

## Review

# Glycogen synthase kinase-3 $\beta$ , or a link between amyloid and tau pathology?

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**Phosphorylation is the most common post-translational modification of cellular proteins, essential for most physiological functions. Deregulation of phosphorylation has been invoked in disease mechanisms, and the case of Alzheimer's disease (AD) is no exception: both in the amyloid pathology and in the tauopathy are kinases deeply implicated. The glycogen synthase kinase-3 (GSK-3) isozymes participate in diverse cellular processes and important signalling pathways and have been implicitly linked to diverse medical problems, i.e. from diabetes and cancer to mood disorders and schizophrenia, and in the neurodegeneration of AD. Here, we review specific aspects of GSK-3 isozymes in the framework of recent data that we obtained in novel transgenic mouse models that robustly recapitulate the pathology and mechanistical problems of AD.**

Keywords: Amyloid, glycogen synthase kinase, tau, transgenic mice

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## Background

Protein phosphorylation at serine, threonine and tyrosine amino acid (aa) side chains is catalysed by a large group of protein kinases, i.e. 518 genes in the human genome were recognized as encoding proteins with potential kinase activity. Phosphorylation is thereby the most common post-translational modification of all cellular proteins and serves to operate as well as regulate very diverse mechanisms and functions, from signal transduction to muscle movement, over gene regulation to endocytosis. Particularly in post-mitotic neurons that are both immobile and plastic, is phosphorylation known to control all major functions: from neurotransmitter release and synaptic signal transduction to axonal transport and formation of new spines.

In the pathology of Alzheimer's disease (AD), besides amyloid peptide formation, the phosphorylation of protein tau is the most important post-translational modification. Numerous kinases can phosphorylate protein tau *in vitro* and in cellular models, but it is not clear which kinases are responsible *in vivo*. Two main types of kinases need to be considered, i.e. the proline-directed kinases such as glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ), cyclin-dependent kinase 5 (cdk5), extracellular signal-regulated kinase 2 (ERK2) and mitogen-associated protein kinase and the non-proline-directed kinases such as protein kinase A, protein kinase C (PKC), calcium-calmodulin dependent protein kinase II (CaMKII), mitogen-associated protein affinity-regulating kinases (MARK). Evidence is accumulating that GSK-3 $\beta$ , also known as tau kinase I (Ishiguro *et al.* 1992), is essential and deeply involved in AD pathology by phosphorylating protein tau. This review concentrates on the GSK-3 isozymes in the framework of recent data that we obtained in novel transgenic mouse models that more robustly recapitulate the pathological and mechanistical problems posed by AD.

Glycogen synthase kinase-3 was originally discovered as a Ser/Thr kinase that regulates glycogen storage by phosphorylation and thereby inhibition of glycogen synthase (GS). Since then, GSK-3 has been implicated in a plethora of cellular processes ranging from cell metabolism and structure to gene expression and cell death or survival. Consequently, any deregulation of GSK-3 activity will affect important signalling pathways in which it participates. Thereby, the kinase has been implicitly linked to the pathophysiology in very diverse medical problems, from diabetes and cancer to mood disorders and schizophrenia, and also in neurodegeneration and AD (Doble & Woodgett 2003; Jope & Johnson 2004; Takashima 2006). Surprisingly, few hard data were reported, however, that implicate GSK-3 directly in specific disease mechanisms, and the foregoing hypothesis is still largely based on conjecture and circumstantial evidence.

Furthermore, and at least in part explaining that situation, we are still lacking thorough understanding of the exact role of either or both of the GSK-3 isozymes in normal physiological processes. In brain, where this review is concentrating on, we do not know if either or both of the GSK-3 isozymes act and how, i.e. specifically or overlapping and redundant, or complementing each other in normal neuronal functions or even acting in different types of neurons or in different cell-types. A major fundamental research effort is needed and actually ongoing to answer these questions. In addition, besides the physiological functions are the pathological roles of GSK-3 studied, in first instance and historically

most prominent with respect to the tau pathology in AD. More recently, the kinase was also proposed to regulate the metabolism of the amyloid precursor protein (APP), drawing particular attention to GSK-3 $\alpha$  (Phiel *et al.* 2003).

Here, we review only some of these aspects in the framework of recent data that we obtained in novel transgenic mouse models that robustly recapitulate the pathological problems posed by AD.

### Characteristics of GSK-3 isozymes

Glycogen synthase kinase-3 is the archaic name for two genomically independent encoded isozymes, GSK-3 $\alpha$  and GSK-3 $\beta$ , that operate as multifunctional serine/threonine kinases in metabolic signalling pathways, cytoskeletal organization and transcriptional control. Consequently, GSK-3 $\alpha$ / $\beta$  isozymes have been implicated in cellular responses as diverse as cell fate decisions in embryonic development and dorsoventral patterning, to glucose metabolism and cadherin-based cell adhesion, as well as in physiological and pathological apoptosis. Most experimental work has been focused on the  $\beta$ -isozyme, although GSK-3 $\alpha$  shares most if not all enzymatic properties with respect to protein phosphorylation, including tau and other neuronal substrates.

The two human GSK-3 isozymes are encoded by two different genes located on chromosome 3 (GSK-3 $\beta$ ) and 19 (GSK-3 $\alpha$ ), but they share 98% sequence identity in the catalytic domain and 84% overall (Woodgett 1990). Glycogen synthase kinase-3 $\beta$  is composed of 420 aa translating into a 47-kDa protein, whereas the 483 aa of GSK-3 $\alpha$  result in the larger 51-kDa protein, with the major difference being a glycine-rich N-terminal domain.

