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Animal models reveal role for tau phosphorylation in human disease

Jürgen Götz [a,*], Amadeus Gladbach [a], Luis Pennanen [b], Janet van Eersel [a], Andreas Schild [c], Della David [d], Lars M. Ittner [a]

[a] Alzheimer's and Parkinson's Disease Laboratory, Brain and Mind Research Institute, University of Sydney, 100 Mallett Street, Camperdown, New South Wales 2050, Australia
[b] University of California, San Francisco, Department of Psychiatry, Rock Hall, 1550 4th Street, San Francisco, CA 94158-2822, USA
[c] Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
[d] University of California, San Francisco, Genentech Hall, 600 16th Street, San Francisco, CA 94158-2517, USA

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ABSTRACT

Many proteins that are implicated in human disease are posttranslationally modified. This includes the microtubule-associated protein tau that is deposited in a hyperphosphorylated form in brains of Alzheimer's disease patients. The focus of this review article is on the physiological and pathological phosphorylation of tau; the relevance of aberrant phosphorylation for disease; the role of kinases and phosphatases in this process; its modeling in transgenic mice, flies, and worms; and implications of phosphorylation for therapeutic intervention.

1. Introduction

The Alzheimer's disease (AD) brain is characterized by massive neuronal and synaptic loss at specific predilection sites [1]. Together with tau-containing neurofibrillary tangles (NFTs) and β-amyloid (Aβ) plaques, they are the defining histopathological hallmarks of AD. Neurofibrillary lesions are found in cell bodies and apical dendrites as NFTs, in distal dendrites as neuropil threads, and in the abnormal neurites that are associated with some β-amyloid plaques (neuritic plaques) [2]. NFTs are also abundant, in the absence of overt plaques, in certain subsets of frontotemporal dementia (FTD) and other tauopathies [3]. The neurofibrillary lesions contain aggregates of the microtubule-associated protein tau that in the course of disease is redistributed from the mainly axonal to the somatodendritic compartment of neurons [4]. In tauopathies such as progressive supranuclear palsy (PSP) or corticobasal degeneration (CBD), tau forms aggregates also in non-neuronal cells [5].

Tau is a phosphoprotein owing to its unusually high serine and threonine content. Under pathological conditions, tau is hyperphosphorylated, meaning that it is phosphorylated to a higher degree at normal, physiological sites, and at additional "pathological" sites [6] (Fig. 1). Phosphorylation of tau tends to dissociate it from microtubules. Tau also undergoes a conformational change which may assist in differential phosphorylation, and vice versa [7].

The *MAPT* (*microtubule-associated protein tau*) gene encoding tau is located on human chromosome 17q21; in the central nervous system it contains 15 exons, with the major tau protein isoform being encoded by 11 exons [8]. By alternative mRNA splicing of exons 2, 3, and 10, six major tau isoforms are produced in the adult human brain. They differ by the presence or absence of one or two short inserts in the amino-terminal half (0N, 1N, or 2N, respectively), and have either three or four microtubule-binding repeat motifs in the carboxy-terminal half (3R or 4R) (Fig. 1). In contrast to humans, mice express only four-repeat tau isoforms (4R0N, 4R1N, or 4R2N) [9].

In AD, no mutations were found in the *MAPT* gene; these were identified in FTD with Parkinsonism linked to chromosome 17 (FTDP-17) [10–12] (Fig. 1). This established that dysfunction of tau in itself can cause neurodegeneration and lead to dementia. In a subgroup of FTD, termed FTLD-U (frontotemporal lobar degeneration with ubiquitin-immunoreactive lesions) or FTDU-17, tau-negative, ubiquitin-positive lesions are prominent. In these patients, mutations were found in the *PGRN* gene encoding progranulin, a growth factor

* Corresponding author. Tel.: +61 2 9351 0789; fax: +61 2 9351 0731.
E-mail address: jgoetz@med.usyd.edu.au (J. Götz).

