10

The Role of Glycogen Synthase Kinase-3 (GSK-3) in Alzheimer's Disease

Miguel Medina[1] and Jesús Avila[2]
[1]*Noscira S.A., Tres Cantos*
[2]*Centro de Biología Molecular "Severo Ochoa" CSIC-UAM, Cantoblanco*
Spain

1. Introduction

Despite its initial discovery as one of five protein kinases activities found to phosphorylate glycogen synthase (GS) in fractioned extracts of rabbit skeletal muscle (Embi et al., 1980; Hemmings et al., 1981), Glycogen Synthase Kinase 3 (GSK-3) is by no means restricted to a role in glycogen metabolism. Indeed, the enzyme targets a wide variety of proteins involved in signalling, metabolism, structural proteins and a remarkable number of transcription factors and plays a far more pleiotropic role than first imagined (Woodgett, 2006). Genetic analyses and the use of selective inhibitors have shown that GSK-3 plays critical roles in development, metabolic homeostasis, neuronal growth and differentiation (Hur & Zhon, 2010), cell polarity, cell fate and apoptosis. Its unique position in modulating the function of a diverse series of proteins in combination with its association with a wide variety of human disorders, from neurodegenerative diseases, stroke, bipolar disorder to diabetes and cancer, has attracted significant attention to the protein both as a therapeutic target and as a means to understand the molecular basis of these disorders.

In particular, the involvement of GSK-3 in several key pathophyisiological pathways leading to Alzheimer's disease (AD) and neurodegenerative diseases has placed this enzyme in a central position in this disorder. Thus, GSK-3 has recently been proposed as a link between the two major pathological pathways in AD, amyloid and tau (Hernández et al., 2010; Muyllaert et al., 2008) and even a "GSK-3 hypothesis of AD", suggesting that GSK-3 might be a casual mediator of the disease, has been put forward (Hooper et al., 2008). This review will focus on describing the key role that GSK-3 plays in AD pathobiology and the use of GSK-3 inhibition as a potential therapeutic approach to treat this disease.

2. GSK-3 structure and regulation

GSK-3 is a highly conserved protein kinase belonging to the CMGC family of serine/threonine protein kinases, as genes encoding the enzyme have been identified in every eukaryotic genome that has been investigated, such as *Dictyostelium discoideum* (Kim et al., 1999), *Xenopus laevis* (Itoh et al., 1995), *Drosophila melanogaster* (Ruel et al., 1993) or parasites such like *Plasmodium falciparum*, *Trypanosoma brucei* or *Leishmania donovani* (Osolodkin et al., 2011). Mammalian GSK-3 is encoded by two genes, *gsk-3a* and *gsk-3β*

(Frame & Cohen, 2001; Grimes & Jope, 2001), that encode proteins of 51 and 47 kDa, respectively and which display 84% overall identity (98% within their catalytic domains), with the main difference being an extra Gly-rich stretch in the N-terminal domain of GSK-3α (Woodgett, 1990). Mammalian GSK-3α and β are each widely expressed although some tissues show preferential levels of some of the two proteins. Furthermore, an alternatively splicing event between exons 8 and 9 of GSK-3β gives rise in neurons to a splice variant (GSK-3β2) containing a 13 amino acids insertion within the kinase domain near to the substrate binding pocket (Mukai et al., 2002). How this insertion affects kinase activity or regulation remains unclear, although some differences between GSK-3β1 and GSK-3β2 isoforms have already been described (see below).

Crystallographic studies have revealed the three-dimensional structure of GSK-3β (Dajani et al., 2001; ter Haar et al., 2001), having the overall shape common to most kinases, with a small N-terminal lobe mostly consisting of β-sheets and a large C-terminal lobe essentially formed of α-helices (Noble et al., 2005). The ATP binding pocket is located between the two lobes and it is well conserved among kinases (Bain et al., 2007). Very recently, a comparison of the human and parasite GSK-3 ATP binding sites has opened the possibility of developing selective drugs specifically affecting parasite GSK-3 (Osolodkin et al., 2011).

Some GSK-3 substrates do not require a very specific sequence but rather a previous (*primed)* phosphorylation by a *priming* kinase on a Ser or Thr residue located four amino acids C-terminal to the Ser or Thr residue to be modified by GSK-3 (see below for regulation through primed phosphorylation). The crystal structure of human GSK-3β has also provided a model for the binding of pre-phosphorylated substrates to the kinase. According to it, primed Ser/Thr is recognized by a positively charged binding pocket formed by residues Arg96, Arg180 and Lys205 that facilitates the binding of the phosphate group of primed substrates. GSK-3β uses the phosphorylated serine or threonine at position +4 of the substrate to align the two domains for optimal catalytic activity (Dajani et al., 2001; ter Haar et al., 2001).

Furthermore, crystal structures of GSK-3β complexes with interacting proteins FRAT/GBP and axin have allowed defining the molecular basis for those interactions, which play critical roles in some signalling pathways (see below for regulation through protein complex formation). These studies confirm the partial overlap of the binding sites of axin and FRAT1/GBP predicted from genetic and biochemical studies (Ferkey & Kimelman, 2002; Fraser et al., 2002) but reveal significant differences in the detailed interactions, and identify key residues mediating the differential interaction with both proteins. This ability of GSK-3β to bind two different proteins with high specificity *via* the same binding site is mediated by the conformational plasticity of the 285-299 loop, while some residues in this versatile binding site are involved in interactions with both axin and FRAT, others are involved uniquely with one or the other (Dajani et al., 2003).

GSK-3 is ubiquitously expressed and, unlike most kinases, has a relatively high activity in resting, unstimulated cells while it is normally reduced in response to a variety of extracellular stimuli (Frame & Cohen, 2001). In mammals, GSK-3α and β are each expressed widely at both the RNA and protein levels, although some tissues show preferential levels of some of the two proteins as for instance, both isoforms are highly expressed in neural tissue. Neither gene appears to be acutely regulated at the transcriptional level whereas the proteins are controlled post-translationally, largely through protein-protein interactions or by post-translational regulation.

Given that chemical inhibitors of GSK-3 are unable to discriminate between the various GSK-3 isoforms, evaluation of isoform-specific functions it is not possible by using these compounds. However, evidence for isoform-specific roles has now emerged from mouse models (see below). For instance, some recent findings suggest that there are tissue- and isoform-specific roles in regulation of glucose metabolism (Patel et al., 2008; Mol Cell Biol), as GSK-3α seems to be the predominant regulator of GS and glycogen synthesis in liver whereas GSK-3β has a more prevalent role within skeletal muscle and pancreas. Also, although the effect of the inserted sequence on kinase activity, substrate specificity or requirement for priming of substrates remains unclear, the neuron-specific alternatively spliced GSK-3β2 isoform appears to phosphorylate unprimed residues on tau and MAP1B to a lesser extent than GSK-3β1 (Mukai et al. 2002, Wood-Kaczmar *et al.* 2009).

Three decades after its discovery as a protein kinase involved in glycogen metabolism, GSK-3 has revealed as a key enzyme in regulating many critical cellular processes, providing a link between many different substrates and various signalling pathways as well as gene expression. Modulation of its activity has also turned out to be much more complex than originally thought. As already mentioned above, one of the main characteristics of GSK-3 is that its activity is high in resting, unstimulated cells while regulated by extracellular signals that typically induce a rapid and reversible decrease in enzymatic activity. Control of GSK-3 activity occurs by complex mechanisms that are each dependent upon specific signalling pathways (for a recent review see Medina & Wandosell, 2011).

Early on, GSK-3 was proved to be a dual specificity kinase differentially regulated by tyrosine and serine/threonine phosphorylation (Wang et al., 1994). The first regulatory mechanism described of GSK-3 activity involved the phosphorylation of specific residues of GSK-3 by other kinases; and more recently through auto-phosphorylation (Frame & Cohen, 2001; Harwood, 2001). Thus, four different regions and residues have been described in the GSK-3 molecule. First, it has been clearly established that phosphorylation of serine residue at positions 21 in GSK-3α and 9 in GSK-3β, correlates with the inhibition of its kinase activity (Frame et al., 2001; Stambolic et al., 1994; Sutherland et al., 1993). Many protein kinases are capable of phosphorylating GSK-3 at this residue, such as Akt, ILK, PKA, p90Rsk (Delcommenne et al., 1998; Fang et al., 2000), and many physiological situations of inhibition of GSK-3 correlate with serine phosphorylation, such as Insulin/IGF1, NGF, or Estradiol treatments, not only in neurons but also in other cell types (Cardona-Gomez et al., 2004). In addition, phosphorylation at threonine 43, present only in the isoform GSK-3β, by ERK also correlates with GSK-3 inhibition (Ding et al., 2005), whereas residues serine 389 and threonine 390 present in GSK-3β have been shown to be phosphorylated by p38 MAPK (Thornton et al., 2008), increasing the capacity of Ser-9 to be phosphorylated rather than promoting a direct inhibition.

In contrast, tyrosine phosphorylation present in positions 279 in GSK-3α or 216 in GSK-3β, correlates with an increase of its kinase activity (Hughes et al., 1993). Different candidate kinases such as Pyk-2 and Fyn have been reported to be able to phosphorylate GSK-3 on this residue *in vitro*, as MEK1/2 has been shown to do it in mammalian fibroblasts (Hartigan et al., 2001; Lesort et al., 1999) or ZAK1 in *Dictyostelium discoideum* (Kim et al., 1999; 2002), although no homologue of this latter kinase has been found in mammals. More recently, it has been suggested that phosphotyrosine in GSK-3 in mammalian systems might arise from a chaperone-dependent intra-molecular autophosphorylation event perhaps regulated by Hsp90 (Cole et al., 2004 Biochem J; Lockhead et al., 2006; Wang et al., 1994). Molecular

dynamics and crystallographic studies clearly suggest that pTyr216 renders the kinase active through interactions with Arg220 and Arg223, stabilizing the activation loop and allowing full substrate accessibility (Buch et al., 2010; Cohen & Goedert, 2004). Very recently, it has been also shown that the extent of phosphorylation at both Ser9 and Tyr216 residues is very similar in both GSK-3β splice variants, β1 and β2 (Soutar et al., 2010).

On the other hand, tyrosine phosphorylation of residue 216 or 279 increased in neuronal cells following exposure to LPA (Sayas et al., 1999) and also upon exposure of neurons to β-amyloid or PrP (Muñoz-Montaño et al., 1997; Perez et al., 2003; Takashima et al., 1998) in a clear correlation with an increase on GSK-3 activity. In addition, in many neuronal cells the pharmacological inhibition of tyrosine phosphatases with *ortho*-vanadate increases the basal level of GSK-3-pTyr (Simon et al., 2008). Thus, taken all together, in addition to the reported tyrosine 216/279 autophosphorylation mechanism proposed, some as-yet-unidentified tyrosine kinases and/or phosphatases may also regulate GSK-3 activity by phosphorylation of this particular residue.

Another mechanism of GSK-3 regulation through post-translational modification involves the removal by calpain of a fragment from the N-terminal region of GSK-3, including the regulatory serines 9/21. After removal of that fragment GSK-3 becomes activated (Goñi-Oliver et al., 2007). The same study showed that both isoforms α and β are cleaved by calpain, although with different susceptibility. Moreover, GSK-3 truncation has been observed in human and mouse post-mortem brain tissue (Goñi-Oliver et al., 2009a). It is noteworthy to consider that a similar mechanism has been described for β-catenin in hippocampal neurons, where after NMDA-receptor-dependent activation; calpain induces the cleavage of β-catenin at the N-terminus, generating stable and truncated forms which maintain its transcriptional capacity (Abe & Takeichi, 2007). Likewise, GSK-3 truncation is mediated by extracellular calcium and can be inhibited by memantine (Goñi-Oliver et al., 2009b), a NMDA antagonist used for the treatment of Alzheimer's disease. Interestingly, GSK-3β has also been recently shown to be cleaved at the N-terminus (and subsequently activated) by matrix metallo-proteinase 2 (MMP-2) in cardiomyoblasts (Kanadasamy & Schulz, 2009).