Glycogen synthase kinase-3 $\beta$  is widely expressed in all tissues but mainly in the developing and adult brain and most abundant in neurons. It is predominantly found in perikarya and proximal portion of the dendrites in adult neurons, while during development, the GSK-3 $\beta$  isozyme is also localized in axons (Leroy & Brion 1999). During embryonic development, GSK-3 $\beta$  acts as an inhibitory component of the Wnt signalling pathway in cell proliferation. Disruption of the murine GSK-3 $\beta$  gene resulted in embryonic lethality caused by severe liver degeneration (reviewed by Doble & Woodgett 2003). Remarkably, neuronal development appeared normal in surviving GSK-3 $\beta$ -deficient mouse embryos analysed between E16 and P0, which was interpreted that GSK-3 $\alpha$  could fully compensate in this respect (Kim *et al.* 2006). The neuron-specific functions of either GSK-3 isozyme in adult brain remain to be discovered *in vivo*.

Explicit involvement of GSK-3 $\beta$  was shown in the formation of neuronal polarity, requiring the phosphorylation of CRMP-2 to define dendrite–axon specification, in both its establishment and its maintenance (Jiang *et al.* 2005; Kim *et al.* 2006; Yoshimura *et al.* 2005). The presumed or proposed critical role for GSK-3 $\beta$  as shown in cellular models needs now to be translated into physiological and pathological problems of adult and ageing brain *in vivo*.

Brain GSK-3 $\beta$  is proposed to be involved in response to cellular stress, regulation of long-term memory, apoptosis and in maintenance of synaptic plasticity by phosphorylation of CREB, compromising its transcriptional activity (Salas *et al.*

2003). More recently, a direct role was proposed for GSK-3 $\beta$  in long-term potentiation (LTP) and long-term depression (LTD) (Hooper *et al.* 2007; Peineau *et al.* 2007), but its direct upstream and downstream cofactors still need to be identified.

Despite being closely related, the GSK-3 isozymes are not functionally identical. Mice deficient for GSK-3 $\beta$  display embryonic lethality, probably because of tumour necrosis factor- $\alpha$ -dependent hepatocytic apoptosis (Hoefflich *et al.* 2000), suggesting that GSK-3 $\alpha$  cannot compensate for its lacking counterpart in that process. More recently, other functional differences between the GSK-3 isozymes began to emerge, e.g. they differently regulate cell survival and transcriptional activation (Liang & Chuang 2006, 2007). Glycogen synthase kinase-3 $\beta$  but not GSK-3 $\alpha$  appears to be a substrate for different PKC isoforms (Goode *et al.* 1992).

Historically, largely neglected, the GSK-3 $\alpha$  isozyme has only recently gained some status of interest in the AD field, in sharp contrast to GSK-3 $\beta$  that is since long implicated in the tau-related pathogenesis of AD. Data were reported suggesting that GSK-3 $\alpha$  regulated the amyloidogenic processing of the APP (Phiel *et al.* 2003). Because this finding could constitute a fundamental event underlying the AD pathology, the report has attracted great interest but has also stirred intense debate.

### Structure–function aspects of GSK-3: activity strictly regulated by phosphorylation

The substrate GSK-3 $\beta$  consensus motif is a (Ser/Thr)-(Pro/X)-X-X-(Ser/Thr) sequence wherein the C-terminal Ser/Thr residue at position P+4 is preferably phosphorylated. This priming is rather unique among kinases, but priming is not absolutely required for all GSK-3 $\beta$  target sites because some substrates, including tau, can be phosphorylated by GSK-3 without priming (Cho & Johnson 2003, 2004; Twomey & McCarthy 2006).

The crystal structure of GSK-3 $\beta$  has provided insight into its catalytic mechanism (Dajani *et al.* 2001; ter Haar *et al.* 2001). The GSK-3 $\beta$  N-terminal domain is mostly  $\beta$ -sheet and resembles tyrosine kinases, while the C-terminal domain contains mainly  $\alpha$ -helices. The overall structure confirmed primary sequence comparisons suggesting that GSK-3 $\beta$  was related to cdk5 and microtubule-associated protein kinases with a similar  $\alpha$ -helical structure, e.g. cdk5 and ERK2 (Mazanetz & Fisher 2007).

The substrate-binding pocket in GSK-3 $\beta$  contains three basic residues, i.e. Arg96, Arg180 and Lys205, that bind the phosphate anion on the primed Ser/Thr residue in the substrate motif (Dajani *et al.* 2001; ter Haar *et al.* 2001). Mutation of these residues in GSK-3 $\beta$  abolishes its activity towards primed substrates but not towards non-primed substrates (Cohen & Frame 2001). Additional interactions with Gln69, Asn95 and possibly Phe67 secure the position of the substrate in the binding pocket (Ilouz *et al.* 2006). Upon positioning of GS primed at Ser656 by casein kinase II, GSK-3 $\beta$  sequentially phosphorylates GS at Ser652, Ser648, Ser644 and Ser640, each acting as priming site for the next phosphorylation step (Cohen & Frame 2001; Fiol *et al.* 1987).

The same mode of action is exerted by GSK-3 on  $\beta$ -catenin at Ser45, Thr41, Ser37, Ser33 and Ser29 to regulate its stability

in the Wnt pathway (Liu *et al.* 2002). As this and other vital signalling pathways appear to converge on GSK-3, or at least include the kinase as essential signal transducer, its activity must be tightly controlled and controllable in different ways. In this respect, GSK-3 is an unusual kinase because its activity appears in large part to be regulated post-translationally by two different types of phosphorylation. Best known is the phosphorylation of Ser9 in GSK-3 $\beta$  and of the equivalent Ser21 in GSK-3 $\alpha$ , whereby the N-terminal phosphorylated domain acts as a pseudosubstrate and blocks the catalytic site (Dajani *et al.* 2001). This single phosphorylation effectively inhibits the GSK-3 kinases, which explains why in most experimental systems, as in our GSK-3 $\beta$  transgenic mice, the mutant GSK-3 $\beta$ [S9A] was used to act as a constitutionally active kinase (Muylle *et al.* 2006; Spittaels *et al.* 2000, 2002).