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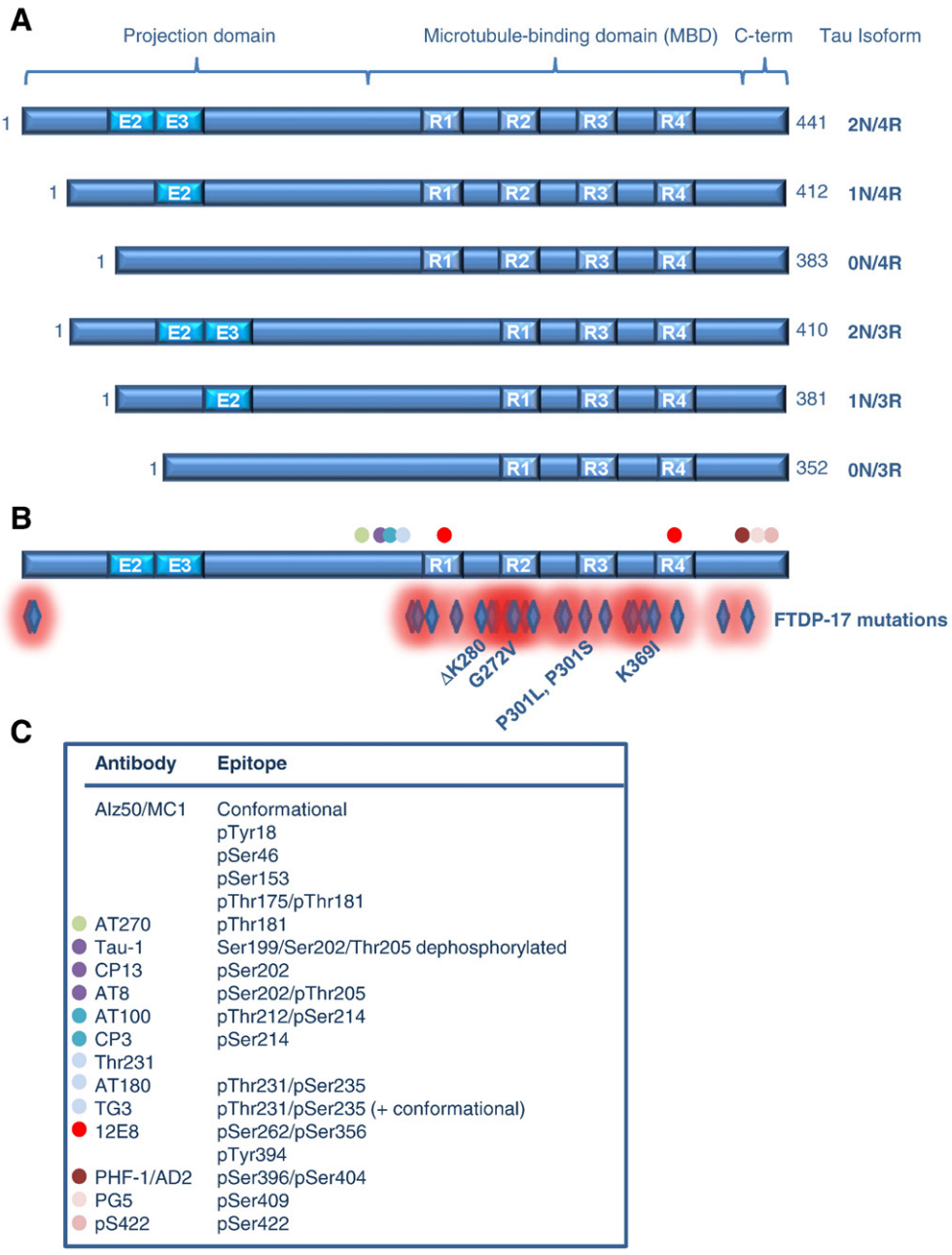

Fig. 1. The microtubule-associated protein tau. (A) Tau is composed of an amino-terminal projection domain, a microtubule-binding domain (MBD), and a carboxy-terminus. Human brain expresses six major tau isoforms. They differ by the presence or absence of one or two 29 amino acid inserts (E2, E3) in the amino-terminal half (0N, 1N, or 2N, respectively), and have either three or four microtubule-binding repeat motifs in the carboxy-terminal half (3R or 4R). (B) Until today, 42 pathogenic exonic and intronic mutations have been identified in the *MAPT* gene encoding tau. Most of the exonic mutations are clustered around the MBD. Of these mutations, several have been expressed in transgenic mice including N279K, ΔK280, G272V, P301L, P301S, V337, and R406W. (C) Selected phospho-epitopes mentioned in this review are shown for clarity along with the name of the phospho-tau-specific antibody. Note: These phospho-epitopes are conserved between humans and mice.

involved in multiple physiological and pathological processes including tumorigenesis [13,14]. It was found that the TAR DNA-binding protein of 43 kDa (TDP-43) is a primary constituent of the ubiquitin-positive inclusions in FTLD-U and amyotrophic lateral sclerosis [15]. Under pathological conditions, TDP-43 is, similar to tau, hyperphosphorylated, ubiquitinated, and carboxy-terminally truncated [16].