Besides post-translational modifications, GSK-3 activity can also be regulated by protein complex association, for instance through its interaction with structural (scaffold) proteins. It is well known that GSK-3 contributes to Wnt signalling by participating in a multiprotein complex formed by axin, β-catenin and adenomatous polyposis coli (APC), among others (for review see, i.e. Moon et al., 2004). Indeed, in the absence of the Wnt ligand, GSK-3 it is able to phosphorylate β-catenin and targeting it for proteasome degradation (Aberle et al., 1997) whereas in its presence GSK-3 is unable to do that, increasing β-catenin cytosolic levels and eventually mediating TCF/LEF-mediated transcription at the nucleus. Recent data suggest that this complex may be specific for the GSK-3β2 splice isoform (Castaño et al., 2010). Recent evidence also supports a neuroprotective role for *Wnt* signaling in neurodegenerative disorders such as AD (Inestrosa & Toledo, 2008).

Moreover, another GSK-3-binding protein (GBP or FRAT) has been reported to regulate GSK-3 enzymatic activity (Itoh et al., 1995; Li et al., 1999). From the three different FRATs that have been cloned and characterized, FRAT1 appears to act as an inhibitory system (Yost et al., 1998) whereas FRAT2 appears to preferentially increase GSK-3-mediated phosphorylation in some residues (Stoothoff et al., 2005). Surprisingly, the triple FRAT-knockout mouse lacks any major defect in brain development (van Amerongen et al., 2005),

which underlines the need to better define the precise role of FRAT in GSK-3 regulation and brain physiology. Furthermore, using the binding site on GSK-3 for FRAT/GBP, a GSK-3-interacting protein (GSKIP) has been identified that can block phosphorylation of different substrates and functions as a negative regulator of GSK-3β (Chou et al., 2006). Other proteins have also been proposed to further contribute to GSK-3 regulation through physical interaction with it. Thus, DISC-1 (Disrupted In Schizophrenia-1) regulates neural progenitor proliferation via the β-catenin/GSK-3β pathway, whereby DISC-1 stabilizes β-catenin by inhibiting GSK-3β activity through a direct binding (Mao et al., 2009). Finally, the dimeric scaffold protein 14-3-3 has been shown to co-elute from brain microtubules together with tau and GSK-3β and this interaction has been proposed to facilitate the interaction of the kinase with some of its substrates (Agarwal-Mawal et al., 2003).

As already mentioned, an unusual property of GSK3 is that most of its substrates require prior phosphorylation (priming) at a residue 4 or 5 amino acids C-terminal to the target residue (Frame & Cohen 2001), thus providing another mechanism of regulation of the GSK-3 activity. Some priming kinases have been identified, such as cdk5 (Alonso et al., 2006; Noble et al., 2003; Sengupta et al., 1997), PAR-1 (Nishimura et al., 2004), casein kinase I (Amit et al. 2002), PKC (Liu et al., 2003) or PKA (Sengupta et al., 1997). That said, there are examples of unprimed substrates reported, although is not entirely clear as yet whether this second set of unprimed substrates may define a different group of functions (Twomey & McCArthy, 2006).

3. GSK-3 activity in AD brain

Compared to age-matched control samples, increased levels of GSK-3 have been found in post-mortem analysis of brains from AD patients (Pei et al., 1997) while a spatial and temporal pattern of increased active GSK-3β expression correlating with the progression of neurofibrillary tangles (NFT) and neurodegeneration has also been shown (Leroy et al., 2002). Thus, GSK-3 has been shown to localize to pre-tangle neurons, dystrophic neurites and NFTs in AD brain (Pei et al., 1997). Neurons actively undergoing granulovacuolar degeneration are also immunopositive for active GSK3β (Leroy et al., 2002). Taken all together, although direct evidence might be lacking, all these studies strongly suggest that GSK-3β activity is increased in the brains of patients suffering from AD.

GSK-3β is the major kinase to phosphorylate tau protein both *in vitro* and *in vivo*. Furthermore, GSK-3β has been proposed as the link between the two major histopathological hallmarks of AD, the extracellular amyloid plaques and the intracellular NFT (Hernández et al., 2010; Ittner and Götz, 2011; Muyllaert et al., 2008). Exposure of primary neuronal cultures to Aβ induces activation of GSK-3β, tau phosphorylation and cell death, whereas blockade of GSK-3 expression by antisense oligonucleotides or its activity by lithium inhibits Aβ-induced toxicity (Alvarez et al., 1999; Hoshi et al., 2003; Wei et al., 2000).

GSK-3β-deficient mice die during embryonic development (Hoeflich *et al.* 2000, Liu *et al.* 2007) whereas GSK-3β heterozygous (+/-) mice are viable, although they show some neurological abnormalities, including reduced aggression, increased anxiety, reduced exploratory activity, poor memory consolidation and reduced responsiveness to amphetamine (O'Brien *et al.* 2004, Kimura *et al.* 2008, Beaulieu *et al.* 2008). Conversely, transgenic mice over-expressing GSK-3β result in behavioural changes that appear to recapitulate hyperactivity observed in the manic phase of bipolar disorder (Prickaerts *et al.* 2006).

On the other hand, mice lacking GSK-3α are viable and develop normally (MacAulay *et al.* 2007), but display enhanced glucose tolerance and insulin sensitivity accompanied by reduced fat mass. Interestingly, GSK-3α knock-out mice show reduced exploratory activity and aggression, similar to the GSK-3β heterozygotes, but in addition have decreased locomotion, impaired co-ordination and a deficit in fear conditioning (Kaidanovich-Beilin *et al.* 2009). These different in phenotypes in mice lacking one isoform or the other suggest non-redundant functions of the GSK-3 genes in the brain, while the overlapping behavioural problems between GSK-3α knockout (KO) and GSK-3β heterozygous (+/-) mice suggest some common substrates.

Furthermore, loss of both GSK-3 isoforms specifically in the brain results in increased self-renewal of neuronal progenitor cells, but reduced neurogenesis (Kim *et al.* 2009), while double GSK3α/β knock-in mice in which endogenous isoforms are replaced by mutant proteins where Ser21/9 have been mutated to Ala21/9 respectively, thus preventing repression by growth factor signalling, exhibit impaired neuronal precursor cell proliferation (Eom & Jope 2009). These data underscore the critical role that proper regulation of expression and activity of GSK-3 play in the maturation of these cells during mammalian brain development.

However, we must be careful when interpreting data from transgenic or KO animals since some strain specificity has been recently observed, at least in the case of improved insulin sensitivity and hepatic glucose homeostasis phenotype observed upon global inactivation of GSK-3α (Patel et al., 2011).

All these observations and the ones described below strongly suggest a central role of GSK-3 in AD pathogenesis and have also led to several efforts trying to identify sequence variations in the *gsk-3* gene and its promoter. Despite early reports of a lack of genetic association between the *gsk-3* coding sequence or its promoter with AD (Russ et al., 2001), several groups have now reported this association. Thus, a polymorphism in the promoter region (-50) of the *gsk-3β* gene appears associated with a two-fold increased risk for sporadic AD when analysing 333 sporadic AD patients and 307 control subjects from Spain (Mateo et al., 2006). More recently, a case-control study has found a rare intronic polymorphism in *gsk-3β* that occurred twice more frequently in AD patients than in aged healthy controls (Schaffer et al., 2010), strongly supporting the notion of a genetic association of the *gsk-3β* gene with AD. Furthermore, two additional independent studies have reported synergistic effects (epistasis) between the *gsk-3β* and either the *MAPT* (tau) genes (Kwok et al., 2008) or the p35 subunit of cdk5 (Mateo et al., 2009) in late-onset AD, further supporting a genetic association between *gsk-3β* and AD. Interestingly, a genetic polymorphism that increases the ratio of GSK-3β1 to GSK-3β2 interacts with tau haplotypes and modifies risk in Parkinson's and Alzheimer's disease (Kwok et al. 2005, 2008).

4. The role of GSK-3 in tau phosphorylation

Tau protein is a microtubule-associated protein (MAP) that in normal physiological conditions binds to microtubules (MT), regulating their assembly, dynamic behaviour, and spatial organization (Drechsel et al., 1992; LoPresti et al., 1995). Later on, tau has also been shown to regulate the axonal transport of organelles, including mitochondria (Ebneth et al., 1998). Tau is primarily, though not exclusively, a neuronal protein encoded by a single gene but with six major isoforms derived by alternative splicing (Goedert et al., 1989; Himmler et

al., 1989). The interaction between tau and tubulin is mediated by four imperfect repeat domains (31-32 residues) encoded by exons 9-12 (Lee et al., 1989). Alternative splicing of exon 10 gives rise to isoforms with 3 or 4 binding domains (3R and 4R tau) (Goedert et al., 1989). Adult human brain shows a 1:1 ratio of 3R and 4R isoforms whereas foetal brain, however, only expresses 3R tau, demonstrating developmental regulation of exon 10 splicing. Different brain regions also differ in the relative levels of 3R and 4R isoforms with granule cells in the hippocampal formation reported to have only 3R tau (Goedert et al., 1989). Disturbances in this ratio are a common feature in most neurodegenerative tauopathies, including AD.

Within neurons, tau is predominantly found in axons as a highly soluble phosphoprotein. As mentioned in the case of alternative splicing, phosphorylation is also developmentally regulated, with a high tau phosphorylation level during embryogenesis and early development, when only the shortest of the isoforms is being expressed. By contrast, adult brain expresses all six isoforms with relatively reduced phosphorylation levels compared with the foetal one (see [Hanger et al., 2009] for a review).

Upon abnormal phosphorylation, the microtubule-associated protein tau reduces its affinity for and dissociates from microtubules. In AD brains tau accumulates in the neuronal perikarya and processes as paired helical filaments (PHF). It has been suggested that at the single-cell level the defects start with a modification of tau by phosphorylation, resulting in a destabilization of microtubules giving rise to a "pre-tangle" stage. After this stage, the destabilization of microtubules leads to loss of dendritic microtubules and synapses, plasma membrane degeneration, and eventually cell death (Iqbal et al., 2009).

The knowledge accumulated in the last years strongly suggest that tau-induced neurodegeneration is most likely a consequence of a combination of loss of (tau) function as well as gain of (toxic) function. On one hand, tau detachment from microtubules after hyperphosphorylation (or mutations) causes impaired microtubule function and axonal transport and eventually synaptic dysfunction and neurodegeneration (Jaworski et al., 2010). On the other hand, hyperphosphorylated tau molecules tend to self-assemble into filaments such as PHF or straight filaments (SF) that form the NFT. But hyperphosphorylated tau seems to also have the capacity of sequestering normal tau molecules (and perhaps other microtubule-associated proteins) into the aggregates, which will also have a negative impact on the normal microtubule function. At some point after detaching from microtubules and getting into the aggregation process, tau molecules also suffer other post-translational modifications such as truncation (Delobel et al., 2008; Gamblin et al., 2003; Nvak et al., 1993), glycosylation (Wang et al., 1996), O-GlcNAcylation (Arnold et al., 1996; Hart et al., 1996), and ubiquitination (Bancher et al., 1991; Mori et al., 1987), which could also contribute to the pathology. For a recent review on the molecular mechanisms by which tau induces neurodegeneration please refer to (Brunden et al., 2009; Iqbal et al., 2009).

