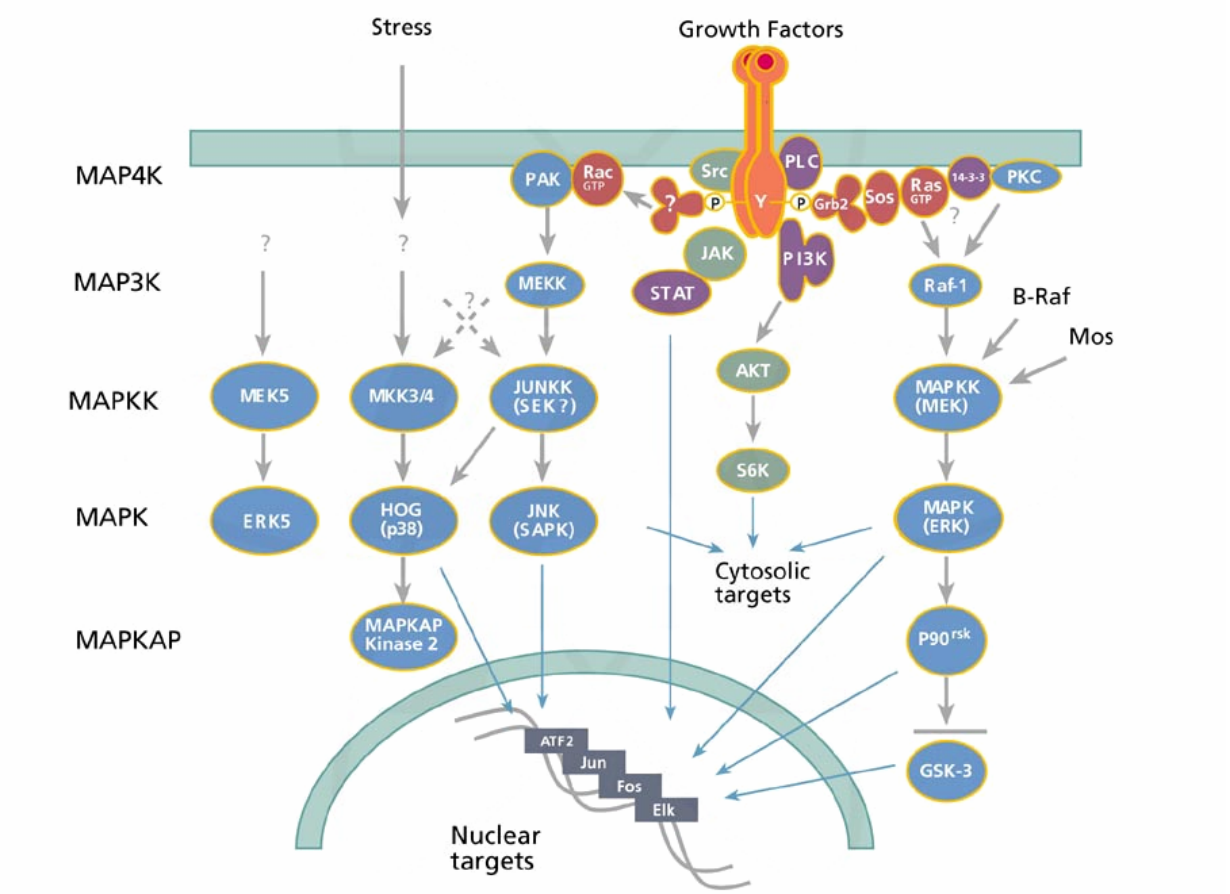

Figure 2: The modules and various components of the MAPK signaling pathways. The cellular response to growth factors, inflammatory cytokines and other mitogens is often mediated by receptors that either are G-protein-linked or are intrinsic protein tyrosine kinases. The binding of the ligand to receptor tyrosine kinases induces dimerization and auto-phosphorylation (activation) of the kinase. The activated tyrosine kinase binds to and phosphorylates an adaptor protein, such as Grb2, that, in turn, activates a guanine nucleotide exchange factor, such as mSOS, that, in turn, activates a small GTP-binding protein, such as Ras or Rac. The GTP-binding proteins then transmit the signal to one of several cascades of protein Ser/Thr kinases that utilize the sequential phosphorylation of kinases to transmit and amplify the signal. These kinase cascades are collectively known as mitogen-activated protein kinase (MAPK) signaling cascades. The best studied of these kinase cascades is the $MAPK^{ERK}$ ($MAPK^{p44/p42}$) signaling cascade. Downstream targets of $MAPK^{ERK}$ include p90rsk (p90 risobomal S6 protein kinase) and the Elk-1 and Stat3 transcription factors. The Jun kinase ($MAPK^{JNK/SAPK}$) and $MAPK^{p38}$ kinase (HOG) pathways are stress-activated MAP kinase cascades. The $MAPK^{JNK}$ cascade is activated by inflammatory cytokines as well as by heat shock and UV irradiation. Downstream targets of $MAPK^{JNK}$ include the transcription factors c-Jun and ATF-2. The $MAPK^{p38}$ pathway is activated by bacterial endotoxins, inflammatory cytokines and osmotic stress. Downstream targets of $MAPK^{p38}$ include the transcription factors ATF-2, Max and CREB. $MAPK^{p38}$ is also involved in the phosphorylation and activation of heat shock proteins.

Maximal MAP kinase activity requires that both tyrosine and threonine residues are phosphorylated. This indicates that MAP kinases act as switch kinase that transmits information of increased intracellular tyrosine phosphorylation to that of serine/threonine phosphorylation (Guan, 1994; Haddad 2001; Haddad 2002d; Ono and Han, 2000; Sugden and Clerk, 1997). Although MAP kinase activation was first observed in

response to activation of EGF, platelet-derived growth factor (PDGF), nerve growth factor (NGF) and insulin and insulin-like receptors, other cellular stimuli such as T-cell activation (which signals through the Lck tyrosine kinase), phorbol esters (that function through activation of protein kinase C (PKC)), thrombin, bombesin and bradykinin (that function through G-proteins), as well as *N*-methyl-$_D$-aspartate (NMDA) receptor activation and electrical stimulation rapidly induce tyrosine phosphorylation of MAP kinases (Avruch et al., 2001; Cano and Mahadevan, 1995; Chakraborty, 2001; Guan, 1994; Kennedy et al., 1999; Lee et al., 2000; Mordet, 1993; Ono and Han, 2000; Pierce et al., 2001; Sugden and Clerk, 1997).

MAP kinases are, however, not the direct substrates for receptor tyrosine kinases (RTKs) or receptor-associated tyrosine kinases but are in fact activated by an additional class of kinases termed MAP kinase kinases (MAPK kinases) and MAPK kinase kinases (MAPKK kinases). One of the MAPK kinases has been identified as the proto-oncogenic serine/threonine kinase, Raf (Bendinelli et al., 1995; Cazaubon et al., 1994; Chen et al., 1996; Cobb et al., 1994; English and Cobb, 2002; Errede et al., 1995; Haddad 2001; Hagemann and Blank, 2001; Kolch, 2000; Lee and McCubrey, 2002; Liebmann, 2001; Marshall, 1995; McCubrey et al., 2000; Morrison, 1995; Oppenheimer et al., 2000; Schlesinger et al., 1998; Sebolt-Leopold, 2000; Tibbles and Woodgett, 1999; Weinstein- Zhu et al., 2001b).

Ultimate targets of the MAP kinases are several transcriptional regulators such as serum response factor (SRF) and the proto-oncogenes Fos, Myc and Jun, as well as members of the steroid/thyroid hormone receptor super family of proteins. The simplified core of a MAPK cascade consists of three protein kinases: A MAP kinase kinase kinase (MAPKKK), a MAP kinase kinase (MAPKK) and MAP kinase (MAPK); these kinases phosphorylate each other in sequence. When activated, MAPKKK phosphorylates the MAPKK at one or two phosphorylation sites, bringing activation of the MAPKK module. MAPKKs are dual-specificity protein kinases that phosphorylate the MAPK at two phosphorylation sites (almost always a threonine and a tyrosine residue), bringing about activation of the MAPK. The active MAPK can then phosphorylate a variety of target proteins throughout the cytoplasm and nucleus (Bardwell, 2006).

On the internal regulatory mechanisms, each of the kinases in the MAPK cascade is opposed by one or more phospho-protein phosphatases. Thus, for an active MAPKK to activate a MAPK, for example, the rate of MAPK phosphorylation must exceed the rate of MAPK de-phosphorylation. The activities of these phosphatases are high enough to make the output of a MAPK cascade depend upon the continual presence of a stimulus feeding into the cascade; if this particular stimulus is withdrawn, for instance, the downstream kinases become inactivated within a very short lapse of time (Figure 2) (Avruch et al., 2001; Cano and Mahadevan, 1995; Chakraborty, 2001; Cullen and Lockyer, 2002; English and Cobb, 2002; Haddad et al., 2003; Kennedy et al., 1999; Lee and McCubrey, 2002; Lee et al., 2000; Mordret, 1993; Pierce et al., 2001).