2. Serine- and threonine-directed phosphorylation of tau

Of all posttranslational modifications, protein phosphorylation in general receives special attention as it is an important cellular regulatory mechanism [6]. It determines enzymatic activity, protein stability, folding properties including protein aggregation, binding of other bio-molecules, and subcellular localization. Phosphorylation occurs mainly on serine, threonine, and tyrosine residues, although proteins can be phosphorylated also on histidine, lysine, and arginine residues. Phosphorylation depends on a balanced interplay between kinases and phosphatases that is disturbed under pathological conditions. Phosphorylation can also be affected by O-glycosylation since some serine and threonine residues can either be O-glycosylated or phosphorylated [17–20].

2.1. Phosphorylation of normal, fetal and 'PHF'-tau

Tau is a phosphoprotein with a remarkably high number of potential phosphorylation sites. The longest human tau isoform in

brain, htau40, contains 45 serines and 35 threonines, of which 17 are followed by prolines (so-called SP/TP-sites). A KXGS motif is found in each microtubule-binding domain making this site a substrate of MARK kinase [21]. In addition, there are four tyrosine residues [6]. Overall, 20% of the protein is composed of amino acids that can be potentially phosphorylated. Therefore, it is no surprise that differential phosphorylation of tau is crucial for its physiological and pathological activities. It is then also logical that phosphorylation of tau within the microtubule-binding domain (e.g., at the KXGS motives recognized by an antibody such as 12E8 specific for pSer262/pSer356[1], see below) has functional consequences that are different from those of phosphorylation of tau within the flanking sequences (Fig. 1).

Fetal brain contains only 3R tau that is phosphorylated at more sites than tau from adult brain (that contains an equal mix of 3R and 4R tau), implying selective dephosphorylation of the short isoforms during brain maturation [22]. In the developing axon, tau forms a smooth proximo-distal phosphorylation gradient: Tau in the soma, immature dendrites and the proximal axon is phosphorylated up to 80% at the tau-1 antibody site (Ser198/Ser199/Ser202 based on htau40), compared to only 20% in the axonal growth cone [23,24].

In the AD brain, tau is hyperphosphorylated meaning that it is phosphorylated to a higher degree at physiological sites as well as, in addition, pathological sites (Fig. 1). This tau associates to form paired helical filaments (PHFs) within NFTs. Thus pathologically phosphorylated tau is also called "PHF"-tau [25,26]. Pathological sites include pSer422, pThr212/pSer214 (AT100), and pThr231/pSer235 (recognized by the conformation- and phosphorylation-dependent antibody PHF-27/TG3, as well as AT180) [7,27–29]. Luckily, the tau research community early on benefited from an excellent collection of epitope-specific antibodies although in recent years the costs for some commercial antibodies have become exorbitantly high (Fig. 1).

Compared to normal tau, PHF-tau displays a reduced electrophoretic mobility reflecting an increased phosphorylation state. As phosphorylation of 'PHF'-tau is very heterogeneous, it runs as a smear on SDS–PAGE gels [30–34]. Upon dephosphorylation, the smear can be resolved into discrete bands of a reduced mobility. PHF-tau also has a lower range of pI values, indicating that it is more negatively charged than normal tau [35]. It has a reduced capacity to bind tubulin and to promote the polymerization and stabilization of microtubules. Upon dephosphorylation, PHF-tau binds to tubulin in a manner that is identical to that of normal tau [36,37]. Using mass spectrometry and phospho-specific antibodies, an increasing number of potential phosphorylation sites have been confirmed in the AD brain, suggesting that as many as 45 of the 84 possible sites of tau are phosphorylated in PHF-tau, compared to 15 in control and up to 20 in fetal brain [38–43]. Obviously, there may be sites that have been missed due to a lack of sensitivity [43]; however, the question is whether these additional phospho-sites are relevant for disease initiation and/or progression.

Many phosphorylation sites have been confirmed with phosphorylation-dependent and epitope-specific anti-tau antibodies (Fig. 1). This assisted in correlating differential phosphorylation with disease progression. It was shown that in the pre-NFT state, when tau has not yet formed filaments, neurons were stained specifically with antibody TG3 (conformational and pThr231/pSer235-specific), and the phosphorylation-dependent antibodies pSer262 and pThr153. Intracellular NFTs were most prominently stained with antibodies against pSer46, pThr175/pThr181, pSer214, pSer262/pSer356 (12E8), and pSer422, whereas extracellular NFTs, the so-called ghost tangles, were most prominently stained with the AT8 (pSer202/pThr205), AT100 (pThr212/pSer214), and PHF-1/AD2 (pSer396/pSer404) antibodies, which also stain intraneuronal NFTs. The different states of tau phosphorylation associated with the different stages in the progression of the disease suggest a sequential phosphorylation process which ultimately leads to NFT formation [44]. Sequential phosphorylation is also evident with regards to individual phospho-epitopes. For example, the AT100 epitope is generated *in vitro* by sequential phosphorylation, first of Ser199, Ser202, and Thr205 (flanking the AT8 epitope), then of Thr212 (by GSK-3β), and finally of Ser214 (by PKA) [45].