Interestingly, recent data strongly indicates that some soluble, oligomeric (pre-filament, immature filaments) tau species, rather than the tangles, are indeed the pathogenic ones (Bretteville & Planel, 2008, Congdon & Duff, 2008), reminiscent of what has happened in recent years in the amyloid field regarding plaques and intermediate Aβ oligomers (Haass & Selkoe, 2007; Walsh & Selkoe, 2007). For a very long time, tangles or fibrils have been considered to be the pathological species, but it has become clear now that, much like amyloid plaques, NFT are the final stages of a pathological process, but the real damage might actually be done by some

intermediate hyperphosphorylated, most likely soluble tau species (Brunden et al., 2009; Iqbal et al., 2009; Jaworski et al., 2010). In fact, there is some evidence suggesting that NFT might be protective indeed, as tangle-bearing neurons seem to survive for long periods of time (Andorfer et al., 2005; de Calignon et al., 2009; Morsch et al., 1999). More recently, some novel mechanisms of propagation of tau protein misfolding from the extracellular to the intracellular space, both *in vitro* (Frost et al., 2009) and *in vivo* (Clavaguera et al., 2010) have been described. The demonstration of a link between tau oligomers and brain pathology in animal models has lately sparked the interest of tau immunotherapies (Boutajangout et al., 2010; Kayed & Jackson, 2009; Medina, 2011; Sigurdsson, 2008).

GSK-3 induces tau phosporylation in several primed and unprimed PHF phosphoepitopes, both *in vitro* and in cell cultures. Activation of the insulin or Wnt signalling pathways increase tau phosphorylation mediated by GSK-3 (Caricasole et al., 2004; Lesort et al., 1999). Furthermore, some genetic studies show an association of Wnt signalling with AD through the low-density lipoprotein receptor-related protein 6 (LRP6), a co-receptor for Wnt signalling, which has been identified as a genetic risk for a subpopulation of late onset AD (De Ferrari et al., 2007). In addition, epidemiological and genetic studies also associate diabetes and insulin resistance with AD (Biessels & Kappelle, 2005; Hamilton et al., 2007; Reiman et al., 2007).

Persistent tau phosphorylation might results in neuritic dystrophy. Lipophosphatidic acid treated neurons result in GSK-3-dependent persistent tau phosphorylation followed by neurite retraction and growth cone collapse (Sayas et al., 2002). Several animal models, which exhibit persistent tau phosphorylation, also display neuritic dystrophy. For instance mice lacking either Reelin, mammalian disabled (mDab1), or VLDLR2 and ApoER2 exhibit persistent tau phosphorylation and have neuritic dystrophy and cytoskeletal abnormalities associated with them (Hiesberger et al., 1999; Sheldon et al., 1997). It is conceivable that persistent phosphorylation by GSK-3 results in neuritic dystrophy and subsequent cytoskeletal breakdown. In *Drosophila,* tau overexpression in combination with phosphorylation by the *Drosophila* GSK-3 homolog *Shaggy*, exacerbated neurodegeneration induced by tau overexpression alone, leading to neurofibrillary pathology (Jackson et al., 2002).

Recent evidence points out to GSK-3 linking tau and neuronal polarity through a protein called CRMP-2 (collapsing response mediator protein-2) which is essential for regulating axon growth and promotes assembly of microtubules (Cole et al., 2004). GSK-3 not only phosphorylates tau but also several CRMPs (Cole et al., 2006), including CRMP-2 (Yoshimura et al., 2005) at Thr514, a residue crucial for controlling its activity. Low levels of phosphorylated CRMP-2 at that residue are present in the growth cone and are associated with axon growth, which is consistent with previous data demonstrating that inhibition of GSK-3 results in enhanced neurite outgrowth (Muñoz-Montaño et al., 1999). These data were substantially backed up by a different group (Jiang et al., 2005) that also found that GSK-3 is spatially regulated, with the ratio of inactive (phosphorylated at S9) versus active (unphosphorylated) being highest in the axon tips, consistent with the fact that higher levels of unphosphorylated CRMP-2 drive axon development, and hence, neural polarity.

5. The role of GSK-3 in Aβ formation and neurotoxicity

While not universally accepted, the so-called amyloid hypothesis of AD has provided the main conceptual framework for studying the causes of the diseases and developing new therapeutic interventions during the last quarter of century. According to it, the gradual

cerebral accumulation of soluble and insoluble assemblies of the amyloid Aβ peptide triggers a cascade of biochemical and cellular alterations that produce the clinical phenotype of AD (Hardy & Higgins, 1992; Hardy & Selkoe, 2002; Selkoe, 1991). The reasons for elevated Aβ levels in most patients with sporadic, late-onset AD are unknown, but recent evidence suggest that these could turn out to include increased neuronal release of Aβ during some kind of synaptic activity (Selkoe, 2002; 2008).

GSK-3 inhibition *per se* decreases Aβ production in cells and in an animal model of amyloidosis, as shown using non-isoform selective pharmacological inhibitors such as lithium, kenpaullone as well as small interfering RNA against the α isoform of GSK-3 (Phiel et al., 2003; Su et al., 2004; Sun et al., 2002). The exact mechanism by which this occurs remains unclear and in fact the isoform specificity of the effect on Aβ production is still highly controversial. However, the observation that amyloid precursor protein (APP) C-terminal fragments accumulate in the presence of these inhibitors suggests that GSK-3 may influence γ-secretase activity. γ-secretase activity is a multiprotein complex that is necessary for the terminal cleavage of APP to generate the Aβ fragment. Interestingly, inhibition of GSK-3 failed to demonstrate accumulation of C-terminal fragments of the Notch protein, which is also a substrate for γ-secretase (Phiel et al., 2003). Actually, GSK-3 has been shown to bind and phosphorylate presenilin 1 (PS1), the catalytic component of the γ-secretase complex, acting perhaps as a docking protein and regulating phosphorylation of some GSK-3 substrates such as tau and β-catenin (Palacino et al., 2001; Su et al., 2004; Takashima et al., 1998; Tesco & Tanzi, 2002; Twomey & McCarthy, 2006). PS1 has been shown to inactivate GSK-3 through PI3K/Akt signalling, preventing tau phosphorylation and apoptosis. Interestingly, PS1 FAD mutations inhibit PS1-dependent PI3K/Akt signalling, facilitating GSK-3 and thus tau phosphorylation (Baki et al., 2004). Furthermore, APP has also been shown to be a substrate for GSK-3 *in vitro* (Aplin et al., 1996) and *in vivo* (Rockenstein et al., 2007), suggesting a role of GSK-3 in APP transport and maturation (da Cruz e Silva & da Cruz e Silva, 2003; Lee et al., 2003) from the early secretory pathway through the axon terminals, perhaps controlling APP processing. Finally, modulation of the GSK-3 signalling pathway by chronic lithium treatment of transgenic animals has been shown to have neuroprotective effects by regulating APP maturation and processing (Rockenstein et al., 2007).

A substantial body of evidence has established the toxic properties of extracellular Aβ peptides on neuronal cells (Selkoe, 2008). Non-neuronal cells however are generally resistant to Aβ treatment, with some exceptions such as endothelial cells and smooth muscle cells (Suhara et al., 2003). On the other hand, oligomers of the Aβ peptide have been reported to act as antagonists for insulin (Towsend et al. 2007) or Wnt (Magdesian et al. 2008) receptors, resulting in an increase in GSK-3 activity. Also, a PS1 lack of function by mutations such as those present in some familial AD patients has been suggested to result in an increase of GSK-3 activity (Baki et al., 2004).

As mentioned, the aggregation of Aβ peptide into soluble oligomers is considered an early event in Alzheimer's disease and the presence of these aggregates seems to lead to neurodegeneration in the context of this disease. However, the mechanisms underlying Aβ-induced neurotoxicity are not completely understood. Although previous studies in mice have suggested that GSK-3 alters Aβ levels via modulation of APP processing (Phiel et al., 2003; Rockenstein et al., 2007), the direct effects of the enzyme on Aβ toxicity, and in the adult nervous system, have not been examined in depth. A recent study has tackled this

particular issue of the specific role of GSK-3 in regulating Aβ42 toxicity in adult neurons *in vivo*, by modulating its activity in an adult-onset *Drosophila* model of Alzheimer's disease (Sofola et al., 2010). This study shows that GSK-3 inhibition ameliorates Aβ42 toxicity in adult flies, and also highlights a novel mechanism of protection by which GSK-3 directly regulates Aβ42 levels in the absence of any effects on APP processing.

6. The role of GSK-3 in synaptic plasticity, learning and memory

GSK-3 has also been shown to phosphorylate and inhibit kinesin-mediated motility. Fast axonal transport misregulation has been hypothesized to play a role in Alzheimer's disease pathogenesis (Morfini et al., 2002). Fibrillar Aβ binds to and induces the clustering of the integrin receptors, leading to the activation of paxillin and focal adhesion kinases. Interestingly, active GSK-3 associates with focal adhesion proteins suggesting the possibility that GSK-3 might mediate neuritic dystrophy via these interactions (Grace & Busciglio, 2003). Abnormal increase in GSK-3 activity has been shown to cause neurodegeneration and interfere with synaptic plasticity (for review see Bhat & Budd, 2002; Bhat & Froelich-Fabre, 2004).

Another important aspect of GSK-3 function is its role in the assembly and disassembly of synapses determining synaptic plasticity. Regarding memory, some states of synaptic plasticity may be considered as a balance between long-term potentiation (LTP) and long-term depression (LTD), with the former strengthening synaptic connections and the latter weakening them. Interestingly, GSK-3 appears to be a key factor in swaying that balance (Hooper et al., 2007; Peineau et al., 2007) since after LTP induction, GSK-3 becomes temporarily inactivated, support for LTD is lost and LTP comes out on top. This is relevant from the drug discovery point of view, since it implies that inhibition of GSK-3 might boost LTP and depress LTD, in principle a good thing for learning and memory. The precise molecular mechanism by which GSK-3 influences these processes remains to be elucidated, although some preliminary data seems to suggest that installation or maintenance of AMPA receptors might play a role (Peinau et al., 2007). Several GSK-3 downstream substrates such as CRMP-2 or the cAMP responsive element-binding protein (CREB) are also involved in synaptic remodelling, a key process required for memory formation. All this evidence has led to propose that GSK-3 acts as a gate through which LTP and memory are established (Hooper et al., 2008) and that memory failure in AD may be due to the inhibition of LTP by GSK-3 overactivity, with neuronal loss ensuing during disease progression.

It is well established that Aβ oligomers inhibit LTP and enhance LTD (Shieh et al., 2003; Walsh et al., 2002; Selkoe, 2008), although the precise mechanisms by which Aβ interferes with long-term plasticity have remained largely unknown. Very recently, GSK-3 has revealed as a key enzyme in mediating Aβ-induced LTP inhibition (Jo et al., 2011). In this study, treatment of rat hippocampal slices with Aβ oligomers induced caspase 3-mediated cleavage of Akt-1, resulting in GSK-3 activation. Consistent with it, treatment with a GSK-3 inhibitor completely prevented Aβ oligomers from inhibiting LTP.

7. Lithium as a GSK-3 inhibitor

The finding that the mood stabilizing drug lithium directly inhibited GSK-3 initially sparked the interest for this enzyme as a potential target for mood disorders. Lithium and valproic

acid are mood stabilizers widely used in the chronic treatment of bipolar disorders. Lithium ions directly inhibit GSK-3 (Klein & Melton, 1996), most likely by competing with magnesium, while valproic acid inhibits GSK-3 activity in relevant therapeutic concentrations in human neuroblastoma cells (Chen et al., 1999), most likely through an indirect mechanism (Rosenberg, 2007).