The best-characterized vertebrate MAPKs fall into three major subgroups (Figure 2). The first subgroup includes the founding members of the MAPK family, extracellular signal-regulated kinase-1 (ERK1; $MAPK^{ERK1/p44}$) and ERK2 ($MAPK^{ERK2/p42}$), and their closest relatives (Bardwell, 2006; Belcheva and Coscia, 2002; Daulhac et al., 1997; Denhardt, 1996; Frye, 1992; Howe et al., 2002; Peyssonnaux and Eychene, 2001; Zhang et al., 2000). This subgroup is often referred to as ERKs, although some ERK proteins are not in fact members of this subgroup family.

The second subgroup is the Jun N-terminal kinases (JNKs), so called because they can activate the Jun transcription factor by phosphorylating two residues near its N-terminus (Barr and Bogoyevitch, 2001; Davis, 2000; Dickens et al., 1997; Dong et al., 2001; Fleming et al., 2000; Harper and LoGrasso, 2001; Ip and Davis, 1998; Leppa and Bohmann, 1999; Mielke and Herdegen, 2000; Noselli, 1998; Noselli and Agnes, 1999; Okazawa and Estus, 2002; Rincon et al., 2000; Weston and Davis, 2002; Yao et al., 1997).

The third subgroup is the p38 MAPKs, so named because of the molecular weight (38 kDa) of the first representative of the subgroup to be discovered (Bulavin et al., 2002; English et al., 1999; Haddad, 2001; Ichijo, 1999; Lee and Young, 1996; Lopez-Ilasaca, 1998; Obata et al., 2000; Rincon, 2001). Members of both the $MAPK^{JNK}$ and $MAPK^{p38}$ pathways are also classified as stress-activated protein kinases (SAPKs), because they are activated in

response to osmotic shock, UV irradiation, inflammatory cytokines and other stressful conditions (Haddad et al., 2003).

In all three subgroups, a large number of MAKKKs feed into the activation of a smaller number of MAPKKs and MAPKs. The diversity of the MAPKKKs thus allows a wide variety of upstream receptors to couple to MAPK cascades (Fig. 2) (Haddad, 2002a; Haddad, 2002f; Haddad and Land, 2002; Lewis et al., 1998; Marshall, 1994; Pearson et al., 2001). MAPKs and their corresponding substrates are given in Table 1.

Table 1: MAPKs and downstream transcription factors and substrates.

KINASE	SUBSTRATES
$MAPK^{ERK}$	Elk-1; SAP-1; Mnk-1/2; MAPKAP-K1/$p90^{Rsk}$; MSK-1
$MAPK^{JNK/SAPK}$	c-Jun; ATF-2; Elk-1
$MAPK^{p38}$	ATF-2; Elk-1; SAP-1; CHOP; MEF2C; MAPKAP-K2/K3; Mnk-1/2; MSK-1; PRAK
MAPKAP-K1/$p90^{Rsk}$	c-Fos; SRF
MSK-1	CREB; Histone H3 and HMG-14

ATF, Activating transcription factor; CHOP, C/EBP homologous protein; CREB, cAMP response element binding protein; HMG-14, High mobility group-14; $MAPK^{ERK}$, mitogen-activated protein kinase/extracellular signal-regulated kinase; MAPKAP-K, MAPK-activated protein kinase; MEF2C, Myocyte enhancer factor 2C; Mnk, MAPK interacting protein kinase; MSK, Mitogen- and stress-activated protein kinase; PRAK, p38-related/activated protein kinase; Rsk, Ribosomal S6 kinase; SAP-1, SRE accessory protein-1; SRF, Serum response factor.

2.2. MAPK signaling as viewed through receptor and non-receptor coupled cofactors

The MAPKs are a grouping of closely related families of Ser/Thr kinases involved in regulating growth, differentiation, and cellular responses to stress or inflammatory cytokines (Haddad and Fahlman, 2002; Haddad et al., 2003). Whereas the $MAPK^{ERK}$ pathway essentially regulates growth, proliferation and differentiation signals, the $MAPK^{JNK/SAPK}$ and $MAPK^{p38}$ pathways regulate cell responses to environmental stress.

The MAPKs link signals generated at the cell surface to transcription factors via a cascade of phosphorylation events basically initiated at the level of the membrane by protein tyrosine kinases (PTKs), of which two classes exist, the receptor PTKs and non-receptor PTKs. Among the PTKs is the Src family of non-receptor PTKs composed of at least nine members. Most are restricted to defined cellular lines, but three (Src, Fyn, Yes) are ubiquitously expressed (Courtneidge et al., 1993; Haddad, 2002d; Haddad et al., 2003). Like their receptor PTK counterparts, the Srcs are at the upper end of the MAPK cascade and serve as initiators for the transduction of signals generated by receptors at the cell surface (Akhand et a., 2002; Pawson, 1994; Parsons and Parsons, 1997).

The Ras G-proteins mediate the mitogen-induced activation of MAP kinases by activating Raf, a family of serine/threonine kinases (Hindley and Kolch, 2002). The MEK1 and MEK2 are the dominant Raf effector proteins, although other Raf substrates have been identified (Pearson et al., 2000). Deactivation of the MAP kinases is an important regulatory feature of the cascade. This is accomplished by phosphatases such as MKP-1/2 PAC-1 that dephosphorylate

$MAPK^{ERK}$ in the nucleus or MPK-3, which dephosphorylate cytosolic $MAPK^{ERK}$ (Haneda et al., 1999). The MAP kinases therefore are a convergence point for a wide variety of extracellular signals. MAPK circuits are a three tiered module consisting of Raf $\rightarrow$ MEK $\rightarrow$ $MAPK^{ERK}$ conveying signals from receptor TKs and G-protein coupled receptors to transcription factor in the cell nucleus (Erickson et al., 1990; Haddad et al., 2003; Ravichandran, 2001; Ray and Sturgill, 1987; Tian and Feig, 2001).

Two other signaling cascades are part of the MAP kinase system. The stress-induced $MAPK^{p38}$ mediates inflammatory or stress responses to cytokines, such as tumor necrosis factor (TNF), genotoxic agents, osmotic shock, bacterial lipopolysaccharides, photo-damage from ultraviolet light, as well as from growth factor withdrawal. The other pathway, $MAPK^{JNK/SAPK-1}$, transduces several stress signals including oxidation/DNA damage along with growth and differentiation signals. Both of these pathways are activated by a Rac-1 and Cdc-42 signals, which can also activate the $MAPK^{ERK1/2}$ system (Vacratsis and Gallo, 2000) (Fig. 3).