[1] For clarity, subsequently first the phosphorylated tau epitope (pS202 or S202) is listed followed by the phospho tau-specific antibody in brackets.

3. Tyrosine-directed phosphorylation of tau

Tau in NFTs is also phosphorylated at tyrosine residues (Fig. 1). There are only 4 tyrosines in tau compared to 80 serines and threonines, which may reflect the relative importance of tyrosine phosphorylation in physiological and pathological functions, as well as the low number of studies. As for the Ser/Thr-epitopes, there are specific antibodies for the Tyr epitopes (reviewed in [6]). c-Abl has been suggested as a candidate kinase for Tyr394 phosphorylation, a site phosphorylated in both fetal and PHF-tau [46].

Tyrosine phosphorylation plays a pivotal role in the association of tau with the plasma membrane, which is mediated by the amino-terminal projection domain of tau [47]. Association of tau with the plasma membrane may play a crucial role in relaying extracellular signals. With its proline rich sequences in the amino-terminus, tau interacts with the SH3 domains of the tyrosine kinase Fyn that phosphorylates tau at Tyr18 [48,49]. In a study that goes far beyond the mapping of phosphorylation sites, Bhaskar and colleagues addressed the question why an altered ratio of 3R to 4R tau might cause disease in FTD [50]. They used the surface plasmon resonance biosensor technique on a panel of tau constructs and found that the interaction between the SH3 domain of Fyn and 3R-tau was 20-fold higher than that for 4R-tau. In addition, the affinity between 4R-tau and Fyn SH3 was increased 25- to 45-fold by phosphorylation-mimicking mutations or by FTDP-17 mutations, supporting a role for the Fyn–tau interaction in neurodegeneration [50].

4. Tau phosphorylation in tau transgenic mice

Classical transgenic approaches continue to be instrumental in dissecting pathogenic mechanisms and testing therapies for AD and FTD [51,52]. There are reports, however, of viral delivery, such as with the Sindbis virus which was employed to express tau in selected hippocampal sites, inducing a pathological conformation of tau as shown by Alz50/MC1-reactivity, which caused an accumulation of insoluble tau and induced a region-specific neurodegeneration [53].

The first published tau transgenic mouse model expressed the longest human four-repeat (2N4R) tau isoform under control of the neuron-specific human Thy1 promoter [54]. Reflecting the approximately 10-fold lower levels of human relative to endogenous murine tau, the phosphorylation-specific anti-tau antibodies AT8 and PHF-1/AD2 revealed strongly labeled neurons in many brain areas, but their numbers were small. However, as in the AD brain, tau was re-localized to the somatodendritic domain, in addition to tau's physiological axonal localization. Gallyas silver impregnation failed to reveal tau filament and NFT formation, and tau staining appeared homogenous or, at the most, granular [54]. A comparable tau phenotype was achieved in mice which expressed the shortest human tau isoform, using the murine 3-hydroxy-methyl-glutaryl CoA reductase promoter. Here, tau was phosphorylated at the AT180 (pThr231/pSer235), AT270 (pThr181), AD2 (pSer396/pSer404), 12E8 (pSer262/pSer356), but not the AT8 (pSer202/pThr205) epitopes [55] (Fig. 1).

Subsequently, a more advanced phenotype was achieved in three wild-type tau transgenic strains, by choosing better expression vectors [56–58]. Tau was found to be phosphorylated at several phospho-epitopes, but NFTs did not develop until the mice reached a very old age [59]. In all three mouse strains, neurofilament-containing