The mechanism of action by which lithium exerts its therapeutic effects is not known but it is conceivable that the acute effects on GSK-3 results in changes in gene regulation and cellular changes which could affect the neuronal plasticity over time (Gould & Manji, 2002; Jope, 1999; Lennox & Hahn, 2000). Lithium also inhibits at least four phosphomonoesterases (including inositol monophosphatase) (York et al., 1995), and phosphoglucomutase (Ray & Szymanki, 1978; Stambolic & Woodgett, 1994), apart from GSK-3 (Klein & Melton, 1996; Li-Smerin et al., 2001). That said, GSK-3 is significantly inhibited at therapeutic lithium concentrations (Gould & Manji, 2002; Phiel et al., 2003; Shaldubina et al., 2001). Thus, if a significant proportion of lithium's therapeutic actions in bipolar disorder results from the inhibition of GSK-3, then this enzyme would be an important target for bipolar disorder (Li et al., 2002; Rowe et al., 2007).

In spite of these attributes, lithium has a narrow therapeutic window (blood serum levels 0.6 to 1.2 mM) above which side effects are intolerable. Overdose can lead to severe neurological dysfunction and in some cases death. Non-CNS side effects of lithium (not uncommonly within therapeutic levels) include tremor, polyuria, polydipsia, nausea, and weight gain. Lithium can have adverse reactions with other drug classes including diuretics, NSAIDS, and other drugs that alter kidney function (see Gould & Manji, 2006 for a review).

There are only a few observational studies that have attempted to address the clinical effect of lithium in patients with AD. A retrospective study with a large sample of patients with dementia resulted in an increased risk of AD in patients who had been treated with lithium within 4 years prior to diagnosis (Ayuso-Mateo et al., 2001), although it is possible that this is partially accounted for by the increased occurrence of depression associated with AD. Moreover, a single case study reported in dementia patient showed that lithium treatment alleviated symptoms of aggression and agitation, while cognition persisted after 1.5 years of treatment (Havens et al., 1982). Furthermore, a significantly increased global cognitive ability as measured by MMSE in non-demented patients appears associated with lithium intake (Terao et al., 2006). The study design and low sample size precludes however to draw any causative conclusion from those studies.

Some pilot studies have been carried out to directly address the effect of lithium treatment in AD patients. An open label feasibility and tolerability study on a small cohort of 22 subjects patients receiving a low dose of lithium was carried out in UK, reported a high discontinuation rate despite few, relatively mild and reversible side effects (MacDonald et al., 2008). A second randomized, single-blind, placebo-controlled, parallel group, multicentre 10-week study was carried out in Germany as a proof-of-principle (Hampel et al., 2009). A total of 71 patients with mild AD (MMSE scores between 21 and 26) were treated with lithium or placebo for 10 weeks after which neuropsychological and neuropsychiatric assessment was performed together with some biomarkers determinations in plasma (Aβ1-42), lymphocytes (GSK-3 activity) and CSF (total tau, phospho-tau, and Aβ1-42). In spite of the fact that lithium plasma levels were within the therapeutic range, no treatment effect was observed in any the cognition assessment scales used or the selected biomarkers. Given the short time of treatment of this study, the possibility that lithium has long-term effects on cognition or any other biomarker in AD remains to be tested.

8. Development of GSK-3 inhibitors and their therapeutic potential

The unique position of GSK-3 as a pivotal and central player in the pathogenesis of both sporadic and familial forms of AD has attracted significant attention to this enzyme as a therapeutic target and also as a means to understand the molecular basis underlying AD and related disorders. This has led to the synthesis of a high number of GSK-3 inhibitors, some of which are currently being tested in phase II proof-of-concept clinical trials (Mangialasche et al., 2010; Medina & Avila, 2010). Inhibition of GSK-3 with small molecules would be expected to slow down progression of neurodegeneration in AD and perhaps other tauopathies as well.

A number of novel potent and fairly selective small-molecule inhibitors of GSK-3 activity from different chemical families have recently been described, including hymenialdisine, indirubins, paullones, maleimides, amino pyrazoles, thiazoles, and 2,4-disubstituted thiadiazolidinones (TDZD) (reviewed in Medina & Castro, 2008). Most of them are ATP-competitive inhibitors, although more recently new small molecule derivatives that exhibit substrate competitive inhibition activity toward GSK-3 have been reported. Since the different GSK-3 isoforms display a high degree of homology within the ATP binding site, inhibitors are unable to exhibit isoform selectivity, as they all show similar potencies towards purified GSK-3α and GSK-3β.

Although the ATP-competitive inhibitors occupy the general area of the highly conserved ATP-binding site, they do explore other available space nearby depending upon their structure and it is possible to obtain selective inhibitors by taking advantage of the small differences that exist between the different kinases. Crystal structures of GSK-3β complexed with a variety of ligands, together with molecular modelling approaches, provide the necessary clues for enhancing selectivity towards GSK-3 (Patel et al., 2007; ter Haar et al., 2001). All ATP-binding site inhibitors make hydrogen bonds with backbone atoms of the kinase domain hinge (residues Asp 133 to Thr 138). The hydrogen bonds are the same as observed with ATP although different inhibitors make different combinations of hydrogen bonds. For instance, the two indirubin complexes (PDB 1UV5 and 1Q41) have four hydrogen bonds. In contrast, the Alsterpaullone complex (PDB 1Q3W) only has three hydrogen bonds (with the two backbone atoms of Val 135).

Some GSK-3 inhibitors also target other areas of the ATP pocket. For instance the nitro-group of the Alsterpaullone (PDB 1Q3W) and the chlorine of I-5 (3-anilino-4-arylmaleimide) interact with the conserved catalytic lysine, Lys 85. The bromine atom of 6-bromoindirubin (PDB 1UV5) is buried in the hydrophobic pocket of GSK-3β between residues Leu 132, Leu 130 and Met 101. This is a pocket that is often targeted to increase the selectivity of the inhibitor since it is one of the most diverse areas in the ATP-binding site of kinases and has been successfully used for instance to increase the selectivity in favour of p38α over ERK2. The GSK-3β ATP-binding site inhibitors do not cover the γ-phosphate transfer area. Targeting this part of the ATP-binding site does not appear to improve the selectivity of the inhibitor, although it may improve the potency as additional contacts between the inhibitor and the protein are established (ter Haar et al., 2006).

Some physiological peptides act as GSK-3 inhibitors, including GBP, a maternal *Xenopus* GSK-3 binding protein homologous to a mammalian T cell proto-oncogene (Yost et al., 1998) and p24, a heat resistant GSK-3 binding protein (Martín et al., 2002). That finding led to a synthetic strategy to develop new inhibitors, such as L803-mts, a peptidic inhibitor that binds to the substrate site (Plotkin et al., 2003). L803-mts has been more recently used to

examine the impact of long-term *in vivo* inhibition of GSK-3 and its effects in specific tissues (Kaidanocih-Beilin & Eldar-Finkelman, 2006).

One classical approach for identifying GSK-3 inhibitors has exploited screening programs specifically aimed at finding new hits among compounds that exhibit other pharmacological profiles. However, the availability of X-ray crystallographic data of GSK-3β and several of its complexes with different inhibitors (ter Haar, 2006) in recent years has enabled the application of rational drug optimisation programs to discover new lead compounds. Molecular docking studies on the inhibitors of GSK-3 kinase in the enzyme binding sites of the X-ray complexes studies provide valuable insights into computational strategies useful for the identification of potential GSK-3 inhibitors (Gadakar et al., 2007). As a result of the great amount of information concerning current GSK-3 inhibitors, there are a huge number of reported empirical structure-activity relationships (SAR) that may guide a rational design of more potent and selective inhibitors. However, only a few studies based on Quantitative Structure-Activity Relationships (QSAR) are available for predicting the inhibitor potency against this specific kinase, and they involve mainly molecular modelling and 3D-QSAR (Medina & Castro, 2008).

The last few years have seen the synthesis of quite a number of fairly selective, potent GSK-3 inhibitors which have started to show *in vivo* efficacy in a diverse array of animal models of human diseases, including Alzheimer's disease. Despite the challenges faced by this approach with respect to safety and specificity, a number of efforts are underway to develop kinase inhibitors and in fact, Noscira's tideglusib (NP12), is already in phase II clinical trials for the treatment of both Alzheimer's disease and progressive supranuclear palsy (PSP), a tauopathy (Medina & Castro, 2008; Medina & Avila, 2010).

9. Conclusion

Three decades after its discovery as a protein kinase involved in glycogen metabolism, GSK-3 has revealed as a cellular nexus, integrating several signalling systems, including several second messengers and a wide selection of cellular stimulants. Modulation of its activity has also turned out to be much more complex than originally thought as control of GSK-3 activity occurs by complex mechanisms that are each dependent upon specific signalling pathways, including post-translational modifications, protein complex formation and subcellular localization. Although there seems to be a good degree of functional overlapping between the different isoforms, some tissue- and isoform-specific functions and substrates are starting to emerge and more will most likely be discovered within the next few years and will open the possibility to design better, more specific inhibitors.

Deregulation or abnormal GSK-3 activity appears to be associated with various relevant pathologies, including Alzheimer's disease, as the enzyme is uniquely positioned as a key, central player in AD pathogenesis, having a critical role in key events such as tau phosphorylation, Aβ formation and neurotoxicity, microtubule dynamics, synaptic plasticity, neuritic dystrophy, cognition, neuronal survival, and neurodegeneration. Furthermore, recent reports point out to a genetic association of the *gsk-3* gene with the risk of AD either by itself or synergistically with tau or cdk5 genes.

Drug discovery and development efforts for AD in the last two decades have primarily focused on targets defined by the amyloid cascade hypothesis, so far with disappointing results, underscoring the need of novel therapeutic approaches and targets. A significant effort has being made in the last few years to synthesize a high number of fairly selective,

potent GSK-3 inhibitors, while some of them have shown *in vivo* efficacy in various animal models of AD. Some of the known drug discovery and development challenges will be faced: lack of good predictive animal models, lack of good validated biomarkers of disease progression, clinical trial design, early diagnosis and treatment, definition of target population, difficulties in demonstrating disease modifying effects, etc. Despite the challenges faced by this approach with respect to safety and specificity, a number of efforts are underway to develop GSK-3 inhibitors as useful drugs for the treatment of AD as some compounds have already reached phase II clinical trials and some proof-of-concept studies are currently ongoing or planned.

10. Acknowledgments

MM acknowledges grant support from EU-FP7 (Neuro.GSK3, project # 223276) and from CDTI's CENIT program (DENDRIA, project # CEN-20101023).

11. References

Abe K, and Takeichi M (2007) NMDA-receptor activation induces calpain-mediated beta-catenin cleavages for triggering gene expression. *Neuron* 53: 387-97.

Aberle H, Bauer A, Stappert J, Kispert A, Kemler R (1997) Beta-catenin is a target for the ubiquitin-proteasome pathway. *EMBO J* 16(13): 3797-804.

Agarwal-Mawal A, Qureshi HY, Cafferty PW, Yuan Z, Han D, Lin R, and Paudel HK (2003) 14-3-3 connects glycogen synthase kinase-3 beta to tau within a brain microtubule-associated tau phosphorylation complex. *J Biol Chem* 278 (15): 12722-8.

Alonso A del C, Li B, Grundke-Iqbal I, and Iqbal K (2006) Polymerization of hyperphosphorylated tau into filaments eliminates its inhibitory activity. *Proc Natl Acad Sci U S A* 103: 8864-9

Alvarez G, Muñoz-Montaño JR, Satrústegui J, Avila J, Bogónez E, Díaz-Nido J. (1999) Lithium protects cultured neurons against beta-amyloid-induced neurodegeneration. *FEBS Lett* 453(3): 260-4.