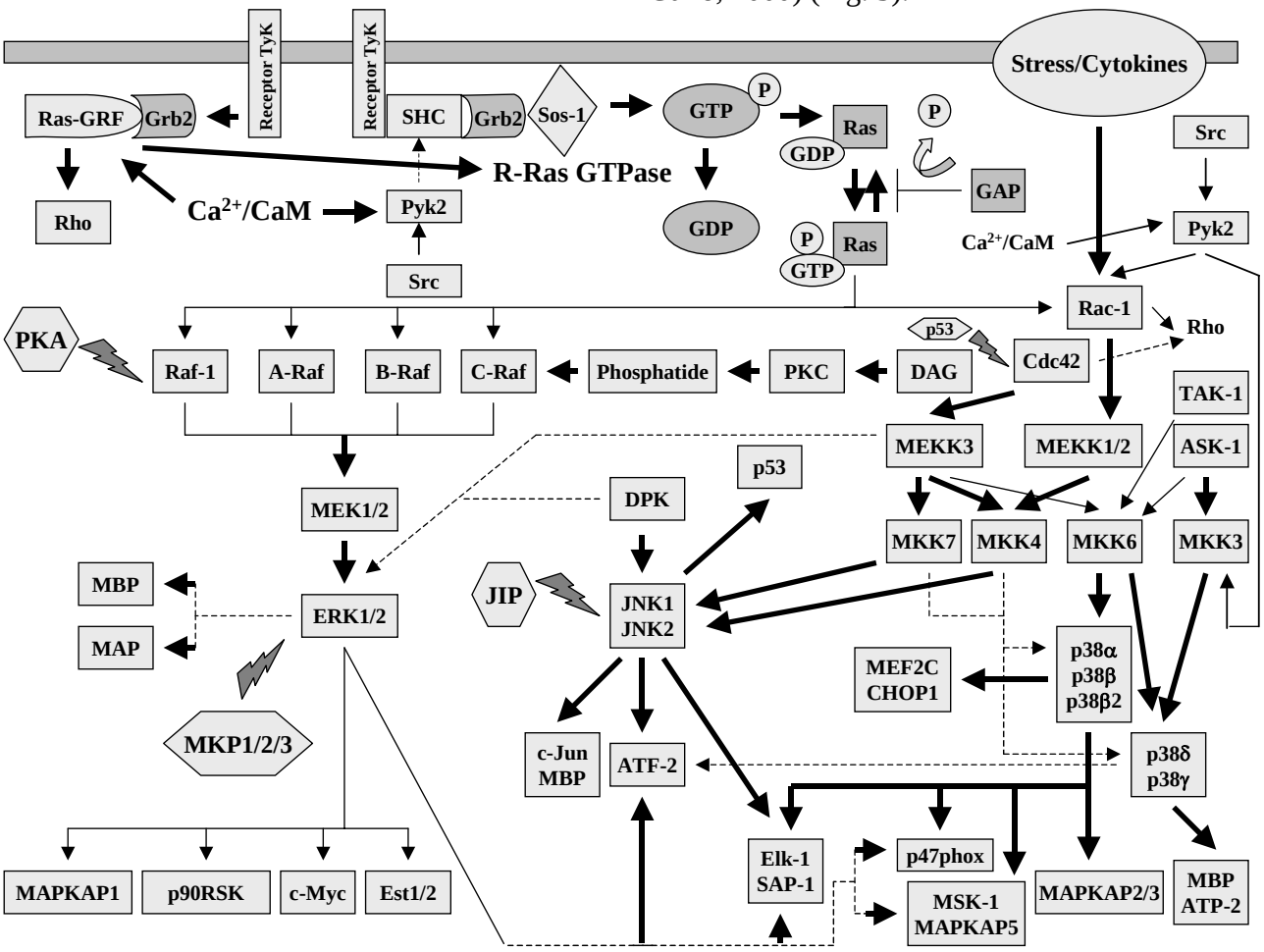

Figure 3: An overview of the complex network of MAPK signaling pathways and their interactions and bifurcations. The sign denotes inhibition, the solid arrows indicate activation (stimulation); P, phosphorylation.

The three MAPK pathways do not act in isolation but in parallel with limited cross-talk possible. MEKK-3 directly activates both MKK-6 (activator of $MAPK^{p38}$) and MKK-7 (activator of $MAPK^{JNK}$), while MKK-4 (activator of $MAPK^{JNK}$ and $MAPK^{p38}$) is activated by MEKK-2 and MEKK-3. The $MAPK^{JNK}$ pathway activates the tumor suppressor gene, p53, which has recently been demonstrated to counteract Cdc-42 (Kumar et al., 1997; Enslen et al., 1998; Herlaar and Brown, 1999; Cuenda, 2000; Mielke and Herdegen,

2000; Gadea et al., 2002; Hazzalin and Mahadevan, 2002; Haddad et al., 2003) (Figure 3). (For further details on MAPKs signaling pathways, refer to Haddad et al., 2003.)

3. Alzheimer's disease: An overwhelming etiological condition or a maneuverable syndrome?

AD is the most common cause of dementia in older people (Li et al., 2001; Marx, 2001). Simply put, it's a medical condition that disrupts the way the brain works. AD most notably affects the parts of the brain that control thought, memory and language (Martindale, 2002). Although the risk of getting the disease increases with age, it is not a normal part of aging (St George-Hyslop, 2000). AD is characterized by adult-onset slowly progressive dementia associated with diffuse cerebral atrophy on neuro-imaging studies. AD is named after Dr. Alois Alzheimer, a German psychiatrist (Bardwell, 2006; Wurtman, 1985).

In 1906, Dr. Alzheimer described changes in the brain tissue of a woman who had died of an unusual mental illness. He found abnormal deposits (called senile or neuritic plaques) and tangled bundles of nerve fibers (called NFTs). These plaques and tangles in the brain have come to be characteristic brain changes due to AD. The diagnosis of AD is based on the histological findings of β-amyloid plaques and intra-neuronal neurofibrillary tangles. A significant association with the e4 allele of apo-lipoprotein E supports the diagnosis of AD in patients with dementia and increases the risk that asymptomatic individuals will eventually develop AD. *ApoE* genotyping, however, is neither fully specific nor sensitive (Bardwell, 2006).

Early and careful evaluation is important because many conditions can cause dementia, some of which are treatable or 'reversible.' Potentially reversible conditions include depression, adverse drug reactions, metabolic changes, and nutritional deficiencies (St George-Hyslop, 2000). A comprehensive patient evaluation includes a complete health history, physical examination, neurological and mental status assessments, and other tests including analysis of blood and urine, electrocardiogram, and an imaging exam, such as CT or MRI (Anderson et al., 2005). While this type of evaluation may provide a diagnosis of possible or probable AD, confirmation requires examination of brain tissue at autopsy.

Although no cure for AD is yet available, medical and social management of the disease can ease the burdens on the patient, and his or her caregiver and family (St George-Hyslop, 2000). To date there are four FDA-approved drugs for the treatment of AD — tacrine (Cognex®), donepezil (Aricept®), rivastigmine (Exelon®) and galantamine (Reminyl®) — and several others in clinical trials. In addition to treating the symptoms, advising the patient and his or her caregiver to initiate health care directives and decisions while the patient still has the capacity to do so can ease the burden for the family as the disease progresses (Beardsley, 1995; St George-Hyslop, 2000; Martindale, 2002).

4. The role of MAPKs in Alzheimer's: Mechanisms and possible therapeutic targets

4.1. Paired helical filaments and Tau phosphorylation

Paired helical filaments (PHFs) are a characteristic pathological feature of AD; their principal component is the microtubule-associated protein *Tau* (Fig. 4). The *Tau* in PHFs (PHF-*Tau*) in AD is hyper-phosphorylated, but the cellular mechanisms responsible for this hyper-phosphorylation are still being elucidated (Ganju et al., 1998; St George-Hyslop, 2000; Zhao et al., 2003). MAPKs are a family of Ser/Thr kinases that link cell surface signals to changes in enzyme activity and gene expression (Shanavas and Papasozomenos, 2000; Haddad, 2002d; Haddad et al., 2003).

Moreover, MAPKs have been shown to phosphorylate *Tau in vitro* at Ser/Thr proline sites, thereby generating a multiply phosphorylated *Tau* protein that is similar to the hyper-phosphorylated *Tau* found in AD neurofibrillary tangles (NFT) (Dalrymple, 2002; Johnson and Bailey, 2003; St George-Hyslop, 2000). For instance, fluorescence resonance energy transfer (FRET) studies demonstrated a tight intermolecular association of activated MAPK with these phospho-*Tau* epitopes (Knowles et al., 1999). The phosphorylation of normal *Tau* by MAPKs such as $MAPK^{ERK}$ induced *Tau* to acquire biochemical properties of AD PHF proteins. In this regards, it was shown that a monoclonal antibody to MAP kinases could recognize $MAPK^{ERK2}$ ($MAPK^{p42}$) in normal and AD cortex, but $MAPK^{ERK2}$ levels were slightly reduced in the AD brain (Trojanowski et al., 1993). The hypothesis was that since $MAPK^{ERK2}$ was

detected in NFTs and senile plaque neurites in the AD hippocampus, $MAPK^{ERK2}$ was positioned to phosphorylate normal *Tau* and could possibly play a role in the generation of PHFs in AD.