Less clear-cut is the role of phosphorylation of Tyr216 in GSK-3 $\beta$  and of the corresponding Tyr279 in GSK-3 $\alpha$  (Hughes *et al.* 1993). On one hand, this was proposed to be an autophosphorylation reaction, and although GSK-3 is known as a typical Ser/Thr kinase, it apparently can act as a tyrosine kinase, at least in *cis* and not in *trans* (Lochhead *et al.* 2006). The tyrosine kinase activity is transient and restricted to a folding intermediate during biosynthesis of GSK-3 $\beta$  and depends on chaperones (Lochhead *et al.* 2006). On the other hand, authentic protein tyrosine kinases could act on the GSK-3 isozymes to phosphorylate Tyr216 or Tyr279 as part of the negative regulation of their kinase activity (Cohen & Frame 2001; Kockeritz *et al.* 2006; Mazanetz & Fisher 2007).

The exact role of the tyrosine phosphorylation has been a matter of controversy because GSK-3 $\beta$  mutated at Tyr216 was reported to retain its active conformation (Dajani *et al.* 2001) as well as its kinase activity (Itoh *et al.* 1995). In contrast, other studies on GSK-3 $\beta$ -Y216 mutants showed almost no kinase activity similar to the kinase-dead mutant GSK-3 $\beta$ [K85M] (Lochhead *et al.* 2006). This is consistent with the rescue of the lethal null phenotype by the active *Drosophila* GSK-3 homologue Shaggy, whereas its kinase-inactive mutant Y214F, equivalent to mammalian GSK-3 $\beta$ [Y216F], was unable to rescue the lethal knockout (Papadopoulou *et al.* 2004).

Structural comparison with the p38 kinase, as its T-loop contains a tyrosine residue that can be phosphorylated, analogous to Tyr216 in GSK-3 $\beta$ , provided insight into this problem. Unphosphorylated Y185 blocked the entry of substrate into the substrate-binding groove of p38 (Bellon *et al.* 1999), and phosphorylation of Y185 reversed this and allowed access of substrate peptides. In analogy, phosphorylation of Tyr216 could act similarly in GSK-3 $\beta$  (ter Haar *et al.* 2001), and evidently Y2798 in GSK-3 $\alpha$ , while a similar mechanism was recently also proposed for cdk5/p25 and ERK2 (Mazanetz & Fisher 2007).

### The lithium connection and relation to axonopathy

For more than 50 years, lithium chloride is in wide use as mood stabilizing agent in patients with bipolar disorders, but it took 30 years before its first molecular target was identified. Today, Li<sup>+</sup> is known to inhibit several enzymes, including

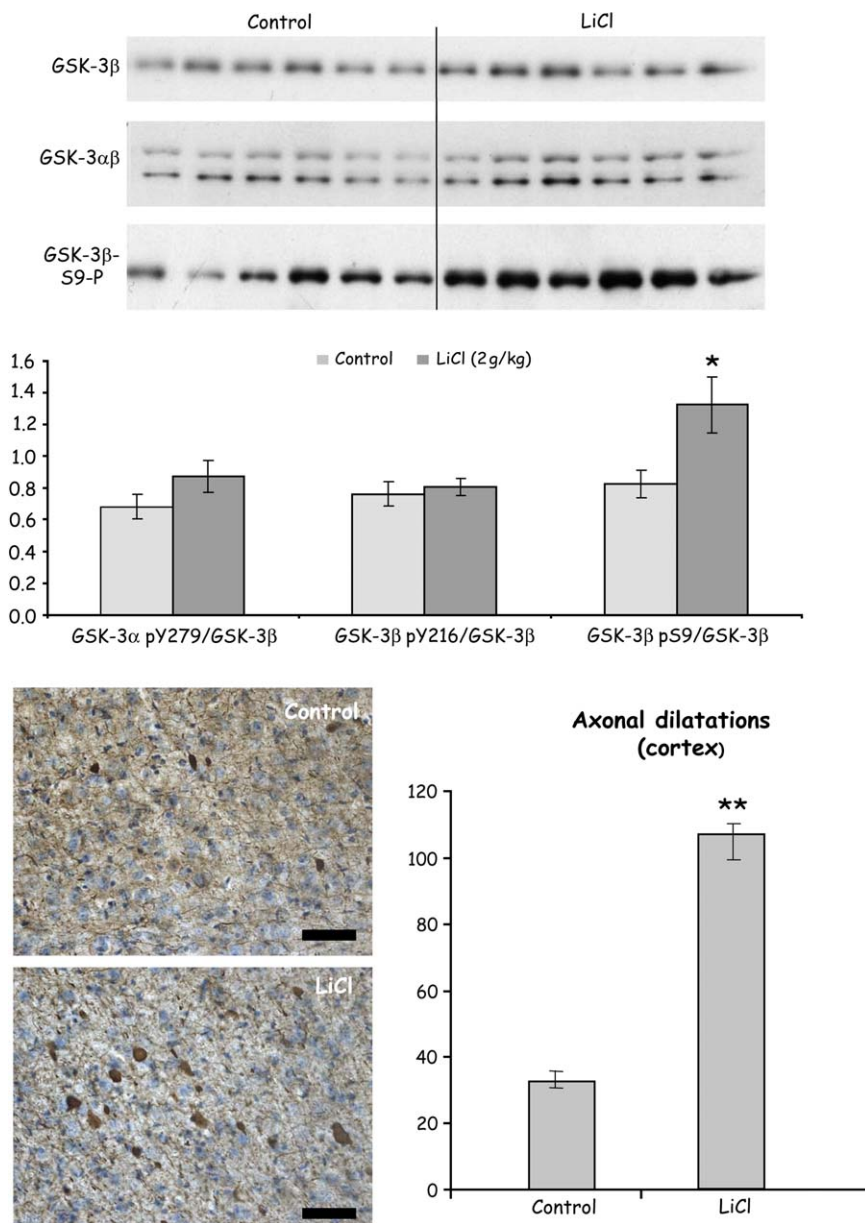
monophosphatase (Berridge *et al.* 1989), phosphomonoesterases (York *et al.* 1995) and GSK-3 (Klein & Melton 1996; Stambolic *et al.* 1996). The inhibition of GSK-3 is exerted directly through competition with magnesium ions (Ryves & Harwood 2001) and indirectly by increasing phosphorylation of GSK-3 on Ser9 by Akt/PKB, which can be reversed by protein phosphatase-1 (De Sarno *et al.* 2002).