Amit S, Hatzubai A, Birman Y, Andersen JS, Ben-Shushan E, Mann M, Ben-Neriah Y, and Alkalay I (2002) Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway. *Genes Dev* 16: 1066-76

Andorfer C, Acker CM, Kress Y, Hof PR, Duff K, Davies P (2005) Cell-cycle reentry and cell death in transgenic mice expressing nonmutant human tau isoforms. *J Neurosci* 25(22): 5446-54.

Aplin AE, Gibb GM, Jacobsen JS, Gallo JM, Anderton BH. (1996) In vitro phosphorylation of the cytoplasmic domain of the amyloid precursor protein by glycogen synthase kinase-3b. *J. Neurochem*. 67(2):699-707

Arnold CS, Johnson GV, Cole RN, Dong DL, Lee M, Hart GW (1996) The microtubule-associated protein tau is extensively modified with O-linked N- acetylglucosamine. *J Biol Chem* 271(46): 28741-4.

Ayuso-Mateos JL, Vázquez-Barquero JL, Dowrick C, Lehtinen V, Dalgard OS, Casey P, Wilkinson C, Lasa L, Page H, Dunn G, Wilkinson G; ODIN Group (2001) Depressive disorders in Europe: prevalence figures from the ODIN study. *Br J Psychiatry* 179: 308-16.

Bain J, Plater L, Elliott M, Shpiro N, Hastie CJ, McLauchlan H, Klevernic I, Arthur JS, Alessi DR, and Cohen P (2007) The selectivity of protein kinase inhibitors: a further update. *Biochem J* 408: 297-315.

Baki L, Shioi J, Wen P, Shao Z, Schwarzman A, Gama-Sosa M, Neve R and Robakis NK (2004) PS1 activates PI3K thus inhibiting GSK-3 activity and tau overphosphorylation: effects of FAD mutations. *EMBO J* 23: 2586-96.

Baki, L., Shioi, J., Wen, P., Shao, Z., Schwarzman, A., Gama-Sosa, M., Neve, R. and Robakis, N.K. (2004) PS1 activates PI3K thus inhibiting GSK-3 activity and tau overphosphorylation: effects of FAD mutations. EMBO J. 23, 2586-96.

Bancher C, Grundke-Iqbal I, Iqbal K, Fried VA, Smith HT, Wisniewski HM (1991) Abnormal phosphorylation of tau precedes ubiquitination in neurofibrillary pathology of Alzheimer disease. *Brain Res* 539(1): 11-8.

Beaulieu JM, Zhang X, Rodriguez RM, Sotnikova TD, Cools MJ, Wetsel WC, Gainetdinov RR and Caron MG (2008) Role of GSK3 beta in behavioralabnormalities induced by serotonin deficiency. *Proc Natl Acad Sci USA* 105: 1333-1338.

Bhat R.V. and Froelich-Fabre S. (2004) Mechanisms of Tauopathies. *Drug Disc. Today Drug Disc.: Dis. Mech.*, 1, 391-8.

Bhat R.V., and Budd S. (2002) GSK3□ signaling: casting a wide net in Alzheimer´s Disease. *Neurosignals*, 11, 251-61.

Biessels GJ and Kappelle LJ (2005) Increased risk of Alzheime's disease in Type II diabetes: insulin resistance of the brain or insulin-induced amyloid pathology? *Biochem. Soc. Trans*. 33, 1041-4.

Boutajangout A, Quartermain D, Sigurdsson EM. (2010) Immunotherapy targeting pathological tau prevents cognitive decline in a new tangle mouse model. *J Neurosci* 30(49): 16559-66.

Bretteville A, Planel E (2008) Tau aggregates: toxic, inert, or protective species? *J Alzheimers Dis* 14(4): 431-6

Brunden KR, Trojanowski JQ, Lee VM (2009) Advances in tau-focused drug discovery for Alzheimer's disease and related tauopathies. *Nat Rev Drug Discov* 8(10): 783-93.

Buch I, Fishelovitch D, London N, Raveh B, Wolfson HJ, and Nussinov R (2010). Allosteric regulation of glycogen synthase kinase 3beta: a theoretical study. *Biochemistry* 49: 10890-901.

Cardona-Gomez P, Perez M, Avila J, Garcia-Segura LM, and Wandosell F (2004) Estradiol inhibits GSK3 and regulates interaction of estrogen receptors, GSK3, and beta-catenin in the hippocampus. *Mol Cell Neurosci* 25: 363-73.

Caricasole A, Copani A, Caraci F, Aronica E, Rozemuller AJ, Caruso A, Storto M, gaviraghi G, Terstappen GC and Nicoletti F (2004) Induction of Dikkopft-1, a negative modulator of the Wnt pathway, is associated with neuronal degeneration in Alzheimer's brain. *J. Neurosc*i. 24, 6021-27.

Castano Z, Gordon-Weeks PR and Kypta RM (2010) The neuron-specific isoform of glycogen synthase kinase-3beta is required for axon growth. *J Neurochem* 113: 117-130.

Chen G, Huang LD, Jiang YM, Manji HK (1999) The mood-stabilizing agent valproate inhibits the activity of glycogen synthase kinase-3. *J Neurochem* 72(3): 1327-30.

Chou HY, Howng SL, Cheng TS, Hsiao YL, Lieu AS, Loh JK, Hwang SL, Lin CC, Hsu CM, Wang C, Lee CI, Lu PJ, Chou CK, Huang CY, and Hong YR (2006) GSKIP is

homologous to the Axin GSK3beta interaction domain and functions as a negative regulator of GSK3beta. *Biochemistry* 45: 11379-89.

Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, Fraser G, *et al*. (2010) Transmission and spreading of tauopathy in transgenic mouse brain. *Nat Cell Biol* 11(7): 909-13.

Cohen P, and Goedert M (2004) GSK3 inhibitors: development and therapeutic potential. *Nat Rev Drug Discov* 3: 479-87.

Cole A, Frame S, and Cohen P (2004) Further evidence that the tyrosine phosphorylation of glycogen synthase kinase-3 (GSK3) in mammalian cells is an autophosphorylation event. *Biochem J* 377: 249-55.

Cole AR, Causeret F, Yadirgi G, Hastie CJ, McLauchlan H, McManus EJ, Hernández F, Eickholt BJ, Nikolic M, and Sutherland C (2006) "Distinct priming kinases contribute to differential regulation of collapsin response mediator proteins by glycogen synthase kinase-3 in vivo" *J. Biol. Chem.* 281(24):16591-8.

Congdon EE, Duff KE (2008) Is tau aggregation toxic or protective? *J Alzheimers Dis* 14(4): 453-7.

da Cruz e Silva EF and da Cruz e Silva OA. (2003) Protein phosphorylation and APP metabolism. *Neurochem. Res*. 28(10): 1553-61.

Dajani R, Fraser E, Roe SM, Yeo M, Good VM, Thompson V, Dale TC, and Pearl LH (2003) Structural basis for recruitment of glycogen synthase kinase 3beta to the axin-APC scaffold complex. *EMBO J* 22: 494-501.

Dajani R, Fraser E, Roe SM, Young N, Good V, Dale TC, and Pearl LH (2001) Crystal structure of glycogen synthase kinase 3 beta: structural basis for phosphate-primed substrate specificity and autoinhibition. *Cell* 105: 721-32.

de Calignon A, Spires-Jones TL, Pitstick R, Carlson GA, Hyman BT (2009) Tangle-bearing neurons survive despite disruption of membrane integrity in a mouse model of tauopathy. *J Neuropathol Exp Neurol* 68(7): 757-61.

De Ferrari G.V., Papassotiropoulos A, Biechele T, Wavrant De-Vrieze F, Avila ME, Major MB, Myers A, Sáez K, Henríquez JP, Zhao A, Wollmer MA, Nitsch RM, Hock C, Morris CM, Hardy J, Moon RT. (2007) Common genetic variation within the low-density lipoprotein receptor-related protein 6 and late-onset Alzheimer's disease. *Proc. Natl. Acad. Sc*i. 104, 9434-39.

Delcommenne M, Tan C, Gray V, Rue L, Woodgett J, and Dedhar S (1998) Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase. *Proc Natl Acad Sci U S A* 95: 11211-6.

Delobel P, Lavenir I, Fraser G, Ingram E, Holzer M, Ghetti B, Spillantini MG, Crowther RA, Goedert M. (2008) Analysis of tau phosphorylation and truncation in a mouse model of human tauopathy. *Am J Pathol* 172(1): 123-31.

Ding Q, Xia W, Liu JC, Yang JY, Lee DF, Xia J, Bartholomeusz G, Li Y, Pan Y, Li Z, Bargou RC, Qin J, Lai CC, Tsai FJ, Tsai CH, and Hung MC (2005) Erk associates with and primes GSK-3beta for its inactivation resulting in upregulation of beta-catenin. *Mol Cell* 19: 159-70.

Drechsel DN, Hyman AA, Cobb MH, Kirschner MW (1992) Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau. *Mol Biol Cell* 3(10): 1141-54.

Ebneth A, Godemann R, Stamer K, Illenberger S, Trinczek B, Mandelkow E (1998) Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: Implications for Alzheimer's disease. *J Cell Biol* 143(3): 777-94.

Embi N, Rylatt DB, and Cohen P (1980) Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. *Eur. J. Biochem.* 107(2):519-27.

Eom TY and Jope RS (2009) Blocked inhibitory serine-phosphorylation of glycogen synthase kinase-3alpha/beta impairs in vivo neural precursor cell proliferation. *Biol Psychiatry* 66: 494-502.

Fang X, Yu SX, Lu Y, Bast RC Jr., Woodgett JR, and Mills GB (2000) Phosphorylation and inactivation of glycogen synthase kinase 3 by protein kinase A. *Proc Natl Acad Sci U S A* 97: 11960-5.

Ferkey DM & Kimelman D (2002) Glycogen synthase kinase-3 beta mutagenesis identifies a common binding domain for GBP and Axin. *J Biol Chem* 277: 16147-52.

Frame S and Cohen P (2001) GSK3 takes centre stage more than 20 years after its discovery. *Biochem J* 359: 1-16.

Frame S, Cohen P, and Biondi RM (2001) A common phosphate binding site explains the unique substrate specificity of GSK3 and its inactivation by phosphorylation. *Mol Cell* 7: 1321-7.

Fraser E, Young N, Dajani R, Franca-Koh J, Ryves J, Williams RS, Yeo M, Webster MT, Richardson C, Smalley CM, Pearl LH, Harwood A, and Dale TC (2002) Identification of the Axin and Frat binding region of glycogen synthase kinase-3. *J Biol Chem* 277: 2176-85.

Frost B, Jacks RL, Diamond MI. Propagation of tau misfolding from the outside to the inside of a cell (2009) *J Biol Chem* 284(19): 12845-52.

Gadakar PK, Phukan S, Dattatreya P, Balaji VN (2007) Pose prediction accuracy in docking studies and enrichment of actives in the active site of GSK-3beta. *J Chem Inf Model* 47(4):446-59.

Gamblin TC, Chen F, Zambrano A, Abraha A, Lagalwar S, Guillozet AL, Lu M, Fu Y, Garcia-Sierra F, LaPointe N, Miller R, Berry RW, Binder LI, Cryns VL (2993) Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease. *Proc Natl Acad Sci USA* 100(17): 10032-7

Goedert M, Spillantini MG, Potier MC, Ulrich J, Crowther RA (1989) Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. *EMBO J* 8(2): 393-9.

Goñi-Oliver P, Avila J, and Hernández F (2009a) Calpain-mediated truncation of GSK-3 in post-mortem brain samples. *J Neurosci Res* 87: 1156-61.