axonal spheroids formed in brain and spinal cord, representing focal axonal dilatations. Orthograde fast axonal transport was reduced as the mice developed a progressive motor phenotype [56]. Phosphorylation of tau was analyzed in more detail in one of the strains, *ALZ17*, where it turned out to be compartmentalized [60]. In the axons of CA1 pyramidal neurons, tau was specifically phosphorylated at the AD2 epitope PSer396/pSer404, concomitant with increased levels of cyclin-dependent kinase-5 (cdk5). In contrast, the 12E8 (pSer262/pSer356) and AT180 (pThr231/pSer235) epitopes were specifically phosphorylated in dendrites, and co-localized with increased levels of GSK-3β [60]. Kinase activities have also been monitored in *htau* mice, which express genomic wild-type tau (the *8c* line [61]), on a tau knockout background [62]. The authors found a significant correlation between specific phosphorylation changes and the amount of aggregated tau. In the *htau* line, but not in non-NFT-forming control mice, there were increased levels in phosphorylated (i.e., activated) p38 and the neuronal cdk5 activators, p35 and p25, with aging, which in turn phosphorylate tau. Changes in tau kinases in the *htau* mice correlated with the amount of tau present in abnormal conformations and with insoluble tau [63]. Together this shows that tau is subject to differential phosphorylation, due to differential activities and compartmentalization of kinases and phosphatases.

The tau field experienced a major advance with the identification of both exonic and intronic mutations in the *MAPT* gene encoding tau in FTDP-17, a familial dementia related to AD [10–12]. The mutations affect the ratio of 3R to 4R tau isoforms and the propensity of tau to form fibrils. By expressing FTDP-17 mutant tau in transgenic mice, many models were established that represented NFT formation in both neuronal and glial cells [64,65]. The transgenic models were examined using transcriptomic and proteomic technology, including the assessment of posttranslational modification [66–70], and they were subjected to behavioral analyses [71,72]. Phosphorylation patterns, however, are difficult to compare as each laboratory uses its own set of phospho-tau-specific antibodies [5]. In P301S mutant tau transgenic mice, neuronal loss was more pronounced than in the P301L tau models, consistent with the early onset of FTD in patients carrying the P301S mutation [73]. In the P301S mice, the largest number of stained neurons was observed with AT8, followed by AP422 (pSer422), AT180 (pThr231/pSer235), and PG5 (pSer409) [73]. Fewer neurons were labeled by AT100 (pThr212/pSer214) and CP3 (pSer214), with the smallest number of neurons being stained by 12E8 (pSer262/pSer356) and PHF-1 (pSer396/pSer404). Western blotting of perchloric acid-soluble tau demonstrated that the human tau band was strongly immunoreactive with all tested antibodies, but AT100 (pThr212/pSer214) and CP3 (pSer214). In contrast, sarkosyl-insoluble human tau protein reacted with all phosphorylation-dependent anti-tau antibodies, including AT100 and CP3. This indicates that immunoreactivity for pSer214 (part of epitope AT100) closely mirrors the presence of filaments, suggesting that phosphorylation of this site occurs in the course of, or after, filament assembly. The first P301L transgenic strain generated, *JNPL3*, is characterized by a pronounced motor phenotype that is not seen in the related P301L transgenic *pR5* mice [74,75].

When we monitored NFT formation and tau phosphorylation in these *pR5* mice for up to 24 months of age we found that NFTs first appeared in the amygdala at 6 months, while at 24 months the only other site with overt NFTs was the CA1 region of the hippocampus [76]. A histological analysis revealed an increase in phosphorylation at the AT180 (pThr231/pSer235), AT270 (pThr181), and 12E8 (pSer262/pSer356) epitopes with aging while the AT8 (pSer202/pThr205) and pS422 epitopes behaved differently. Firstly, whereas AT8 reactivity was found in both the soma and dendritic branch of CA1 pyramidal neurons, pS422 staining was more confined to the soma. Secondly, numbers of AT8-positive neurons increased from 3 to 6 months of age, but at 20 months of age, the only neurons left with AT8-reactivity were those which had undergone NFT formation. In contrast, pS422-reactivity came up only late and concomitantly with NFT formation [76]. As far as the disappearance in our *pR5* mice of the AT8 epitope in all but NFT-bearing neurons is concerned, one explanation may be that with advanced pathology, neurons learn to cope with AT8 phosphorylation and the activities of the kinase and/or phosphatase regulating phosphorylation of this epitope become balanced resulting in a zero net phosphorylation at this epitope [6,77]. This balancing act seems to be specific for the AT8 epitope and the enzymes governing its phosphorylation, as other epitopes such as AT180 or AT270 continue to be phosphorylated. In NFT-bearing neurons, however, the AT8 phospho-epitope becomes stabilized suggesting that either a tau phosphatase may not be able to access the phosphorylation site or the kinase/phosphatase equilibrium in the dysfunctional neuron may be out of balance. Our AT8 findings are supported by data in *P301S* transgenic mice which, although analyzed only until 6 months of age, reveal an increased pS422, AT8, and AT180 phosphorylation in RIPA-extracted brains that peaks at 4–5 months and then declines significantly at 6 months. This decline was not seen with AT270 or phosphorylation-independent tau antibodies [78]. The authors state that the reduction at 5 months was particularly marked for the AT8 epitope [78]. The importance of both the AT8 and pS422 epitope in tauopathies is also demonstrated by the fact that in both the *JNPL3* and the *rTg4510* strain discussed below, a 170 kDa band linked to clinical features was preferentially detected by AT8 and pS422, and this, possibly oligomeric, species negatively correlated with memory [79].