In transfected cells, GSK-3-dependent decrease in amyloid peptide production, both A $\beta$ 40 and A $\beta$ 42, was observed after treatment with Li<sup>+</sup>, without indications for cellular toxicity (Su *et al.* 2004; Sun *et al.* 2002). Li<sup>+</sup> appeared to protect neurons against amyloid peptide-mediated cellular toxicity and to prevent A $\beta$ -induced phosphorylation of protein tau (Alvarez *et al.* 1999). Conversely, increased A $\beta$  production by Li<sup>+</sup> was reported to be independent of GSK-3 inhibition (Feyt *et al.* 2005). *In vivo*, in transgenic mice expressing mutant protein tau, Li<sup>+</sup> reduced the hyperphosphorylation of tau and its aggregation, but it could not, however, dissociate already formed tangles (Engel *et al.* 2006; Nakashima *et al.* 2005; Noble *et al.* 2005). In addition, Li<sup>+</sup> was shown to promote ubiquitination of tau in these model systems.

Although Li<sup>+</sup>-mediated inhibition of GSK-3 $\beta$  appeared as a valuable therapeutic target in AD, doubts have been raised on its beneficial effects. In patients treated with Li<sup>+</sup>, the incidence of dementia was actually increased compared with the group that received none or other treatments (Dunn 2005). As Li<sup>+</sup> does more than just inhibiting GSK-3, other beneficial or harmful effects need to be examined. These require more research before a firm recommendation of lithium ions as a preventive or curative drug, and thereby of GSK-3 $\beta$  as a valuable target in AD, can be issued (Terao *et al.* 2006).

To test the effectiveness of LiCl in an experimental model, we have treated our tau-4R transgenic mice (Spittaels *et al.* 1999, 2000) with lithium chloride added to the chow (2 g/kg) for 4 months. The tau-4R mice develop a major axonopathy with wallerian degeneration of axons distal to the dilations, resulting in severe motor impairment (Spittaels *et al.* 1999). This clinical phenotype was explained by the excessive binding of the human transgenic tau-4R to microtubuli, thereby hindering and eventually blocking the passage of kinesin and dynein motor proteins. This is in line with our observation that the entire pathology is rescued by co-expression of human GSK-3 $\beta$ , which is mechanistically explained by the phosphorylation of tau-4R preventing it from binding to the microtubuli, restoring normal axonal transport (Spittaels *et al.* 2000).

In agreement with these early transgenic findings, our current pharmacological data show that Li<sup>+</sup> aggravated the axonopathy of tau-4R mice treated *in vivo* for a prolonged time period (Fig. 1). In parallel with a threefold increase in the number of axonal dilations, the Li<sup>+</sup>-treated tau-4R mice developed a more severe motor impairment (rotarod). In addition, the phosphorylation of Ser9 in GSK-3 $\beta$  was increased by treatment with LiCl relative to untreated tau-4R mice (Fig. 1). Therefore, besides the direct inhibition of GSK-3 $\beta$  by Li<sup>+</sup>, this extra indirect inhibitory effect appears to be exerted by activation of Akt/PKB or by inhibition of protein phosphatases (Caccamo *et al.* 2007; Hong *et al.* 1997; Noble *et al.* 2005).



**Figure 1: Treatment with LiCl inhibits GSK-3β activity and aggravates axonal dilatations in Tau-4R mice.** Upper panel: biochemical analysis of GSK-3β in brain of Tau-4R mice (Spittaels *et al.* 1999) that received normal chow (control) or chow containing LiCl (2 g/kg) for 4 months. Western blotting was performed with antibodies specific for: (1) GSK-3α and GSK-3β isozymes phosphorylated, respectively, on tyrosine Y279 (upper band approximately 51 kDa) and tyrosine 216 (lower band approximately 47 kDa) (Upstate, Temecula, CA, USA) and for (2) GSK-3β-phospho-Ser9 (Cell Signalling, Danvers, MA, USA). This analysis showed only a significant increased phosphorylation of GSK-3β at Ser9 (mean ± SEM,  $n = 6$ ). Lower panel: immunohistochemistry for neurofilaments with antibody SMI-31 (Sternberger, Lutherville, MD, USA) shows a threefold increase in axonal dilatations in Tau-4R mice treated with LiCl (2 g/kg) for 4 months. Significant statistical differences: \* $P < 0.05$ ; \*\* $P < 0.01$ . Scale bars: 50 μm.