Goñi-Oliver P, Avila J, and Hernández F (2009b) Memantine inhibits calpain-mediated truncation of GSK-3 induced by NMDA: implications in Alzheimer's disease. *J Alzheimers Dis*. 18(4): 843-8.

Goñi-Oliver P, Lucas JJ, Avila J, and Hernández F (2007) N-terminal cleavage of GSK-3 by calpain: a new form of GSK-3 regulation. *J Biol Chem* 282: 22406-13.

Gould TD and Manji HK (2006) Glycogen synthase kinase 3: a target for novel mood disorder treatments. In: *Glycogen Synthase Kinase 3 (GSK-3) and Its Inhibitors-Drug*

discovery and Development. Wang B (Ed), Ch 7, pp. 125-154. Wiley Series in Drug Discovery and Development. New Jersey, USA..

Gould TD, Manji HK (1995) The Wnt signaling pathway in bipolar disorder. *Neuroscientist* 8(5): 497-511.

Grace EA and Busciglio J (2003) Aberrant activation of focal adhesion proteins mediates fibrillar amyloid β-induced neuronal dystrophy. *J. Neurosci*. 23, 493-502.

Grimes CA, and Jope RS (2001) The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. *Prog Neurobiol* 65: 391-426.

Haass C, Selkoe DJ (2007) protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* 8(2): 101-12.

Hamilton G, Proitsi P, Jehu L, Morgan A, Williams J, O'Donovan M, Owen MJ, Powell JF and Lovestone S (2007) Candidate gene association study of insulin signalling genes and Alzheimer's disease: evidence for SOS2, PCK1, and PPARgamma as susceptibility loci. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 144, 508-16.

Hampel H, Ewers M, Bürger K, Annas P, Mörtberg A, Bogstedt A, Frölich L, Schröder J, Schönknecht P, Riepe MW, Kraft I, Gasser T, Leyhe T, Möller HJ, Kurz A, Basun H (2009) Lithium trial in Alzheimer's disease: a randomized, single-blind, placebo-controlled, multicenter 10-week study. *J Clin Psychiatry* 70(6): 922-31.

Hanger DP, Anderton BH, Noble W (2009) Tau phosphorylation: The therapeutic challenge for neurodegenerative disease. *Trends Mol Med* 15(3): 112-9.

Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297: 353–356.

Hardy JA, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256: 184–185.

Hart GW, Kreppel LK, Comer FI, Arnold CS, Snow DM, Ye Z, Cheng X, DellaManna D, Caine DS, Earles BJ, Akimoto Y, Cole RN, Hayes BK (1996) O-GlcNAcylation of key nuclear and cytoskeletal proteins: reciprocity with O-phosphorylation and putative roles in protein multimerization. *Glycobiology* 6(7): 711-6.

Hartigan JA, Xiong WC, and Johnson GV (2001) Glycogen synthase kinase 3beta is tyrosine phosphorylated by PYK2. *Biochem Biophys Res Commun* 284: 485-9.

Harwood AJ (2001) Regulation of GSK-3: a cellular multiprocessor. *Cell* 105: 821-4.

Havens WW 2nd, Cole J (1982) Successful treatment of dementia with lithium. *J Clin Psychopharmacol* 2(1): 71-2.

Hemmings BA, Yellowlees D, Kernohan JC, and Cohen P (1981) Purification of glycogen synthase kinase 3 from rabbit skeletal muscle. Copurification with the activating factor (FA) of the (Mg-ATP) dependent protein phosphatase. *Eur. J. Biochem*. 119(3):443-51.

Hernández F, Gómez de Barreda E, Fuster-Matanzo A, Lucas JJ, Avila J (2010) GSK3: a possible link between beta amyloid peptide and tau protein. *Exp Neurol*. 223(2): 322-5.

Hiesberger T., Trommsdorff M., Howell B. W., Goffinet A., Mumby M. C., Cooper J. A. and Herz J. (1999) Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron* 24, 481-9.

Himmler A, Drechsel D, Kirschner MW, Martin DW Jr (1989) Tau consists of a set of proteins with repeated C-terminal microtubule-binding domains and variable N-terminal domains. *Mol Cell Biol* 9(4): 1381-8.

Hoeflich KP, Luo J, Rubie EA, Tsao M-S, Jin O and Woodgett JR (2000) Requirement for GSK3beta in cell surival and NFkB activation. *Nature* 406: 86-90.

Hooper C, Killick R, and Lovestone S (2008) The GSK3 hypothesis of Alzheimer's disease. *J. Neurochem*. 104: 1433-9.

Hooper C, Markevich V, Plattner F, Killick R, Schofield E, Engel T, Hernandez F, Anderton B, Rosenblum K, Bliss T, Cooke SF, Avila J, Lucas JJ, Giese KP, Stephenson J, and Lovestone S (2007) Glycogen synthase kinase-3 inhibition is integral to long-term potentiation. Eur. J. Neurosci. 25(1): 81-6.

Hoshi M, Sato M, Matsumoto S, Noguchi A, Yasutake K, Yoshida N, Sato K (2003) Spherical aggregates of beta-amyloid (amylospheroid) show high neurotoxicity and activate tau protein kinase I/glycogen synthase kinase-3beta. *Proc Natl Acad Sci U S A* 100(11): 6370-6375.

Hsieh H, Boehm J, Sato C, Iwatsubo T, Tomita T, Sisodia S, Malinow R (2006) AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. Neuron 52(5): 831-43.

Hughes K, Nikolakaki E, Plyte SE, Totty NF, Woodgett JR (1993) Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. *EMBO J* 12(2): 803-8.

Hur EM and Zhon FP (2010) GSK3 signalling in neural development. *Nat Rev Neurosci* 11: 539-51.

Inestrosa NC, Toledo EM (2008) The role of Wnt signaling in neuronal dysfunction in Alzheimer's Disease. *Mol Neurodegener* 24: 3-9.

Iqbal K, Liu F, Gong CX, Alonso Adel C, Grundke-Iqbal I (2009) Mechanisms of tau-induced neurdegeneration. *Acta Neuropathol* 118(1): 53-69.

Itoh K, Tang TL, Neel BG, and Sokol SY (1995) Specific modulation of ectodermal cell fates in Xenopus embryos by glycogen synthase kinase. *Development* 121, 3979-88.

Ittner LM, Götz J (2011) Amyloid-β and tau--a toxic pas de deux in Alzheimer's disease. *Nat Rev Neurosci* 12(2): 65-72.

Jackson G. R., Wiedau-Pazos M., Sang T. K., Wagle N., Brown C. A., Massachi S. and Geschwind D. H. (2002) Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in Drosophila. *Neuron* 34, 509-19

Jaworski T, Kügler S, Van Leuven F (2010) Modeling of tau-mediated synaptic and neuronal degeneration in Alzheimer's disease. *Int J Alzheimers Dis*. pii: 573138.

Jiang H, Guo W, Liang X, Rao Y. (2005) Both the establishment and the maintenance of neuronal polarity require active mechanisms: critical roles of GSK-3β and its upstream regulators. Cell. 120:123-35.

Jo J, Whitcomb DJ, Olsen KM, Kerrigan TL, Lo SC, Bru-Mercier G, Dickinson B, Scullion S, Sheng M, Collingridge G, Cho K (2011) Aβ(1-42) inhibition of LTP is mediated by a signaling pathway involving caspase-3, Akt1 and GSK-3β. Nat Neurosci [Epub ahead of print]. PMID: 21441921.

Jope RS (1999) Anti-bipolar therapy: mechanism of action of lithium. *Mol Psychiatry* 4(2): 117-28.

Kaidanovich-Beilin O, Eldar-Finkelman H (2006) Long-term treatment with novel glycogen synthase kinase-3 inhibitor improves glucose homeostasis in ob/ob mice: molecular characterization in liver and muscle. *J Pharmacol Exp Ther* 316(1): 17-24.

Kaidanovich-Beilin O, Lipina TV, Takao K van Eede M, Hattori S, Laliberté C, Khan M, Okamoto K, Chambers JW, Fletcher PJ, MacAulay K, Doble BW, Henkelman M, Miyakawa T, Roder J, Woodgett JR. (2009) Abnormalities in brain structure and behavior in GSK-3alpha mutant mice. *Mol Brain* 2: 35.

Kandasamy AD, and Schulz R (2009) Glycogen synthase kinase-3beta is activated by matrix metalloproteinase-2 mediated proteolysis in cardiomyoblasts. *Cardiovasc Res* 83: 698-706.

Kayed R, Jackson GR. Prefilament tau species as potential targets for immunotherapy for Alzheimer disease and related disorders (2009) *Curr Opin Immunol* 21(3): 359-63.

Kim L, Harwood A, and Kimmel AR (2002) Receptor-dependent and tyrosine phosphatase-mediated inhibition of GSK3 regulates cell fate choice. *Dev Cell* 3: 23-32.

Kim L, Liu J, and Kimmel AR (1999).The novel tyrosine kinase ZAK1 activates GSK3 to direct cell fate specification. *Cell* 99: 399-408.

Kim WY, Wang X, Wu Y, Doble BW, Patel S, Woodgett JR and Snider WD (2009) GSK-3 is a master regulator of neural progenitor homeostasis. *Nat Neurosci* 12: 1390-1397.

Kimura T, Yamashita S, Nakao S, Park JM, Murayama M, Mizoroki T, Yoshiike Y, Sahara N and Takashima A (2008) GSK-3beta is required for memory reconsolidation in adult brain. *PLoS One,* 3(10): e3540.

Klein PS, Melton DA (1996) A molecular mechanism for the effect of lithium on development. *Proc Natl Acad Sci USA* 93(16): 8455-9.

Kwok JB, Hallupp M, Loy CT, Chan DK, Woo J, Mellick GD, Buchanan DD, Silburn PA, Halliday GM, Schofield PR (2005) GSK3B polymorphisms alter transcription and splicing in Parkinson's disease. *Ann Neurol* 58(6): 829-39.

Kwok JB, Loy CT, Hamilton G, Lau E, Hallupp M, Williams J, Owen MJ, Broe GA, Tang N, Lam L, Powell JF, Lovestone S, Schofield PR (2008) Glycogen synthase kinase-3beta and tau genes interact in Alzheimer's disease. *Ann Neurol* 64(4): 446-54.

Lee G, Neve RL, Kosik KS (1989) The microtubule binding domain of tau protein. *Neuron* 2(6): 1615-24.

Lee JH, Lau KF, Perkinton MS, Standen CL, Shemilt SJ, Mercken L, Cooper JD, McLoughlin DM, Miller CC. (2003) The neuronal adaptor protein X11a reduces Aβ levels in the brains of Alzheimer's APPswe Tg2576 transgenic mice. *J Biol Chem*. 278(47): 47025-9.

Lenox RH, Hahn CG (2000) Overview of the mechanism of action of lithium in the brain: fifty-year update. *J Clin Psychiatry* 61(Suppl 9): 5-15.

Leroy K, Boutajangout A, Authelet M, Woodgett JR, Anderton BH, and Brion JP (2002) The active form of glycogen synthase kinase-3b is associated with granulovacuolar degeneration in neurons in Alzheimer's disease. *Acta Neuropathol*. 103(2):91-9.

Lesort M, Jope RS, and Johnson GV (1999) Insulin transiently increases tau phosphorylation: involvement of glycogen synthase kinase-3beta and Fyn tyrosine kinase. *J Neurochem* 72: 576-84.

Li L, Yuan H, Weaver CD, Mao J, Farr GH 3rd, Sussman DJ, Jonkers J, Kimelman D, and Wu D (1999) Axin and Frat1 interact with dvl and GSK, bridging Dvl to GSK in Wnt-mediated regulation of LEF-1. *EMBO J* 18: 4233-40.