To determine whether NFTs are central to the neurotoxic cascade in AD or represent a protective neuronal response, P301L transgenic *rTg4510* mice were generated [80]. Here, transgene overexpression could be reduced by adding doxycycline to the drinking water [80]. Despite the fact that even under suppressed conditions tau levels were higher than what is generally achieved by conventional transgenic approaches, memory function recovered and number of neurons stabilized, while NFTs continued to accumulate under these conditions. These data show that dysfunction of tau impairs memory, when mutant tau is massively overexpressed. They further imply that soluble tau rather than NFTs themselves are neurotoxic [80]. Tau phosphorylation in the *rTg4510* brain was addressed in an accompanying paper, monitoring the mice from 1.3 to 8.5 months of age [81]. Differences were found between the hippocampus and cortex as AT8 reactivity was preceded by TG3 in the hippocampal CA1 region in an order which is opposite to that found in the cortex. In the CA1 region the epitopes appeared in the following order: CP13 (pSer202), MC1 (conformational), and TG3 (conformational and pThr231/pSer235) at 1.3 months, followed by PG5 (pSer409), AT8 (pSer202/pThr205), PHF-1 (pSer396/pSer404), followed by Bielschowsky reactivity at 4–5.5 months. For the cortex, MC1, CP-13, AT8, and PHF-1 appeared first, followed by TG3, PG5, and Bielschowsky reactivity [81]. This appearance of phosphorylation sites raises the question as to whether for tau to aggregate there needs to be a specific order of phosphorylation events, an issue discussed below in more detail.

An interesting model of the specific phosphorylation profile that characterizes Pick's disease is the K3 strain that expresses human tau carrying the FTD mutation K369I [82]. K3 mice develop a progressive histopathology that is reminiscent of human FTD with the K369I mutation [83]. Specifically, as in human Pick's disease [84] and in the human K369I patient where the tau-containing Pick bodies are 12E8 (pSer262/pSer356)-negative [83], K3 mice also develop ovoid tau inclusions that are 12E8-negative, while additional phospho-epitopes of tau (as in humans) are strongly phosphorylated [82]. K3 mice show an early-onset memory impairment and amyotrophy, in the absence of overt neurodegeneration. However, as the mice age, neurodegeneration becomes evident. Different from our previously generated tau transgenic strains, the K3 mice express the transgene in the substantia nigra (SN) and show an early-onset motor phenotype that reproduces

Parkinsonism with tremor, bradykinesia, abnormal gait, and postural instability. Interestingly, motor performance of young but not old K3 mice improved upon L-dopa treatment, which bears similarities to Parkinsonism in FTD. The early-onset symptoms in the K3 mice are mechanistically related to selectively impaired anterograde axonal transport of distinct cargos, which precedes the loss of dopaminergic SN neurons that occurs in aged mice [82]. The impaired axonal transport in SN neurons affects, among others, vesicles containing the dopamine-synthesizing enzyme tyrosine hydroxylase (TH) [82]. We found that phosphorylated tau interacts pathologically with the kinesin-associated adapter protein JIP1 both in the mice and in AD brain [82,85]. We proved that phosphorylation of tau is required for this pathogenic effect [85] suggesting a pathological interaction of JIP1 and phosphorylated tau as a general pathomechanism in tauopathies including AD.

Using an elegant stereotaxic injection approach, tau toxicity was shown to bear resemblance to prions and to spread through the brain [86]. The study used two mouse strains: *ALZ17* mice that express high levels of wild-type human tau but reveal only a modest pathology: amyotrophy in the absence of obvious neuronal cell loss and, despite a massive hyperphosphorylation of tau, no formation of NFTs [58,60]. In contrast, *P301S* mice express, at levels comparable to the *ALZ17* mice, a mutant form of tau found in familial cases of FTD; the mice present with a particularly robust phenotype, characterized by neurodegeneration in the spinal cord and an abundance of NFTs [73]. When diluted brain extracts from 6-month-old *P301S* mice were intracerebrally injected into 3-month-old *ALZ17* mice and the injected mice were analyzed up to 15 months post-injection, NFT formation was found as revealed by Gallyas silver impregnation, and reactivity with antibody AT100 (pThr212/pSer 214).