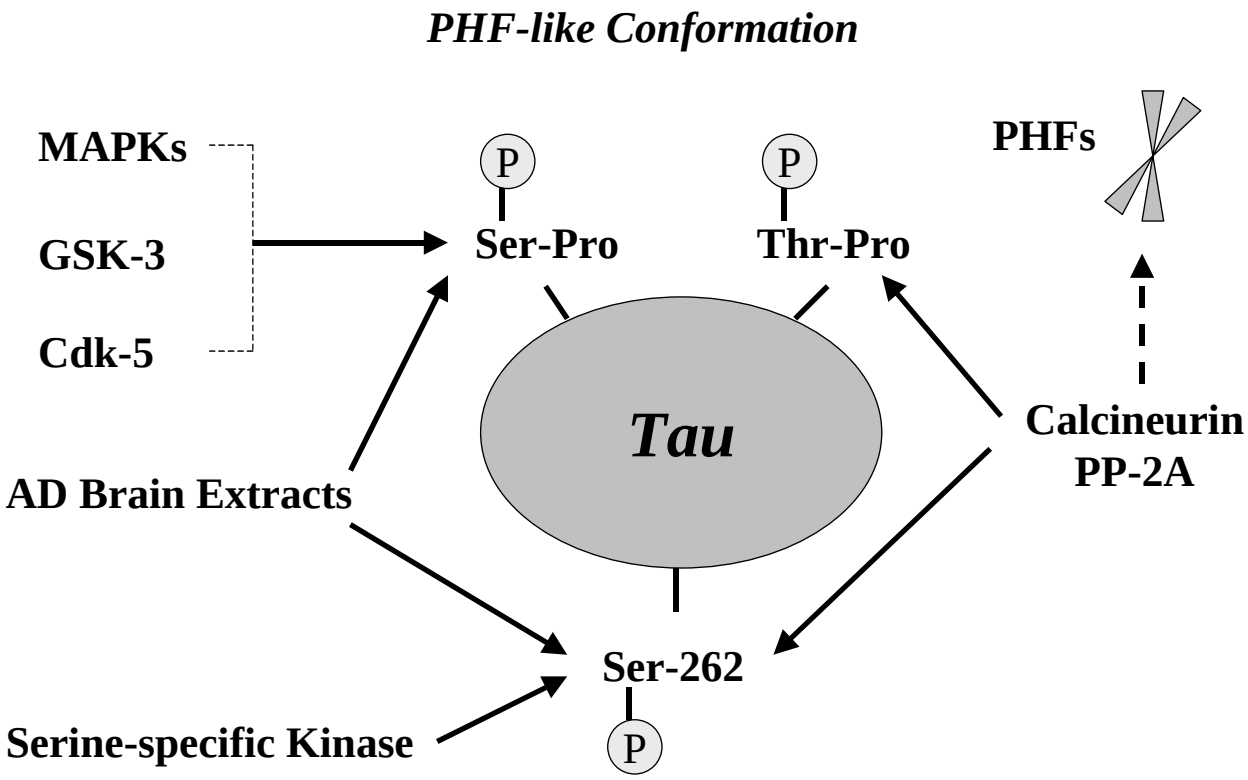

Figure 4: Schematic diagram depicting the role of kinases and phosphatases acing on *Tau* protein in AD. *Tau* contains specific phosphorylation sites that are apparently critical for hyper-phosphorylation in AD. One class comprises the Ser-Pro and Thr-Pro motifs that determine the reactivity with diagnostic antibodies; the other class contains, at least, Ser-262 and has a strong effect on microtubule binding. Proline-directed kinases such as MAPKs, GSK-3 and Cdk-5 basically phosphorylate Ser-Pro or Thr-Pro motifs, and the Serine-specific kinase phosphorylates Ser-262 in the first repeat and the corresponding serines in the other repeats. AD-derived brain extracts usually contain a mixture of the aforementioned kinases. The phosphatases calcineurin and PP-2A can remove the phosphates from Tau as well as from AD PHFs, thereby acting in an endogenous negative feedback mechanism.

In contrast, activating MAPK in cultures of primary neurons or transfected COS cells expressing *Tau* isoforms did not increase the level of phosphorylation for any PHF-*Tau* epitope investigated (Lovestone et al., 1994). However, elevating glycogen synthase kinase (GSK)-3 activity in the COS cells by co-transfection with GSK-3α or GSK-3β decreased the electrophoretic mobility of *Tau* so that it resembled that of PHF-*Tau*, and induced reactivity with PHF-*Tau*-selective monoclonal antibodies (Aplin et al., 1996; Lovestone et al., 1994).

These observations were reinforced by the construction of mammalian cell culture systems producing high levels of intracellular phosphorylated *Tau*. COS-1 fibroblast-like cells were transiently transfected to simultaneously express *Tau*, MAPK and MAPKK, or alternatively to express *Tau* and GSK-3

(Aplin et al., 1996; Baum et al., 1995). B103 neuron-like cells (which contain MAPK but little *Tau* or GSK-3) were stably transfected to express *Tau* or *Tau* and GSK-3. In both systems, GSK-3 transfected cells contained *Tau* AT-8/M (defined by AT-8 staining and *Tau* PHF-like mobility), but MAPK-transfected cells required phosphatase inhibitors, such as okadaic acid (OKA) or calyculin (CAL), to produce *Tau* AT-8/M. *In vitro*, CAL and OKA inhibited protein phosphatase (PP)-1 and PP-2A.

In addition, inducing *Tau* phosphorylation at the AT-8 site in MAPK-transfected cells required 2-10 times more OKA than CAL, suggesting both PP-1 and PP-2A helped block the phosphorylation (Baum et al., 1995; Tanaka et al., 1998). Though levels of *Tau* AT-8/M increased in COS-1 cells, the ratio of particulate to supernatant *Tau* levels did not change, and no tangles were observed; it was suggested that post-translational modifications or co-aggregating proteins were likely needed to induce PHF (Ghribi et al., 2003).

4.2. Tau phosphorylation and MAPK inhibition

Mobility shift, phospho-epitope analysis and direct measurement of kinase activity indicated that the MEK-1 inhibitor (PD-098059) dose-dependently blocked basal and OKA-induced MAPK activation (Ekinci and Shea, 1999; Ho et al., 1997; Rapoport and Ferreira, 1999, 2000; Schneider et al., 1999). Despite a block of MAPK activation by this inhibitor, robust *Tau* hyper-phosphorylation was observed in response to OKA. In addition, activation of MAPK by phorbol 12-myristate 13-acetate (PMA) did not result in *Tau* phosphorylation, indicating that in primary cultures of cortical neurons elevated MAPK activity was not sufficient to induce *Tau* hyper-phosphorylation.

Similarly, using immuno-histochemistry, Yamaguchi et al. (1996) examined the localization of four types of proline-directed kinases in the brains of control rats and in the brains of non-demented aged human subjects, subjects with AD and those with Down's syndrome. The four kinases were: cyclin-dependent kinase (cdk)-5, a component of *Tau* protein kinase (TPK)-II, TPK-I/GSK-3β, GSK-3α and $MAPK^{ERK2}$. Antiserum for cdk-5 showed the most preferential and consistent labeling of intra-neuronal NFT. Antiserum for TPK I/GSK-3β also labeled intra-neuronal NFT. Double immuno-labeling for TPK I/GSK-3β and *Tau*-1 showed that TPK I/GSK-3β was closely associated with NFT.

Antiserum for GSK-3α labeled neurons weakly and the intensity of labeling did not differ between neurons with and without NFT. In addition, antiserum for MAPK labeled neurons in superficial cortical layers, but NFT appeared in both superficial and deep cortical layers (Reynolds et al., 2000; Yamaguchi et al., 1996). These findings suggested that cdk-5 and TPK I/GSK-3β are the critically important kinases for the generation *in vivo* of hyper-phosphorylated *Tau*, the main component of the paired helical filaments in NFT.

In concert with these observations, the novel brain protein kinase PK-40 was characterized by its ability to phosphorylate Lys-Ser-Pro sites in neuro-filament and *Tau* proteins (Mandelkow et al., 1993). PK-40 is recognized as a member of the family of $MAPK^{ERK}$ by its reactivity with $MAPK^{ERK}$-specific antibodies ($PK\text{-}40^{ERK}$). Particularly, protein sequence analysis suggested that PK-40 is a form of $MAPK^{ERK2}$. Bovine *Tau* or recombinant human *Tau* proteins can be hyper-phosphorylated by $PK\text{-}40^{ERK}$ to produce the electrophoretic mobility shifts and certain immunochemical properties characteristic of PHF-*Tau* isolated from AD brain tissue (Trojanowski et al., 1993).

On the mechanisms related to $PK\text{-}40^{ERK}$, a cAMP-dependent protein kinase A (PKA)-mediated pathway is involved. It was observed that PKA phosphorylates *Tau* to a lesser extent; however, the product is not like the hyper-phosphorylated *Tau* of AD in several important respects. Subsequently, it was reported that *in vitro* $PK\text{-}40^{ERK}$ further phosphorylates *Tau* that was previously saturated by PKA, provided that the concentrations of free un-complexed ATP are low (Blanchard et al., 1994; Raghunandan and Ingram, 1995). Interestingly, the actions of different kinases on *Tau* are not independent, but may depend on the order in which they work on *Tau*; i.e., prior phosphorylation by PKA partially inhibits the action of $PK\text{-}40^{ERK}$.

Furthermore, a subpopulation of $MAPK^{ERK2}$ species in soluble brain fractions can be efficiently phosphorylated and activated in cell-free systems, simply by adding Mg^{2+}-ATP. Two phospho-isoforms of $PK\text{-}40^{ERK2}$ are formed in this process, which have

reduced gel mobility, very much like the $MAPK^{ERK2}$ form obtained in cell culture by stimulation with growth factors (Roder et al., 1993; Roder et al., 1995). One of these low-mobility forms cannot be inactivated with PP-2A or with tyrosine phosphatases; the second form can be slowly inactivated by PP-2A. In this case two Ser/Thr phosphates are removed at different rates during inactivation: One phosphate is very quickly removed to result in the formation of a high-mobility 39-kDa $MAPK^{ERK2}$ species without consequence for activity; the other, slowly removed Ser/Thr phosphate controls the activity but has no effect on the gel mobility of $MAPK^{ERK2}$. These results unequivocally showed that forms of $MAPK^{ERK2}$ exist with properties different from the previously characterized $MAPK^{ERK2}$ ($MAPK^{p42}$) from stimulated cell cultures. The active $MAPK^{ERK2}$ forms produced in the presence of Mg^{2+}-ATP alone could provide an explanation for the existence of constitutive $MAPK^{ERK2}$-like NFT phosphorylation *in vivo*. Excessive formation of an $MAPK^{ERK2}$ species resistant to inactivation by PP-2A might be relevant to the persistent pathological *Tau* hyper-phosphorylation in AD. It was subsequently postulated that $PK\text{-}40^{ERK}$ might play a crucial role in the etiology of this disease (Roder et al., 1993; Roder et al., 1995).