In essence, the combined data prove that in tau-4R mice, the action of GSK-3β on axonopathy is evident in both directions, i.e. increasing the GSK-3β activity rescued the axonopathy (Spittaels *et al.* 2000), while inhibition of GSK-3β aggravates the axonopathy. Besides the evident therapeutic implications, discussed further below, the data establish GSK-

3β in its physiological function of controlling the phosphorylation of tau *in vivo*, and thereby its binding to microtubuli and axonal transport.

Similar treatment of our APP-V717I mice (age 10–12 months at the start) for 4 months with lithium chloride added to their chow (2 g/kg) did not affect the levels of the

amyloid peptides A $\beta$ 40 or A $\beta$ 42 in their brain or the amyloid plaque load (results not shown). A higher dose of LiCl (10 mg/kg chow) proved toxic for the amyloid mice on longer treatment, while in short-term treatments (1 week), the effect on brain amyloid levels was significant, i.e. the brain A $\beta$ 40 and A $\beta$ 42 levels decreased to 53 and 71% of those in age- and sex-matched untreated APP-V717I mice (results not shown). Obviously, the effects of LiCl on APP processing are less pronounced, and the needed higher dosage would even imply that different mechanisms are being targeted by Li<sup>+</sup> in the amyloid than in the tau pathology.

### Relation to tauopathy

Glycogen synthase kinase-3 $\beta$  has the longest history in the tau pathology in AD as the more important tau kinase I based on *in vitro* and cellular models (Buee *et al.* 2000; Grimes & Jope 2001; Iqbal & Grundke-Iqbal 2005; Ishiguro *et al.* 1992; Johnson & Stoothoff 2004; Mandelkow 1993). Pathological evidence is, however, limited to colocalization of GSK-3 $\beta$  with different pathological structures, e.g. tangles or argyrophilic grains (Ishizawa *et al.* 2003; Leroy & Brion 1999; Takashima 2006). In this case deregulation/activation of GSK-3 $\beta$  by amyloid has been invoked in AD to explain its role in the ontogenesis of the tauopathy (Welsh *et al.* 1996; reviews by Balaraman *et al.* 2006; Doble & Woodgett 2003; Jope & Johnson 2004; Takashima 2006; Terwel *et al.* 2002), but solid experimental evidence is lacking *in vivo*.

The constitutional overexpression of GSK-3 $\beta$  *in vivo* has, in itself, not yielded a valid model for tauopathy. On the contrary, and unexpectedly, increasing the GSK-3 $\beta$  activity in brain actually rescued the severe axonopathy of tau-4R mice (Spittaels *et al.* 1999, 2000, 2002), as discussed in the previous section. Conditional high-level overexpression of GSK-3 $\beta$  increased the phosphorylation of tau and caused neurodegeneration without authentic tauopathy, with all defects fully reversible (review by Avila *et al.* 2006).

Glycogen synthase kinase-3 $\beta$  mice have impaired LTP in CA1, and conversely, induction of LTP appears to decrease kinase activity, as indicated indirectly by increased phosphorylation of GSK-3 $\beta$  at Ser9 (Hooper *et al.* 2007). In Tau and APP (TAPP) double transgenic mice (Ishizawa *et al.* 2003) and in triple transgenic mice (Billings *et al.* 2007), indications for increased GSK-3 $\beta$  activity were observed, without resolving the relation to or from amyloid and tau pathology.

Active GSK-3 $\beta$  partially localizes in tangle-bearing neurons in brain of Alzheimer patients (Pei *et al.* 1999). Monoclonal antibodies, AD2 and PHF1, that are directed against Ser396/Ser404 and antibody AT100 specific for Thr212/Ser214 identified these residues as specific phosphorylation sites for GSK-3 $\beta$  *in vivo* and *in vitro* (Spittaels *et al.* 1999, 2000; Terwel *et al.* 2005). The appearance of the AT100 epitope correlated with enhanced neuronal toxicity and increased aggregation of tau into fibrils (Jackson *et al.* 2002).

Glycogen synthase kinase-3 $\alpha$ , in contrast to the  $\beta$ -isozyme, has not been studied extensively and was rather unexpectedly claimed as implicated in the amyloid pathogenesis of AD (Phiel *et al.* 2003), as discussed above. No data are known to us that relate GSK-3 $\alpha$  to the tauopathy in AD.

### Overview of recent findings on GSK-3 in relation to AD

Following its first and still most important implication in the hyperphosphorylation of protein tau (Ishiguro *et al.* 1992), GSK-3 $\beta$  has been proposed over the past years to function also in many other, if not all, pathological features of AD from tauopathy to amyloid and from inflammation to memory. Although all are potentially interesting, it can be anticipated that only some hypotheses will withstand experimental scrutiny. It is impossible to cover all aspects of GSK-3 isozymes that might be related to AD in the space provided here, and we have included only some issues that appear close to the problems at hand in this section.