Interestingly, Clavaguera and colleagues found that Gallyas reactivity (i.e., NFT formation) was not confined to the site of injection, but rather induced up to 2 mm distant of the injection site [86]. A time-course analysis suggests a stereotypical mode of spreading (a feature characteristic of AD). Another remarkable finding was that insoluble rather than soluble tau was responsible for the induction of a tau pathology. Also, induction of the tau pathology seemed to be mediated by oligodendroglia (a cell type not affected by tau pathology in the parental *P301S* mice).

Additional tau transgenic models have been discussed by us in detail elsewhere [5,65,87]. Taken together, tau transgenic models have proven *in vivo* that the presence of familial FTD-associated tau mutations causes tau hyperphosphorylation, aggregation, nerve cell dysfunction, as well as neuronal and glial cell loss. They have revealed that a glial tau pathology can affect neuronal functioning and that a tau pathology in general causes behavioral impairment [88]. Finally, the models highlight distinct phosphorylation sites such as pS422, pThr212/pSer214 (AT100), pThr231/pSer235 (TG3), and pSer202/pThr205 (AT8) in disease initiation and progression and, hence, provide a means to target distinct kinases for therapeutic intervention.

5. Tau phosphorylation in kinase transgenic mice

In principal, tau phosphorylation can be brought about by the upregulation of kinases, the downregulation of phosphatases, or both. A major question asked in the field is which kinase(s) and phosphatase(s) in brain bring about the pathological changes that characterize AD-tau and how these are related to neuronal demise. The human genome encodes a total of 516 protein kinases, with numbers comparable in mice. Of these kinases, several have been identified as potential tau kinases, mostly by *in vitro* assays incubating recombinant tau protein with the respective kinases. These include GSK-3β; Cdk5; the MAP kinases JNK, ERK, and p38; MARK; CK2; DYRK1A; TTBK1; and P70S6 kinase [43,89–99]. These experiments assisted in determining which kinases phosphorylate which phospho-epitopes of tau and whether there is a sequential phosphorylation of sites, such as for phosphorylation of Thr231, Ser396, and Ser400 by GSK-3β that depends on a previous phosphorylation of Ser235, Ser400, and Ser404, respectively, to provide one example [100,101]. A few of these candidate kinases have been expressed in transgenic mice and tau phosphorylation has been analyzed, either in single-transgenic mice or after crossbreeding with tau transgenic mice. The findings confirmed that kinases play an important role in NFT formation and neurodegeneration. The bulk of studies concentrated on two kinases, GSK-3β and Cdk5, as outlined below.

The kinase GSK-3β is inactivated by phosphorylation of residue Ser9. Mice with a constitutive active S9A form of GSK-3β showed increased activity, in the absence of neurofibrillary pathology. Interestingly, crossing of these mice with human wild-type tau transgenic mice markedly improved the axonopathy and motor deficits that characterize the latter strain [102]. These findings would therefore suggest that GSK-3β is protective. However, additional studies using different models reached the opposite conclusion. When mice with an inducible expression of GSK-3β were crossed with mice that express tau with three FTDP-17 mutations combined (*VLW* mice), the double-transgenic mice developed thioflavin S-positive tau aggregates and tau filaments. Moreover, the atrophy of the dentate gyrus of the hippocampus, which was present in the single-transgenic GSK-3β mice, was accelerated in the double-transgenic mice [103]. To explore whether the phenotype resulting from increased GSK-3β activity could be reverted following restoration of normal GSK-3β levels, transgene expression was shut down in symptomatic mice. This led to normal GSK-3β activity, normal phospho-tau levels, diminished neuronal cell death, and suppression of the cognitive deficits [104]. When an inducible system was used to express a dominant negative mutant form of GSK-3β, this caused apoptosis and a reversible motor deficit [105]. In these mice, AT8 phosphorylation (pSer202/pThr205) was reduced by 72% and PHF-1 phosphorylation (pSer396/pSer404) by 46%. So in conclusion, these data identify GSK-3β as a tau kinase *in vivo*.