Furthermore, incubation of rat, human and rhesus monkey temporal neo-cortex slices with the phosphatase inhibitor OKA induced epitopes of *Tau* similar to those found in PHFs. OKA induced variant forms of *Tau* at 60-68 kDa, which were recognized by the monoclonal antibodies Alz-50 (in humans only) and 5E2 and two polyclonal anti-peptide antisera, OK-1 and OK-2 (Garver et al., 1995). The phosphorylation-sensitive monoclonal antibody *Tau*-1 failed to recognize the slowest mobility forms of *Tau* after okadaic acid treatment. Moreover, FK-520 (1-10 μM), a potent inhibitor of calcineurin activity, was tested in brain slices and found not to alter *Tau* mobility. However, combinations of FK-520 and OKA caused *Tau* mobility shifts similar to those seen after OKA treatment; similar results were seen using the calcineurin-selective inhibitor cypermethrin (Garver et al., 1995). Treatment of human slices with OKA decreased both PP-2A and calcineurin activity; FK-520 inhibited only PP-2B activity. A proposed *Tau*-directed kinase, 42-kDa $MAPK^{ERK2}$, was activated by OKA but not FK-520. Of note, NGF activated $MAPK^{ERK2}$, particularly when used in combination with OKA; changes in *Tau* mobility were seen when this kinase was activated. Forskolin, in addition, antagonized the effects of NGF on both $MAPK^{ERK2}$ activity and *Tau* phosphorylation and forskolin alone had little effect on PHF-like *Tau* formation induced by phosphatase inhibitors, outlining complex interactions between *Tau*-directed protein kinases and protein phosphatases and suggesting potential sites for therapeutic targeting.

4.3. Tau structural analysis and MAPK-related protein phosphorylation

The interactions of *Tau* protein with microtubules could be visualized from two points of view, phosphorylation and domain structure (Anderton et al., 2001; Angulo et al., 2003; Floyd, 1999; James et al., 1996; Jenkins et al., 2000; Mandelkow et al., 1995; Stein and Johnson, 2003). *Tau* can be phosphorylated at many sites and by several kinases, notably by proline-directed kinases (MAPK, GSK-3, cdk-5), which generate Alzheimer-like antibody epitopes. Other kinases phosphorylate Ser 262, a site that has a particularly pronounced influence on the affinity of *Tau* for microtubules (Fig. 4). All of these sites can be cleared by phosphatases PP-2A and CAL. The site Ser-262 lies within the repeat domain of *Tau*. However, when probing the domains of *Tau* for their effects on microtubule binding, nucleation, assembly, or bundling, the repeat domain has only a weak influence. Whereas the repeat domain of *Tau* binds to microtubules with low affinity, repeat-less *Tau* binds strongly yet unproductively in terms of microtubule assembly.

Productive binding of *Tau* to microtubules depends on the combination of repeats with the flanking regions, as if the flanking regions acted as ‘jaws’ for the proper positioning of *Tau* on the microtubule surface. Analysis of phosphorylation of *Tau* is often performed using phosphorylation-sensitive monoclonal antibodies thought to report the presence or absence of one or two specific phosphorylations (cognate sites). Using several such antibodies, it was found that a much more complicated relationship existed between phosphorylation at specific sites, as monitored by two-dimensional phospho-peptide mapping, and antibody recognition of these sites (Roder et al., 1997). Multiple phosphorylation of *Tau* in several stages by PK-40, for instance, suggested that phosphorylation at cognate sites is sometimes necessary, but not sufficient, to induce a change of antibody reactivity and in some cases is not even necessary in the

background of multiple phosphorylation at other sites. No single phosphorylation site was found to be responsible for any level of gel mobility shift associated with phosphorylation. Moreover, *Tau* acquired its maximal gel mobility retardation and final immunochemical profile at sub-stoichiometric phosphorylation of most sites. This suggested that many alternate phosphorylation patterns could produce the same conformational and immunochemical presentation on SDS-PAGE. Although PK-40^{ERK2} prefers some phosphorylation sites, most notably Ser-235, followed by Ser-199 or Ser-202 and Thr-205, the phosphorylation of multiple Ser/Thr-Pro sites is not highly sequential (Fig. 4). Ser-396 is one of the least preferred sites and seems to require prior phosphorylation at Ser-404.

Moreover, MAPK immunoreactivity and *in situ* hybridization patterns of the two major genes that comprise MAPK activity, MAPK^{ERK1} and MAPK^{ERK2}, were reported in the human hippocampal formation (Hyman et al., 1994). The goal was to determine whether the pattern of MAPK^{ERK} expression was consistent with the hypothesis that MAPKs directly contribute to NFT formation. MAPK^{ERK1} mRNA was detected in small amounts and confined primarily to dentate gyrus granule cells (Hyman et al., 1994). MAPK^{ERK2} mRNA, by contrast, gave a much stronger hybridization signal and was present in dentate gyrus granule cells and pyramidal cells throughout all hippocampal sub-fields and adjacent temporal neo-cortex. Quantitative measures of MAPK^{ERK2} mRNA revealed that neuro-filament bearing neurons contained approximately 15% less MAPK^{ERK2} mRNA than nearest normal neighbors (Hyman et al., 1994). NFT-bearing neurons contained approximately 25% less poly-A mRNA, suggesting a relative preservation of MAPK^{ERK2} mRNA even in metabolically compromised cells.

MAPK immunoreactivity (which represented both MAPK^{ERK1} and MAPK^{ERK2}) was reported in neuronal soma, dendrites, axons and in reactive astrocytes (Hyman et al., 1994). In AD, neurons that contain NFTs are also MAPK immunoreactive, but neurons that contain the highest amounts of MAPK immunoreactivity are not necessarily vulnerable for NFTs. MAPK immunoreactivity was present in the same neurons as NFTs and in the same subcellular compartments as *Tau*, supporting a role for MAPKs in *Tau* phosphorylation in AD. However, the presence of MAPK^{ERK} immunoreactivity was not sufficient to predispose neurons to NFT formation (Korneyev, 1998).

4.4. Tau/β-APP-mediated phosphorylation mechanisms and MAPK regulation

Biological effects related to cell growth, as well as a role in the pathogenesis of AD, have been ascribed to the β-amyloid precursor protein (β-APP). The aberrant expression or processing of β-APP is the only known genetic basis for presenile familial AD, and the molecular connection between β-APP and *Tau* has always been perplexing (Grant et al., 1999; Jones et al., 1999; Wirths et al., 2006). Transgenic experiments, for instance, have revealed that long-term memory is dependent on cAMP-response element binding protein, CREB (Sato et al., 1997). CREB phosphorylation at Ser-133 is essential for its transcriptional activity. In this respect, it was demonstrated that β-APP induced CREB phosphorylation at Ser-133 in rat pheochromocytoma PC-12 cells. β-APP, furthermore, induced the phosphorylation of MAPK^{ERK1} and MAPK^{ERK2} at Tyr-204, and PD-98059, a MEK1 inhibitor, inhibited β-APP-induced CREB phosphorylation at serine-133 (Sato et al., 1997). It was concluded that elevated β-APP level induces CREB phosphorylation at Ser-133 via MAPK$^{ERK1/2}$-dependent pathway.

In addition, it was reported that the secreted form of β-APP potently stimulates MAPKs. For instance, brief exposure of PC-12 pheochromocytoma cells to β-APP secreted by transfected Chinese hamster ovary cells stimulated the 43-kDa form of MAPK (Greenberg et al., 1994). Induction of a dominant inhibitory form of Ras in a PC-12-derived cell line prevented the stimulation of MAPK by secreted β-APP, demonstrating the dependence of the effect upon p21-Ras. Moreover, it was found that enhancement in *Tau* phosphorylation was associated with the β-APP-induced MAPK stimulation (Greenberg et al., 1994). In the Ras dominant inhibitory cell line, β-APP failed to enhance phosphorylation of *Tau*, thereby providing a link between secreted β-APP and the phosphorylation state of *Tau*. Furthermore, β-APP has been shown to serve as a G_0-coupled receptor in cell-free systems (Okamoto et al., 1995). It was shown that stimulation of β-APP by anti-APP antibody as well as by a mutation found in familial AD resulted in the activation of a specific set of MAPK in multiple

vertebrate cells (Murayama et al., 1996), thereby concluding that β-APP might act as a cell surface receptor of biological relevance that turns on specific Ser/Thr kinases, and suggested that the signaling function of β-APP is a potential target of familial AD mutations (Oakley et al., 2006).