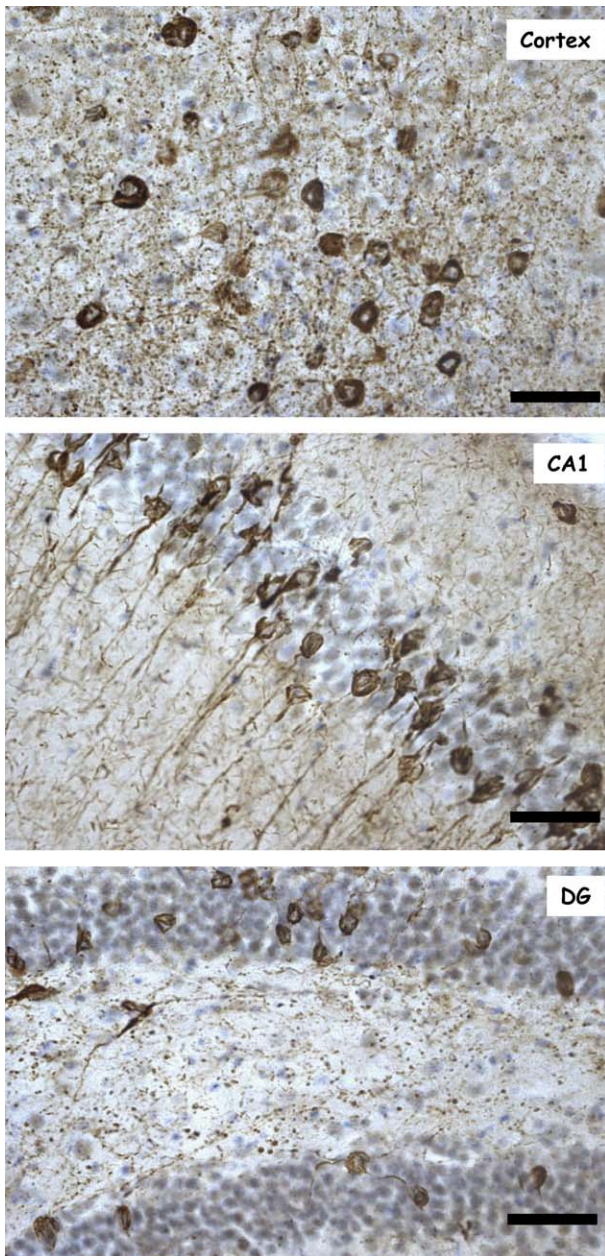
Besides phosphorylation of protein tau, GSK-3 $\beta$  could be involved in tau messenger RNA splicing. Inhibition of GSK-3 $\beta$  activity resulted in colocalization with splicing regulator SC35 in the nucleus. Conversely, the absence of SC35 increased the splice variant of tau containing exon 10, and thereby the tau-4R protein isoform (Hernandez *et al.* 2004). Elevated GSK-3 $\beta$  activity, however, resulted in more tau-3R isoform with reduced microtubule-binding capacity. These mechanisms might be operational during development when the tau-3R isoform prevails, while in adult brain, the tau-4R isoform is more important (Sennvik *et al.* 2007, references therein).

Glycogen synthase kinase-3 was claimed to promote the production as well as the toxicity of A $\beta$  (Takashima *et al.* 1993) and regulate several aspects of APP metabolism (Aplin *et al.* 1997; Kirschenbaum *et al.* 2001; Takashima *et al.* 1998). Recently, it was reported that inhibition of GSK-3 $\beta$  is neuroprotective in APP transgenic mice (Rockenstein *et al.* 2007) and that GSK-3 $\beta$  activity modified the localization and function of prysenilin-1 (PS1), while reducing neuronal viability and synaptic plasticity (Uemura *et al.* 2007).

The combined data comply with the suggestion that GSK-3 $\beta$  is a potential therapeutic target acting on both major pathologies in AD, which has gained further ground by the findings that lithium ions can reduce tauopathy, as well as amyloid pathology *in vivo* (Noble *et al.* 2005; Rockenstein *et al.* 2007).

Nevertheless, inhibition of vesicular trafficking of APP by protein tau in a cellular model did not affect the amyloidogenic processing (Goldsbury *et al.* 2006), indicating that APP is transported by other routes and/or that production of amyloid peptides occurs in other cellular compartments. Moreover, the caveat that lithium ions are by no means specific for GSK-3 $\beta$  needs to be kept in mind.

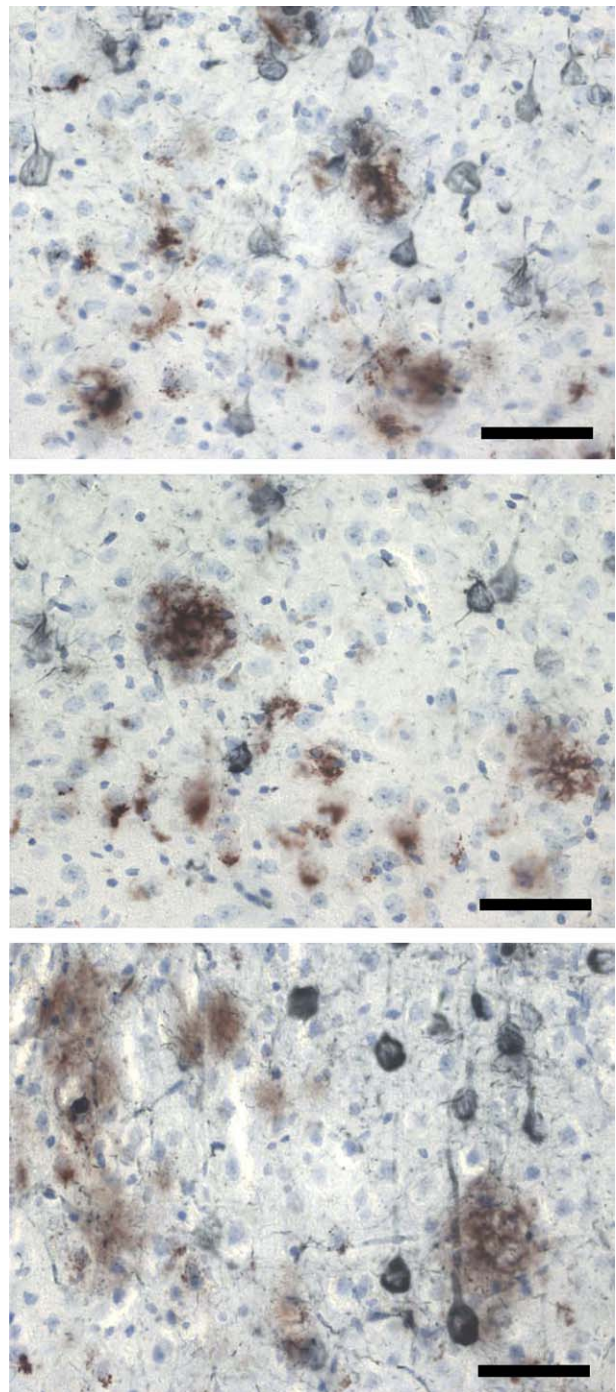
Finally, GSK-3 could function directly in processes that underlie memory and learning. Learning decreased the activity of GSK-3 $\beta$  in triple transgenic mice, concurrent with delaying the formation of amyloid plaques and the hyperphosphorylation of tau, leading to the suggestion that inactive GSK-3 could favour learning capacity (Billings *et al.* 2007). In a more fundamental approach, inhibition of GSK-3 $\beta$  was increased upon induction of LTP in the hippocampus, while expression of GSK-3 $\beta$  impaired the induction of LTP (Hooper *et al.* 2007; our unpublished results). While these data could potentially help clarify the cognitive defects in diseases with aberrant GSK-3 activation, such as AD, the relation of GSK-3 $\beta$  to hippocampal LTP and LTD does appear to be more complex (Peineau *et al.* 2007).