Li X, Bijur GN, Jope RS (2002) Glycogen synthase kinase-3beta, mood stabilizers, and neuroprotection. *Bipolar Disord* 4(2): 137-44.

Li-Smerin, Y, Levitan ES and Johnson JW (2001) Free intracellular Mg(2+) concentration and inhibition of NMDA responses in cultured rat neurons. *J Physiol* 533: 729-43.

Liu KJ, Arron JR, Stankunas K, Crabtree GR and Longaker MT (2007) Chemical rescue of cleft palate and midline defects in conditional GSK3b mice. *Nature* 446: 79-82.

Liu SJ, AH Zhang, Li HL, Wang Q, Deng HM, Netzer WJ, Xu H, and Wang JZ (2003) Overactivation of glycogen synthase kinase-3 by inhibition of phosphoinositol-3 kinase and protein kinase C leads to hyperphosphorylation of tau and impairment of spatial memory. *J Neurochem* 87: 1333-44.

Lochhead PA, Kinstrie R, Sibbet G, Rawjee T, Morrice N and Cleghon V (2006) A chaperone-dependent GSK3beta transitional intermediate mediates activation-loop autophosphorylation. *Mol Cell* 24: 627-633.

LoPresti P, Szuchet S, Papasozomenos SC, Zinkowski RP, Binder LI (1995) Functional implications for the microtubule-associated protein tau: Localization in oligodendrocytes. *Proc Natl Acad Sci USA* 92(22): 10369-73.

MacAulay K, Doble BW, Patel S, Hansotia T, Sinclair EM, Drucker DJ, Nagy A and Woodgett JR (2007) Glycogen synthase kinase 3alpha-specific regulation of murine hepatic glycogen metabolism. *Cell Metab* 6: 329-337.

Macdonald A, Briggs K, Poppe M, Higgins A, Velayudhan L, Lovestone S (2008) A feasibility and tolerability study of lithium in Alzheimer's disease. *Int J Geriatr Psychiatry* 23(7): 704-11.

Magdesian MH, Carvalho MM, Mendes FA, Saraiva LM, Juliano MA, Juliano L, Garcia-Abreu J, and Ferreira ST (2008) Amyloid-beta binds to the extracellular cysteine-rich domain of Frizzled and inhibits Wnt/beta-catenin signaling. *J Biol Chem*. 283(14): 9359-68

Mangialasche F, Solomon A, Winblad B, Mecocci P, Kivipelto M (2010) Alzheimer's disease: clinical trials and drug development. *Lancet Neurol* 9(7): 702-16.

Mao Y, Ge X, Frank CL, Madison JM, Koehler AN, Doud MK, Tassa C, Berry EM, Soda T, Singh KK, Biechele T, Petryshen TL, Moon RT, Haggarty SJ, and Tsai LH (2009) Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3beta/beta-catenin signaling. *Cell* 136 (6), 1017-31.

Martín CP, Vázquez J, Avila J, Moreno FJ (2002) P24, a glycogen synthase kinase 3 (GSK 3) inhibitor. Biochim Biophys Acta. 2002 Jan 2;1586(1):113-22.

Mateo I, Infante J, Llorca J, Rodríguez E, Berciano J, Combarros O (2006) Association between glycogen synthase kinase-3beta genetic polymorphism and late-onset Alzheimer's disease. *Dement Geriatr Cogn Disord* 21(4): 228-32.

Mateo I, Vázquez-Higuera JL, Sánchez-Juan P, Rodríguez-Rodríguez E, Infante J, García-Gorostiaga I, Berciano J, Combarros O (2009) Epistasis between tau phosphorylation regulating genes (CDK5R1 and GSK-3beta) and Alzheimer's disease risk. *Acta Neurol Scand* 120(2): 130-3.

Medina M & Wandosell F (2011) Deconstructing GSK-3: the fine regulation of its activity) *Int J Alz Dis* (in press).

Medina M (2011) Recent developments in tau-based therapeutics for neurodegenerative diseases. *Recent Pat CNS Drug Discov* 6(1): 20-30

Medina M, Avila J (2010) Glycogen synthase kinase-3 (GSK-3) inhibitors for the treatment of Alzheimer's disease. *Curr Pharm Des* 16(25): 2790-8.

Medina M, Castro A (2008) Glycogen synthase kinase-3 (GSK-3) inhibitors reach the clinic. *Curr Opin Drug Discov Devel* 11(4): 533-43.

Moon RT, Kohn AD, De Ferrari GV, and Kaykas A (2004) Wnt and beta-catenin signalling: diseases and therapies. *Nat Rev Genet* 5: 691-701.

Morfini G., Pigino G., Beffert U., Busciglio J. and Brady S. T. (2002) Fast axonal transport misregulation and Alzheimer's disease. *Neuromol. Med*. 2, 89-99.

Mori H, Kondo J, Ihara Y. (1987) Ubiquitin is a component of paired helical filaments in Alzheimer's disease. *Science* 235(4796): 1641-4.

Morsch R, Simon W, Coleman PD (1999) Neurons may live for decades with neurofibrillary tangles. *J Neuropathol Exp Neurol* 58(2): 188-97.

Mukai F, Ishiguro K, Sano Y, Fujita SC (2002) Alternative splicing isoform of tau protein kinase I/glycogen synthase kinase 3 beta. *J Neurochem.* 81(5):1073-83.

Muñoz-Montaño JR, Lim F, Moreno FJ, Avila J, and Díaz-Nido J. (1999) Glycogen Synthase Kinase-3 Modulates Neurite Outgrowth in Cultured Neurons: Possible Implications for Neurite Pathology in Alzheimer's Disease. *J. Alzheimers Dis*. 1(6):361-78.

Munoz-Montano JR, Moreno FJ, Avila J, and Diaz-Nido J (1997) Lithium inhibits Alzheimer's disease-like tau protein phosphorylation in neurons. *FEBS Lett* 411: 183-8.

Muyllaert D, Kremer A, Jaworski T, Borghgraef P, Devijver H, Croes S, Dewatcher I, and Van Leuven F. (2008) Glycogen synthase kinase-3beta, or a link between amyloid and tau pathology? *Genes Brain Behav*. 7 (suppl. 1): 57-66.

Nishimura I, Yang Y, and Lu B (2004) PAR-1 kinase plays an initiator role in a temporally ordered phosphorylation process that confers tau toxicity in Drosophila. *Cell* 116: 671-82.

Noble W, Olm V, Takata K, Casey E, Mary O, Meyerson J, Gaynor K, LaFrancois J, Wang L, Kondo T, Davies P, Burns M, Veeranna, Nixon R, Dickson D, Matsuoka Y, Ahlijanian M, Lau LF, and Duff K (2003) Cdk5 is a key factor in tau aggregation and tangle formation in vivo. *Neuron* 38: 555-65.

Noble W, Planel E, Zehr C, Olm V, Meyerson J, Suleman F, Gaynor K, Wang L, LaFrancois J, Feinstein B, Burns M, Krishnamurthy P, Wen Y, Bhat R, Lewis J, Dickson D, and Duff K (2005) Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo. *Proc Natl Acad Sci U S A* 102: 6990-5.

O'Brien WT, Harper AD, Jove F, Woodgett JR, Maretto S, Piccolo S and Klein PS (2004) Glycogen synthase kinase-3beta haploinsufficiency mimics the behavioral and molecular effects of lithium. *J Neurosci* 24: 6791-6798.

Osolodkin DI, Zakharevich NV, Palyulin VA, Danilenko VN, and Zefirov NS (2011) Bioinformatic analysis of glycogen synthase kinase 3: human versus parasite kinases.*Parasitology* Feb 24: 1-11.

Palacino J.J., Murphy M.P., Murayama O., Iwasaki K., Fujiwara M., Takashima A., Golde T.E., and Wolozin B. (2001) Presenilin 1 regulates β-catenin-mediated transcription in a glycogen synthase kinase-3-independent fashion. *J. Biol. Chem*. 276(42):38563-9.

Patel DS, Dessalew N, Iqbal P, Bharatam PV (2007) Structure-based approaches in the design of GSK-3 selective inhibitors. *Curr Protein Pept Sci* 8(4):352-64.

Patel S, Doble BW, MacAulay K, Sinclair EM, Drucker DJ, Woodgett JR (2008) Tissue-specific role of glycogen synthase kinase 3beta in glucose homeostasis and insulin action. *Mol Cell Biol* 28(20): 6314-28.

Patel S, Macaulay K, Woodgett JR (2011) Tissue-specific analysis of glycogen synthase kinase-3α (GSK-3α) in glucose metabolism: effect of strain variation. *PLoS One* 6(1): e15845.

Pei JJ, Tanaka T, Tung YC, Braak E, Iqbal K, and Grundke-Iqbal I (1997) Distribution, levels, and activity of glycogen synthase kinase-3 in the Alzheimer disease brain. J *Neuropathol Exp Neurol*. 56(1):70-8.

Peineau S, Taghibiglou C, Bradley C, Wong TP, Liu L, Lu J, Lo E, Wu D, Saule E, Bouschet T, Matthews P, Isaac JT, Bortolotto ZA, Wang YT, and Collingridge GL (2007) LTP inhibits LTD in the hippocampus via regulation of GSK-3ba. *Neuron*. 53(5):703-17.

Perez M, Rojo AI, Wandosell F, Diaz-Nido J, and Avila J (2003) Prion peptide induces neuronal cell death through a pathway involving glycogen synthase kinase 3. *Biochem J* 372: 129-36.

Phiel CJ, Wilson CA, Lee VM, and Klein PS (2003) GSK-3alpha regulates production of Alzheimer's disease amyloid-b peptides *Nature*. 423(6938):435-9.

Plotkin B, Kaidanovich O, Talior I, Eldar-Finkelman H (2003) Insulin mimetic action of synthetic phosphorylated peptide inhibitors of glycogen synthase kinase-3. J Pharmacol Exp Ther 305(3): 974-80.

Prickaerts J, Moechars D, Cryns K, Lenaerts I, van Craenendonck H, Goris I, Daneels G, Bouwknecht JA and Steckler T (2006) Transgenic mice overexpressing glycogen synthase kinase 3beta: a putative model of hyperactivity and mania. *J Neurosci* 26: 9022-9029.

Ray WJ Jr, Szymanki ES, Ng L (1978) The binding of lithium and of anionic metabolites to phosphoglucomutase. *Biochim Biophys Acta* 522(2): 434-42.

Reiman EM, Webster JA, Myers AL, Hardy J, Dunckley T, Zismann VL, Joshipura KD, Pearson JV, Hu-Lince D, Huentelman MJ, Craig DW, Coon KD, Liang WS, Herbert RH, Beach T, Rohrer KC, Zhao AS, Leung D, Bryden L, Marlowe L, Kaleem M, Mastroeni D, Grover A, Heward CB, Ravid R, Rogers J, Hutton ML, Melquist S, Petersen RC, Alexander GE, Caselli RJ, Kukull W, Papassotiropoulos A, Stephan DA. (2007) GAB2 alleles modify Alzheimer's risk in ApoE4 carriers. *Neuron* 54, 713-20.

Rockenstein E, Torrance M, Adame A, Mante M, Bar-on P, Rose JB, Crews L, and Masliah E. (2007) Neuroprotective effects of regulators of the glycogen synthase kinase-3b signaling pathway in a transgenic model of Alzheimer's disease are associated with reduced amyloid precursor protein phosphorylation. *J. Neurosci*. 27(8):1981-91.

Rosenberg (2007) The mechanisms of action of valproate in neuropsychiatric disorders: can we see the forest for the trees? *Cell Mol Life Sci* 64(16): 2090-103.

Ruel L, Bourouis M, Heitzler P, Pantesco V, and Simpson P (1993) Drosophila shaggy kinase and rat glycogen synthase kinase-3 have conserved activities and act downstream of Notch. *Nature* 362 57-60.