On the mechanisms reported, β-APP, which accumulates extracellularly in AD brain, induces Ca^{2+} influx in culture via the $_{L}$-gated voltage-sensitive Ca^{2+} channel. Since this channel is normally activated by PKA-mediated phosphorylation, kinase activities recruited following β-APP treatment of cortical neurons and SH-SY-5Y neuroblastoma were examined. β-APP increased channel phosphorylation; this increase was unaffected by the PKA inhibitor, H89, but was reduced by PD-98059 (Abe and Saito, 2000; Ekinci et al., 1999; Guise et al., 2001).

Pharmacological and antisense oligonucleotide-mediated reduction of MAPK activity also reduced β-APP-induced accumulation of Ca^{2+}, reactive oxygen species (ROS), phospho-*Tau* immunoreactivity and apoptosis, indicating that MAPK mediates multiple aspects of β-APP-induced neuro-toxicity and suggesting that Ca^{2+} influx initiates neuro-degeneration in AD (Zhang and Jope, 1999). In addition, β-APP increased MAPK-mediated phosphorylation of membrane-associated proteins and reduced phosphorylation of cytosolic proteins without increasing overall MAPK activity (Ekinci et al., 1999). Increasing MAPK activity with EGF did not increase channel phosphorylation, to indicate that redirection, rather than increased activation, of MAPK activity mediates β-APP-induced neuro-toxicity (Canet-Aviles et al., 2002).

Another mechanism known involves phosphatidylinositol 3-kinase (PI3-K) (Shaw et al., 2001). Because of the physiological role of PI3-K in the translocation of glucose transporter-containing vesicles, it was speculated that PI3-K involvement in β-APP metabolism might act at the level of vesicular trafficking (Solano et al., 2000). In accord with the aforementioned observations, exposure of primary rat microglia and human THP-1 monocytes to β-APP resulted in the tyrosine kinase-dependent activation of two parallel signal transduction cascades involving members of the MAPK superfamily. β-APP stimulated the rapid, transient activation of $MAPK^{ERK1/2}$ in microglia and only $MAPK^{ERK2}$ in THP-1 monocytes (McDonald et al., 1998).

A second superfamily member, $MAPK^{p38}$, was also activated with similar kinetics. Scavenger receptor and receptor for advanced glycated end products (RAGE) ligands failed to activate $MAPK^{ERK}$ and $MAPK^{p38}$ in the absence of significant increases in protein tyrosine phosphorylation, demonstrating that scavenger receptors and RAGE are not linked to these pathways (McDonald et al., 1998; Sun et al., 2003). Importantly, $MAPK^{JNK}$ SAPKs were not significantly activated in response to β-APP. Moreover, exposure of microglia and THP-1 monocytes to β-APP resulted in the activation of RSK-1 and RSK-2 and phosphorylation of CREB at Ser-133, providing a mechanism for β-APP -induced changes in gene expression (McDonald et al., 1998).

In reinforcement of these reports, Shin et al. (1999) investigated the role of presenilin-1 (PS-1) in the secretion of α-secretase derived sAPP-α and associated intracellular signaling pathways. Human embryonic kidney (HEK) 293 cells, in this respect, were transfected with exon-9 deletion (ΔE9) mutant PS-1 cDNA in an ecdyson-inducible system. sAPP-α secretion was lower in the mutant PS-1 expressing cells compared with non-expressing cells (Shin et al., 1999). When activated by PDBu, secretion of sAPP-α and the level of phosphorylated MAPK were enhanced in ΔE9 PS-1 un-induced cells, but not in the mutant PS-1 induced cells. PD-98059, in addition, blocked PDBu induced sAPP-α secretion from ΔE9 PS-1 un-induced cells, but had no effect on the mutant PS-1 induced cells, indicating that PS-1 mediates PDBu-induced sAPP-α secretion and MAPK activation.

In contrast to the neutral role of $MAPK^{JNK}$ and $MAPK^{p38}$ in AD, using nano-electrospray mass spectrometry, an extensive phosphorylation *in vitro* by several candidate *Tau* kinases, namely, $MAPK^{JNK}$, $MAPK^{p38}$, $MAPK^{ERK2}$ and GSK-3β was observed (between 10 and 15 sites were identified for each kinase). The three MAPKs phosphorylated Ser-202 and Thr-205 but not detectably Ser-199, whereas conversely GSK-3β phosphorylated Ser-199 but not detectably Ser-202 or Thr-205 (Otth et al., 2003; Reynolds et al., 2000; Troy et al., 2001). In addition, phosphorylated Ser-404 was found with all of these

kinases except $MAPK^{JNK}$. To recapitulate, the MAP kinases may not be strictly proline specific: $MAPK^{p38}$ phosphorylated the non-proline sites Ser-185, Thr-245, Ser-305 and Ser-356, whereas $MAPK^{ERK2}$ was the strictest. Furthermore, all of the sites detected except Thr-245 and Ser-305 are known or suspected phosphorylation sites in paired helical filament-*Tau* extracted from AD brains, suggesting that the three MAPKs are importantly all strong candidates as *Tau* kinases that may be involved in the pathogenic hyper-phosphorylation of *Tau* in AD (Reynolds et al., 2000; Zhu et al., 2000).

In addition, Thr-668 within the carboxy-terminus of the AD β-APP is a known *in vivo* phosphorylation site. Phosphorylation of β-APP/Thr-668 is believed to regulate β-APP function and metabolism (Standen et al., 2001). Thr-668 precedes a proline, which suggests that it is targeted for phosphorylation by proline-directed kinase(s). The ability of four major neuronally active proline-directed kinases, cdk-5, GSK-3β, $MAPK^{ERK2}$ and $MAPK^{JNK1}$ to phosphorylate β-APP/Thr-668 indicated a robust phosphorylation of this site both *in vitro* and *in vivo* (Figure 4) (Matsuda et al., 2001; Troy et al., 2001).

Another interesting mechanism reported for the action of β-APP in AD indicated a major role for glutamate, the principal excitatory neurotransmitter in the mammalian brain. Several lines of evidence suggested that glutamatergic hypo-activity exists in the AD brain, where it may contribute to both brain amyloid burden and cognitive dysfunction (Mills and Reiner, 1999). Although metabotropic glutamate receptors (Haddad, 2005) have been shown to alter cleavage of β-APP, little attention has been paid to the role of NMDA receptors in this process. It was reported that the activation of NMDA receptors in transiently transfected human embryonic kidney 293 cells increased the production of sAPP (Mills and Reiner, 1999).

Moreover, using both pharmacological and gene transfer techniques, it was shown that this effect was largely due to activation of the MAPK, specifically $MAPK^{ERK}$. These observations further our understanding of the pathways that regulate APP cleavage and buttress the notion that regulation of β-APP is critically dependent upon MAPKs (Bi et al., 2000).

Conversely, β-APP has been reported to induce the phosphorylation of MAPKs. For instance, using acute and organotypic hippocampal slice preparations, Dineley et al. (2001) demonstrated that β-APP peptide 1-42 (β-APP42) couples to the MAPK cascade via α7 nicotinic acetylcholine receptors (nAChR). *In vivo* elevation of β-APP, such as that exhibited in animal models for AD, leads to the upregulation of α7-nAChR protein. This upregulation occurred concomitantly with the down-regulation of $MAPK^{ERK2}$ in hippocampi of aged animals (Dineley et al., 2001).

The phosphorylation state of a transcriptional mediator of long-term potentiation and a downstream target of the $MAPK^{ERK}$ cascade, CREB protein, were affected also, supporting the model that derangement of hippocampus signal transduction cascades in AD arises as a consequence of increased β-APP burden and chronic activation of $MAPK^{ERK}$ in an α7-nAChR-dependent manner that eventually leads to the down-regulation of $MAPKE^{RK2}$ and decreased phosphorylation of CREB protein. Potential pathways involving cell death-related cofactors and AD are schematized in Fig. 5.

4.5. Redox/oxidative mechanisms and MAPK-mediated Tau phosphorylation

Oxidative stress play a major role on the pathogenesis of AD (Chen et al., 2003; de la Monte et al., 2000; Floyd, 1999; Tamagno et al., 2003). For example, increased expression of heme oxygenase-1 (HO-1) is a common feature in a number of neuro-degenerative diseases, including AD (Floyd, 1999; Haddad, 2002d; Haddad, 2003; Haddad, 2006; Haddad and Land, 2000a; Haddad and Land, 2000b; Haddad et al., 2000; Takeda et al., 2000; Zhang and Jope, 1999). Interestingly, the spatial distribution of HO-1 expression in diseased brain is essentially identical to that of pathological expression of *Tau*.