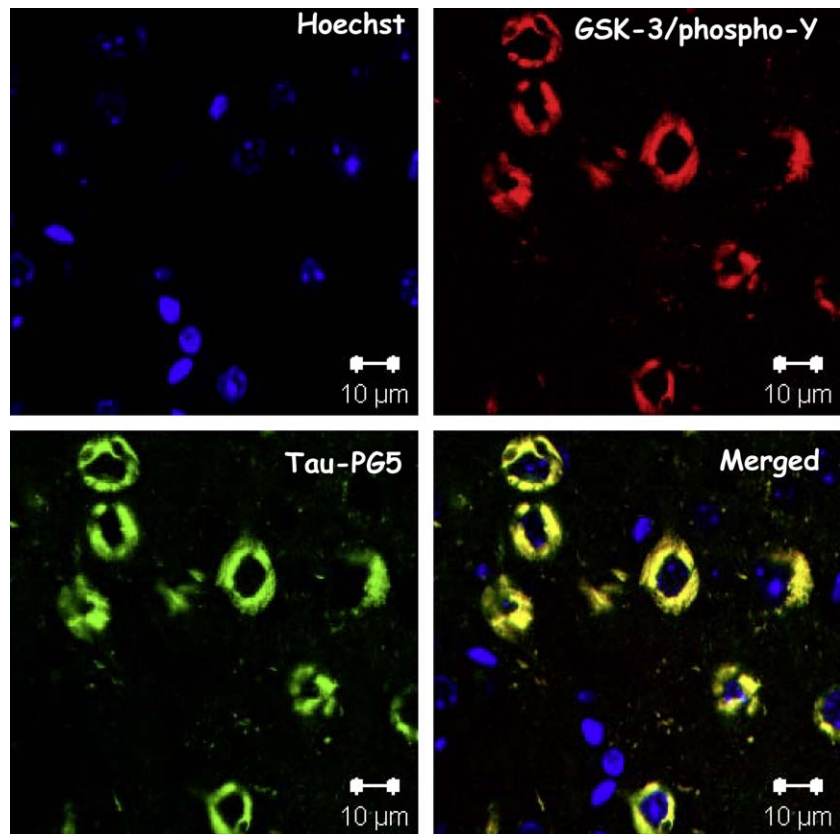
**Figure 2: Severe tauopathy in brain of GSK-3 $\beta$   $\times$  Tau-P301L bigenic mice.** Tauopathy in GSK-3 $\beta$   $\times$  Tau-P301L bigenic mice (20-month-old) showed by staining with antibody AT100 (Pierce, Rockford, IL, USA) in different brain regions: cortex (upper panel) and hippocampus CA1 (middle panel) and dentate gyrus (lower panel). Scale bars: 50  $\mu$ m.

### Combined pathology in AD: contribution of GSK-3 $\beta$ as 'the link'

Neurofibrillary tangles (NFT) are as essential as amyloid plaques for the diagnosis of AD. According to the amyloid cascade hypothesis, amyloid comes first, but the nature of



**Figure 3: Combined amyloid and tau pathology in APP-V717  $\times$  Tau-P301L bigenic mice.** Double immunohistochemistry with antibodies Pan-A $\beta$  (brown, Clontech, Saint-Germain-en-Laye, France) and AT100 (black, Pierce, Rockford, IL, USA) shows combined amyloid and tau pathology, respectively, in cortex of a 17-month-old APP-V717  $\times$  Tau-P301L bigenic mouse. Scale bars: 50  $\mu$ m.