Russ C, Lovestone S, Powell JF (2001) Identification of sequence variants and analysis of the role of the glycogen synthase kinase 3 beta gene and promoter in late onset Alzheimer's disease. *Mol Psychiatry* 6(3): 320-4

Sayas C. L., Avila J. and Wandosell F. (2002) Regulation of neuronal cytoskeleton by lysophosphatidic acid: role of GSK-3. *Biochim. Biophys. Acta* 1582, 144-53.

Sayas CL, Moreno-Flores MT, Avila J, Wandosell F (1999) The neurite retraction induced by lysophosphatidic acid increases Alzheimer's disease-like Tau phosphorylation. *J Biol Chem* 274(52): 37046-52.

Schaffer BA, Bertram L, Miller BL, Mullin K, Weintraub S, Johnson N, Bigio EH, Mesulam M, Wiedau-Pazos M, Jackson GR, Cummings JL, Cantor RM, Levey AI, Tanzi RE, Geschwind DH (2008) Association of GSK3B with Alzheimer disease and frontotemporal dementia. *Arch Neurol* 65(10): 1368-74.

Selkoe DJ (1991) The molecular pathology of Alzheimer's disease. *Neuron* 6: 487–498.

Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. *Science* 298: 789–791.

Selkoe DJ (2008) Soluble oligomers of the amyloid β-protein impair synaptic plasticity and behaviour. *Behav Brain Res* 192(1): 106-13.

Sengupta A, Wu Q, Grundke-Iqbal I, Iqbal K, and Singh TJ (1997) Potentiation of GSK-3-catalyzed Alzheimer-like phosphorylation of human tau by cdk5. *Mol Cell Biochem* 167: 99-105.

Shaldubina A, Agam G, Belmaker RH (2001) The mechanism of lithium action: state of the art, ten years later. *Prog Neuropsychopharmacol Biol Psychiatry* 25(4): 855-66.

Sheldon M., Rice D. S., D'Arcangelo G., Yoneshima H., Nakajima K., Mikoshiba K., Howell B. W., Cooper J. A., Goldowitz D., and Curran T. (1997) Scrambler and yotari disrupt the disabled gene and produce a reeler-like phenotype in mice. *Nature* 389, 730-3.

Sigurdsson EM (2008). Immunotherapy targeting pathological tau protein in Alzheimer's disease and related tauopathies *J Alzheimers Dis* 15(2): 157-68.

Simon D, Benitez MJ, Gimenez-Cassina A, Garrido JJ, Bhat RV, Diaz-Nido J, and Wandosell F (2008) Pharmacological inhibition of GSK-3 is not strictly correlated with a decrease in tyrosine phosphorylation of residues 216/279. *J Neurosci Res* 86: 668-74.

Sofola O, Kerr F, Rogers I, Killick R, Augustin H, Gandy C, Allen MJ, Hardy J, Lovestone S, Partridge L (2010) Inhibition of GSK-3 ameliorates Abeta pathology in an adult-onset Drosophila model of Alzheimer's disease. *PLoS Genet* 6(9). pii: e1001087.

Soutar MP, Kim WY, Williamson R, Peggie M, Hastie CJ, McLauchlan H, Snider WD, Gordon-Weeks PR, and Sutherland C (2010) Evidence that glycogen synthase kinase-3 isoforms have distinct substrate preference in the brain. *J Neurochem* 115: 974-83.

Stambolic V, and Woodgett JR (1994) Mitogen inactivation of glycogen synthase kinase-3 beta in intact cells via serine 9 phosphorylation. *Biochem J* 303 (3): 701-4.

Stambolic V, Ruel L, Woodgett JR (1997) Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells. *Curr Biol* 6(12): 1664-8.

Stoothoff WH, Cho JH, McDonald RP, and Johnson GV (2005) FRAT-2 preferentially increases glycogen synthase kinase 3 beta-mediated phosphorylation of primed sites, which results in enhanced tau phosphorylation. *J Biol Chem* 280: 270-6.

Su Y Ryder J., Li B., Wu X., Fox N., Solenberg, P., Brune K., Paul S., Zhou Y., Liu F., and Ni B. (2004) Lithium, a common drug for bipolar disorder treatment, regulates amyloid-β precursor protein processing. *Biochemistry* 43(22):6899-908.

Suhara T, Magrané J, Rosen K, Christensen R, Kim HS, Zheng B, McPhie DL, Walsh K, Querfurth H (2003) Abeta42 generation is toxic to endothelial cells and inhibits

eNOS function through an Akt/GSK-3beta signaling-dependent mechanism. *Neurobiol Aging* 24(3): 437-51.

Sun X., Sato S., Murayama O., Murayama M., Park J.M., Yamaguchi H., and Takashima A. (2002) Lithium inhibits amyloid secretion in COS7 cells transfected with amyloid precursor protein C100. *Neurosci. Lett.* 321(1-2):61-4.

Sutherland C, Leighton IA, and Cohen P (1993) Inactivation of glycogen synthase kinase-3 beta by phosphorylation: new kinase connections in insulin and growth-factor signalling. *Biochem J* 296: 15-9.

Takashima A, Honda T, Yasutake K, Michel G, Murayama O, Murayama M, Ishiguro K, and Yamaguchi H (1998a) Activation of tau protein kinase I/glycogen synthase kinase-3beta by amyloid beta peptide (25-35) enhances phosphorylation of tau in hippocampal neurons. *Neurosci Res* 31: 317-23.

Takashima A., Murayama O., Kohno T., Honda T., Yasutake K., Nihonmatsu N., Mercken M., Yamaguchi H., Sugihara S., and Wolozin B. (1998b) Presenilin 1 associates with glycogen synthase kinase-3β and its substrate tau. *Proc. Natl. Acad. Sci. U S A.* 95(16):9637-41.

ter Haar E (2006) The crystal structure of glycogen synthase kinase 3. In: *Glycogen Synthase Kinase 3 (GSK-3) and Its Inhibitors-Drug discovery and Development.* Wang B (Ed), Ch 5, pp. 61-82. Wiley Series in Drug Discovery and Development. New Jersey, USA..

ter Haar E, Coll JT, Austen DA, Hsiao HM, Swenson L, and Jain J (2001) Structure of GSK3beta reveals a primed phosphorylation mechanism. *Nat Struct Biol* 8: 593-6.

Terao T, Nakano H, Inoue Y, Okamoto T, Nakamura J, Iwata N (2006) Lithium and dementia: a preliminary study. *Prog Neuropsychopharmacol Biol Psychiatry*30(6): 1125-8.

Tesco G. and Tanzi R.E. (2000) GSK-3β forms a tetrameric complex with endogenous PS1-CTF/NTF and β-catenin. Effects of the D257/D385A and FAD-linked mutations. *Ann. NY Acad. Sci*. 920:227-32.

Thornton TM, Pedraza-Alva G, Deng B, Wood CD, Aronshtam A, Clements JL, Sabio G, Davis RJ, Matthews DE, Doble B, and Rincon M (2008) Phosphorylation by p38 MAPK as an alternative pathway for GSK3beta inactivation. *Science* 320: 667-70.

Townsend M, Mehta T, and Selkoe DJ (2007) Soluble Abeta inhibits specific signal transduction cascades common to the insulin receptor pathway. *J Biol Chem* 282 (46): 33305-12.

Twomey C and McCarthy JV (2006) Presenilin-1 is an unprimed glycogen synthase kinase-3beta substrate. *FEBS Lett* 580: 4015-20.

van Amerongen R, Nawijn M, Franca-Koh J, Zevenhoven J, van der Gulden H, Jonkers J, and Berns A (2005) Frat is dispensable for canonical Wnt signaling in mammals. *Genes Dev* 19: 425-30.

Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416(6880): 535-9.

Walsh DM, Selkoe DJ (2007) A beta oligomers - a decade of discovery. *J Neurochem* 101(5): 1172-84

Wang JZ, Grundke-Iqbal I, Iqbal K (1996) Glycosylation of microtubule-associated protein tau: an abnormal posttranslational modification in Alzheimer's disease. *Nat Med* 2(8): 871-5.

Wang QM, Fiol CJ, DePaoli-Roach AA, and Roach PJ (1994) Glycogen synthase kinase-3 beta is a dual specificity kinase differentially regulated by tyrosine and serine/threonine phosphorylation. *J Biol Chem* 269: 14566-74.

Wei H, Leeds PR, Qian Y, Wei W, Chen R, Chuang D (2000) Beta-amyloid peptide-induced death of PC 12 cells and cerebellar granule cell neurons is inhibited by long-term lithium treatment. *Eur J Pharmacol* 392(3): 117-23.

Woodgett JR (2006) Glycogen synthase Kinase 3: an introductory synopsis. In: *Glycogen Synthase Kinase 3 (GSK-3) and Its Inhibitors-Drug discovery and Development.* Wang B (Ed), Ch 1, pp. 3-24. Wiley Series in Drug Discovery and Development. New Jersey, USA..

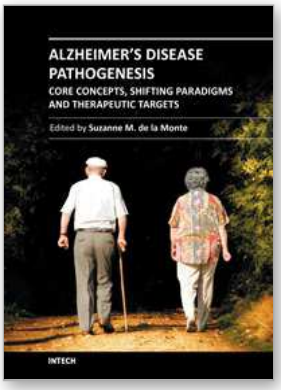
Woodgett JR: Molecular cloning and expression of glycogen synthase kinase-3/factor A. *EMBO J.* (1990) 9(8):2431-8.

Wood-Kaczmar A, Kraus M, Ishiguro K, Philpott KL and Gordon-Weeks PR (2009) An alternatively spliced form of glycogen synthase kinase-3beta is targeted to growing neurites and growth cones. *Mol Cell Neurosci* 42: 184-194.

York JD, Ponder JW, Majerus PW (1995) Definition of a metal-dependent/Li(+)-inhibited phosphomonoesterase protein family based upon a conserved three-dimensional core structure. *Proc Natl Acad Sci USA* 92(11): 5149-53.

Yoshimura T, Kawano Y, Arimura N, Kawabata S, Kikuchi A, Kaibuchi K. (2005) GSK-3β regulates phosphorylation of CRMP-2 and neuronal polarity. Cell 120:137-49.

Yost C, Farr GH 3rd, Pierce SB, Ferkey DM, Chen MM, and Kimelman D (1998)GBP, an inhibitor of GSK-3, is implicated in Xenopus development and oncogenesis. *Cell* 93: 1031-41.



Alzheimer's Disease Pathogenesis-Core Concepts, Shifting Paradigms and Therapeutic Targets

Edited by Dr. Suzanne De La Monte

ISBN 978-953-307-690-4
Hard cover, 686 pages
Publisher InTech
Published online 12, September, 2011
Published in print edition September, 2011

Alzheimer's Disease Pathogenesis: Core Concepts, Shifting Paradigms, and Therapeutic Targets, delivers the concepts embodied within its title. This exciting book presents the full array of theories about the causes of Alzheimer's, including fresh concepts that have gained ground among both professionals and the lay public. Acknowledged experts provide highly informative yet critical reviews of the factors that most likely contribute to Alzheimer's, including genetics, metabolic deficiencies, oxidative stress, and possibly environmental exposures. Evidence that Alzheimer's resembles a brain form of diabetes is discussed from different perspectives, ranging from disease mechanisms to therapeutics. This book is further energized by discussions of how neurotransmitter deficits, neuro-inflammation, and oxidative stress impair neuronal plasticity and contribute to Alzheimer's neurodegeneration. The diversity of topics presented in just the right depth will interest clinicians and researchers alike. This book inspires confidence that effective treatments could be developed based upon the expanding list of potential therapeutic targets.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821