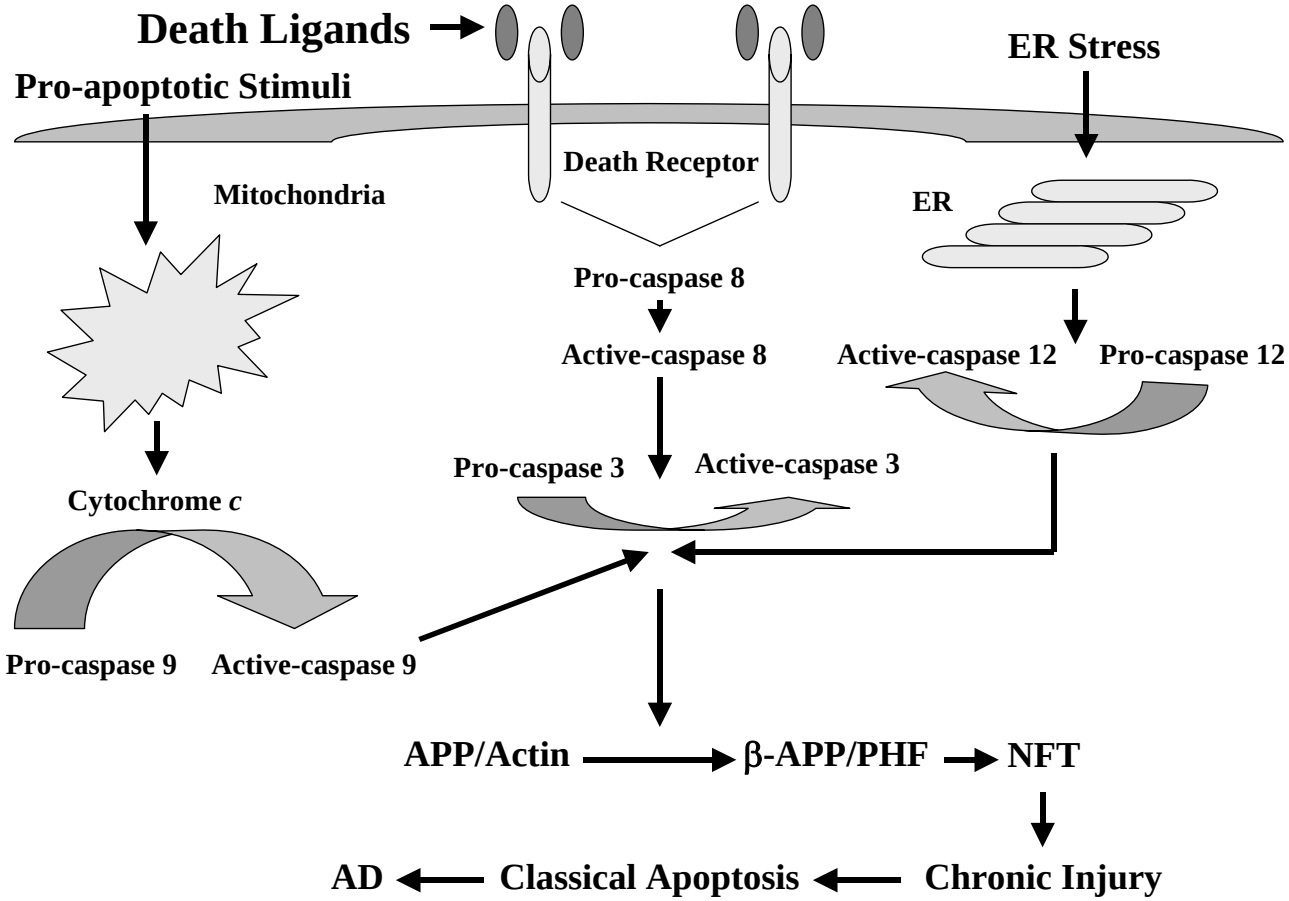

Figure 5: A diagrammatic scheme showing potential caspase/apoptosis pathways in the development of AD due to chronic injury caused by the excessive deposition of β-APP and NFT. AD, Alzheimer's; APP, Amyloid precursor protein; ER, Endoplasmic reticulum; NFT, Neurofibrillary tangle; PHF, Paired helical formation.

The relationship between HO-1 and *Tau* was explored, using neuroblastoma cells stably transfected with sense and antisense HO-1 constructs, as well as with the vector alone. In transfected cells over-expressing HO-1, the activity of heme oxygenase was increased, and conversely, the level of *Tau* protein was dramatically decreased, when compared with antisense HO-1 or CEP transfected cells (Haddad, 2002c; Haddad, 2002d; Takeda et al., 2000). The suppression of *Tau* protein expression was almost completely reversed by zinc-deuteroporphyrin, a specific inhibitor of HO activity. The activated forms of $MAPK^{ERK}$ were also decreased in cells over-expressing HO-1, although no changes in the expression of total $MAPK^{ERK1/2}$ proteins were observed (Takeda et al., 2000).

These data are in agreement with the finding that the expression of *Tau* is regulated through signal cascades including the $MAPK^{ERK}$, whose activities are modulated by oxidative stresses (Fig. 6). The expression of *Tau* and HO-1, then, may be regulated by oxidative stresses (Haddad, 2002b; Haddad, 2002c) in a coordinated manner and play a pivotal role in the cytoprotection of neuronal cells (Pei et al., 2003).

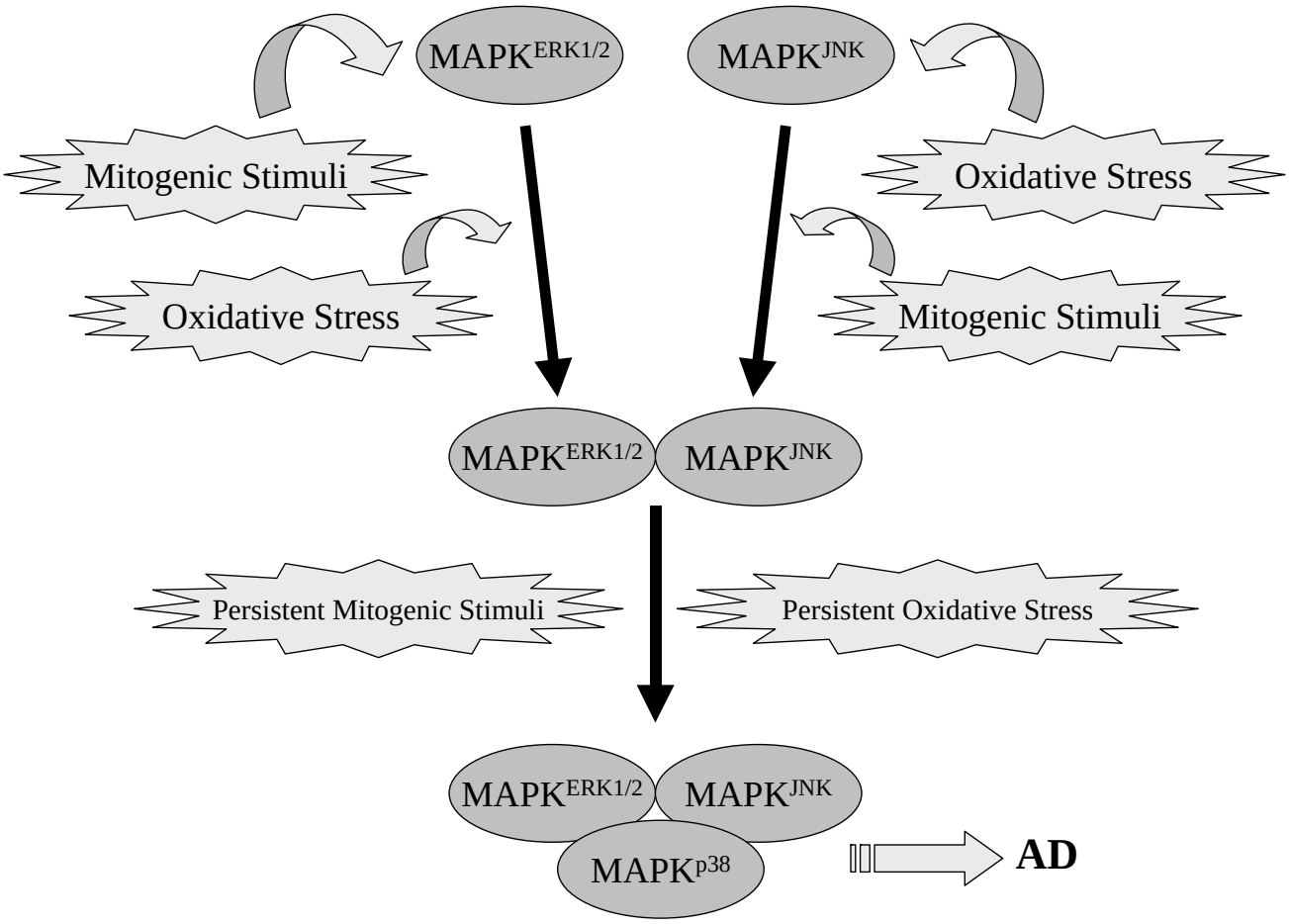

Figure 6: The role of selective MAPKs, mitogenic stimuli and oxidative stress in the development and evolution of AD.