**Figure 4: Colocalization of tauopathy and GSK-3.** Confocal microscopy images of double labelling for GSK-3 $\alpha$  (pY279)/ $\beta$  (pY216) (red, Upstate) and Tau-PG5 (green, P. Davies, New York, NY, USA) in GSK-3 $\beta$   $\times$  Tau-P301L bigenic mice showing colocalization (merged, yellow). Hoechst (blue) is used as nuclear staining. Scale bars: 10  $\mu$ m.

the pathways leading from amyloid peptides to the hyperphosphorylation and aggregation of protein tau remain unresolved. Moreover, whereas GSK-3 $\beta$  is accepted to phosphorylate tau, precise data for its exact contribution in brain *in vivo* remain circumstantial (reviews by Buee *et al.* 2000; Goedert & Ghetti 2007; Grimes & Jope 2001; Johnson & Stoothoff 2004; Terwel *et al.* 2002).

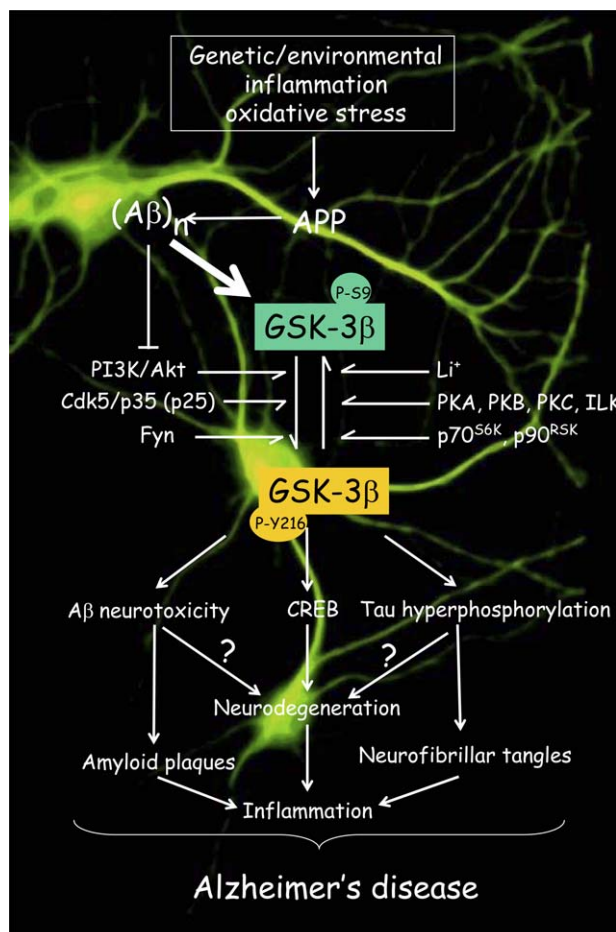
Based on our Tau-P301L transgenic mice that develop a moribund tauopathy with hyperphosphorylation and aggregation of protein tau (Terwel *et al.* 2005), we have now developed double transgenic Tau-P301L  $\times$  GSK-3 $\beta$ [S9A] mice. These mice develop a dramatic forebrain tauopathy with NFT in cortical and hippocampal neurons, which become literally packed with filamentous tangles (Fig. 2). The Tau-P301L  $\times$  GSK-3 $\beta$  bigenic model thereby provides the missing pathological and biochemical evidence that the contribution of GSK-3 $\beta$  is essential for the hyperphosphorylation of protein tau that causes aggregation in neurons *in vivo*.

In parallel, we generated APP-V717I  $\times$  Tau-P301L double transgenic mice that present full-blown combined AD-like pathology, consisting of amyloid plaques and NFT (Fig. 3). The amyloid pathology is similar in aspect and timing but more intense than in the parental APP-V717I mice, which lack overt tauopathy like all amyloid-only mouse models (Moechars *et al.* 1999; Van Dorpe *et al.* 2000; Van Leuven 2000). In the APP-V717I  $\times$  Tau-P301L double transgenic

mice, the tauopathy is dramatically and robustly enhanced in the hippocampus and cortex relative to the parental Tau-P301L mice, exposing a marked synergism between both major pathologies of AD in this model (Muylleert *et al.* 2006). Moreover, GSK-3 colocalizes with the tangles in sick neurons (Fig. 4) similar as in AD brain (Leroy & Brion 1999; and references therein).

Combining our data obtained in single and the novel double transgenic mice underlines the role of GSK-3 $\beta$  as the most potent tau kinase *in vivo* and, most importantly, establish the GSK-3 isozymes firmly as molecular link from the amyloid to the tau pathology, thereby exerting a pivotal role in the pathogenesis of AD (Fig. 5).

We conclude that the combined data show a direct and mechanistic involvement of GSK-3 $\beta$  in the pathogenesis in AD, whereby the apparent sequence of events is starting with an amyloid overload that activates GSK-3 $\beta$  to increase the phosphorylation of protein tau. This does not appear to be a side reaction of the pathogenesis because tauopathy is an integral part of the problem in all AD cases, including early-onset familial cases. Moreover, the most recent experimental evidence shows an essential role for tau in the cognitive defects induced by amyloid in a mouse model (Roberson *et al.* 2007). The mouse models will now be instrumental to define the molecular factors that are upstream and downstream of GSK-3 $\beta$  in the signalling pathway from amyloid to AD (Fig. 5).



**Figure 5: Schematic representation of GSK-3 fitting into the overall pathogenesis in AD.**

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### Conflicts of interest

This article was presented at a symposium on *Alzheimer's disease – new insights from animal models and molecular pathways, to be translated into human pathology*, which took place at the Genes, Brain and Behavior 2007 Society Annual Meeting, 21–25 May 2007, Doorwerth, the Netherlands. The symposium was sponsored by the European Commission [Marie Curie Early Stage Training (MEST)-CT-2005-020013, neurodegeneration in Alzheimer's Disease (NEURAD), Alzheimer Ph.D Graduate School].

The authors declare no conflicts of interest.