4.6. Inflammatory mediators and MAPK-mediated Tau phosphorylation

Inflammatory mediators (Haddad, 2002d; Haddad, 2002e; Haddad and Harb, 2005a; Haddad and Harb, 2005b) have been implicated in the pathophysiology of neuro-degenerative diseases, including AD (Paris et al., 2003; Xia and Hyman, 2002). For instance, the chemokine receptor CXCR3 and its ligand, IP-10, were detected in AD brains (Xia et al., 2000). CXCR3 was detected constitutively on neurons and neuronal processes in various cortical and sub-cortical regions; IP-10 was observed in a subpopulation of astrocytes in normal brain and was markedly elevated in astrocytes in AD brains. Many IP-10^{+} astrocytes were also associated with senile plaques and had an apparently coordinated upregulation of MIP-1β (Xia et al., 2000). Moreover, it was shown that CXCR3 ligands, IP-10 and Mig, were able to activate $MAPK^{ERK1/2}$ pathway in mouse cortical neurons, suggesting a novel mechanism of neuronal-glial interaction.

Similarly, one of the CXCR2 ligands GRO-α/KC can be a potent trigger for the $MAPK^{ERK1/2}$ and PI-3 kinase pathways, as well as *Tau* hyper-phosphorylation in the mouse primary cortical neurons (Xia and Hyman, 2002). GRO-α immunoreactivity can be also detected in a subpopulation of neurons in normal and AD. Therefore, the CXCR2-ligand pair may have a potent pathophysiological role in neuro-degenerative diseases.

In concert with this, reactive microglia have been suggested to play a role in AD process, and previous

studies have shown that expression of CD45, a membrane-bound protein-tyrosine phosphatase (PTP), is elevated in microglia in AD brains (Tan et al., 2000). To investigate the possible role of CD45 in microglial responsiveness to β-APP, primary cultured microglia were co-treated with a tyrosine phosphatase inhibitor [potassium bisperoxo (1,10-phenanthroline) oxovanadate (phen), 5 micrometer] and freshly solubilized β-APP peptides (1000 nm). Data showed synergistic induction of microglial activation as evidenced by TNF-α production and nitric oxide (NO) release, both of which were dependent on the activation of $MAPK^{ERK1/2}$. Furthermore, co-treatment with phen and β-APP peptides resulted in microglia-induced neuronal cell injury (Fig. 5). Stimulation of microglial CD45 by anti-CD45 antibody markedly inhibited these effects via inhibition of $MAPK^{ERK1/2}$, suggesting that CD45 is a negative regulator of microglial activation (Tan et al., 2000). Accordingly, primary cultured microglia from CD45-deficient mice demonstrated hyper-responsiveness to β-APP, as evidenced by TNF-α release, NO production and neuronal injury after stimulation with β-APP peptides.

As a validation of these findings *in vivo*, brains from a transgenic mouse model of AD [transgenic Swedish APP-over-expressing (Tg APP(Sw)) mice] deficient for CD45 demonstrated markedly increased production of TNF-α, compared with Tg APP(Sw) mice (Chong et al. 2001; Smits et al., 2001; Tan et al., 2000; Tomidokoro et al., 2001; Del Villar and Miller, 2004). Taken together, these results suggested that therapeutic agents that stimulate the CD45/PTP signaling pathway might be effective in suppressing microglial activation associated with AD.

Moreover, a similar mechanism has been proposed for IL-1. For instance, activated $MAPK^{p38}$ and IL-1 have both been implicated in the hyper-phosphorylation of *Tau* (Sheng et al., 2001). This, together with findings showing that IL-1 activates $MAPK^{p38}$ *in vitro* and is markedly over-expressed in AD brain, suggested a role for IL-1-induced $MAPK^{p38}$ activation in the genesis of NFT in AD. Frequent co-localization of hyper-phosphorylated *Tau* protein (AT8 antibody) and activated $MAPK^{p38}$ in neurons and in dystrophic neurites in AD and frequent association of these structures with activated microglia over-expressing IL-1 were reported (Sheng et al., 2001). Tissue levels of IL-1 mRNA as well as of both phosphorylated and non-phosphorylated isoforms of *Tau* were elevated in those brains.

Furthermore, significant correlations were found between the numbers of AT8- and $MAPK^{p38}$-immunoreactive neurons, and between the numbers of activated microglia over-expressing IL-1 and the numbers of both AT8- and $MAPK^{p38}$-immunoreactive neurons. In addition, rats bearing IL-1-impregnated pellets showed a six- to seven-fold increase in the levels of $MAPK^{p38}$ mRNA, compared with rats with vehicle-only pellets (Sheng et al., 2001). These results suggested that microglial activation and IL-1 overexpression are part of a feedback cascade in which $MAPK^{p38}$ overexpression and activation leads to *Tau* hyper-phosphorylation and NFT pathology in AD (Atzori et al., 2001; Daniels et al., 2001; Ferrer et al., 2001; Williamson et al., 2002; Hoozemans et al., 2004). More recently, IL-6 was also reported to induce Alzheimer-type phosphorylation of *Tau* protein by deregulating the cdk5/p35 pathway (Quintanilla et al., 2004).

5. Summary, conclusions and future prospects

There are multiple lines of evidence showing that oxidative stress (Haddad, 2004a; Haddad, 2004b) and aberrant mitogenic signaling (Zhu et al., 2002) play an important role in the pathogenesis of AD (Churcher, 2006; Haddad and Land, 2000a; Haddad and Land, 2000b; Haddad et al., 2000; Zhu et al., 2001a; 2001b) (Fig. 6). However, the chronological relationship between these and other events associated with disease pathogenesis is not strictly elucidated. Given the crucial role that MAPK pathways play in mitogenic signaling ($MAPK^{ERK}$) and cellular stress signaling ($MAPK^{JNK/SAPK}$ and $MAPK^{p38}$), the chronological and spatial relationship between activated MAPKs during disease progression is warranted (Savage et al., 2002; Taru et al., 2002). While all three kinases are activated in the same susceptible neurons in mild and severe cases, in non-demented cases with limited pathology, both $MAPK^{ERK}$ and $MAPK^{JNK}$ are activated but $MAPK^{p38}$ is not (Ferrer et al., 2005). However, in non-demented cases lacking any sign of pathology, either $MAPK^{ERK}$ alone or $MAPK^{JNK}$ alone can be activated, indicating that MAPK pathways are differentially activated during the course of AD and, by inference, suggesting that both oxidative stress/inflammatory and abnormalities in mitotic signaling can independently serve to initiate, but both are necessary to propagate,

disease pathogenesis (Daniels et al., 2001; Koistinaho et al., 2002).

The complex molecular interactions between MAPKs and proteins (plagues) associated with the evolution of AD form a cornerstone in the knowledge of a burgeoning field of neurodegenerative, inflammatory diseases and ageing (Ho et al., 2005). MAPKs, therefore, may constitute a neurogenetic, therapeutic target for the diagnosis of a preventative medical strategy for early detection, and possible treatment, of Alzheimer's.

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Abbreviations

Alzheimer's disease, AD; β-Amyloid precursor protein, β-APP; Calmodulin, CaM; Calyculin, CAL; cAMP-response element binding protein, CREB; Cyclin-dependent kinase, cdk; Early-onset familial AD, EOFAD; Epidermal growth factor, EGF; EGF receptor threonine kinase, ERT kinase; Extracellular receptor kinase, ERK; Fluorescence resonance energy transfer, FRET; Glutathione S-transferase P*i*, GSTp; Glycogen synthase kinase, GSK; GTPase activating protein, GAP; Guanine nuclear exchange factor, GEF; Heme oxygenase, HO; Human embryonic kidney, HEK; Interleukin, IL; Jun N-terminal kinase, JNK; JNK-interacting protein, JIP; *N*-Methyl-$_D$-aspartate, NMDA; Microtubule associated protein, MAP; MAP kinase phosphatase, MKP; Mitogen-activated protein kinase, MAPK; MAP kinase kinase kinase, MAPKKK; MAP kinase kinase, MAPKK; Mixed lineage kinase, MLK; Myelin basic protein, MBP; Nerve growth factor, NGF; Neurofibrillary tangles, NFT; Nicotinic acetylcholine receptors, nAChR; Nitric oxide, NO; Okadaic acid, OKA; Paired helical filament PHF; Phorbol 12-myristate 13-acetate, PMA; Phosphatidylinositol 3 kinase, PI3-K; Platelet-derived growth factor, PDGF; Presenilin, PS; Protein kinase A, PKA; Protein kinase C, PKC; Protein phosphatase, PP; Protein tyrosine kinase, PTK; Protein-tyrosine phosphatase, PTP; Reactive oxygen species, ROS; Receptor for advanced glycated end products, RAGE; Receptor tyrosine kinase, RTK; Ribosomal S6 protein kinase, RSK; Serum response factor, SRF; Stress-activated protein kinase, SAPK; *Tau* protein kinase, TPK; Transforming growth factor, TGF; Tumor necrosis factor, TNF.

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