Another kinase that has been expressed in transgenic mice is Cdk5. To address its role in tau pathogenesis, P301L tau transgenic *JNPL3* mice were crossed with mice transgenic for the Cdk5 activator p25, the latter being expressed under control of the *NSE* promoter. This caused a fivefold increase in NFT numbers in double-transgenic mice, along with hyperphosphorylation of tau at the putative cdk5 epitopes pThr181 (AT270), pSer202, pThr231, and pSer396/pSer404 (AD2/PHF-1) [106]. On the other hand, a p25-mediated overactivation of Cdk5 in a *CaMKII* promoter-driven p25 transgenic strain did not induce tau hyperphosphorylation at a young age per se, possibly because Cdk5 overactivation inhibited GSK-3β, by phosphorylating its inhibitory Ser9 site [107]. However, as the mice became older, this inhibition was lost, resulting in increased GSK-3β activity that was associated with tau hyperphosphorylation at the AT8 (pSer202/pThr205) and PHF-1 (pSer396/pSer404) sites. Together with pharmacological and co-immunoprecipitation experiments this would suggest that GSK-3β is a key mediator of tau hyperphosphorylation, while Cdk5 acts as a modulator of tau hyperphosphorylation via the inhibitory regulation of GSK-3β [107]. These findings were confirmed in a second p25 transgenic model [108]. It was found that phosphorylation of residue Ser9 of GSK-3β was mediated by an enhanced activity of the neuregulin receptor complex, ErbB, and by activation of the downstream phosphatidylinositol 3 kinase/Akt pathway. While young p25 mice had elevated Aβ levels, levels of phosphorylated tau were decreased. Thus, Cdk5 appears to play a dominant role in the regulation of amyloidogenic APP processing, whereas GSK-3β seems to play a dominant role in overall tau phosphorylation [108].

Inducible systems were employed that caused pronounced phenotypic changes. For example, transgenic mice with an inducible p25 expression in the postnatal forebrain revealed a massive neuronal loss in the cortex and hippocampus, along with forebrain

atrophy, astrogliosis, and caspase-3 activation [109]. At only 5 weeks of age, endogenous tau was hyperphosphorylated at many epitopes, as shown for AT8 (pSer202/pThr205) and PHF-1 (pSer396/pSer404). In 1-year-old mice, immuno-electron microscopy of sarkosyl-insoluble fractions revealed tau filaments that were phosphorylated at the AT8, PHF-1, TG3 (pThr231/pSer235), and AT100 (pThr212/pSer214) epitopes. Also, Gallyas silver impregnation revealed NFT formation in these mice [109]. In a second inducible mouse strain, neuronal p25 triggered a similar neurodegeneration and marked neuronal loss, causing brain atrophy with a 40% loss at 5 months of age resulting in an almost complete elimination of the hippocampus. Interestingly, this type of neurodegeneration was not associated with hyperphosphorylation of tau or Aβ generation [110]. One likely explanation is that p25 neurotoxicity is related to substrates other than tau or APP.

The tau-tubulin kinase (TTBK) family consists of TTBK1 and TTBK2 that belong to the casein kinase 1 superfamily. Different from TTBK2, TTBK1 is specifically expressed in neurons. It can phosphorylate tau directly at multiple Ser/Thr residues that are found in PHF-tau in AD brain. Furthermore, TTBK1 is expressed in NFT-bearing neurons in the AD cortex [99]. Transgenic mice expressing full-length human TTBK1 show an age-dependent memory impairment accompanied by increased phosphorylation of tau and neurofilaments, and increased levels of p25 and p35 [111].

Finally, another interesting kinase is DYRK1A (dual specificity tyrosine-regulated kinase-1A) that has been implicated in Down's syndrome (DS), due to the location of the *Dyrk1a* gene in the critical region of human chromosome 21 [96]. DS is a genetic disorder in humans caused by partial or complete trisomy of chromosome 21. People with DS show a tendency towards premature aging and an increased risk for AD [112]. DS patients show an early-onset tauopathy that resembles AD in many ways. In addition to mental problems, motor dysfunction is highly prevalent in DS. *Dyrk1a* transgenic mice express the transgene in several areas of the mid- and hindbrain [113]. When assessed in motor tests such as the treadmill, the mice showed impairment in some parameters; in particular, they required longer training periods [113]. A transcriptomic analysis revealed upregulation of the NMDA receptor subunit 2A which may explain the altered excitatory transmission reported in humans with DS and in DS mouse models [114]. Altered synaptic plasticity along with learning and memory deficits was reported for a second strain, that used a BAC clone of *Dyrk1a* [115]. A similar memory phenotype was found in *Dyrk1a* haplo-insufficient mice [116]. In one of the mouse models, tau phosphorylation was addressed: tau in *Dyrk1a* transgenic mice was hyperphosphorylated at Thr212, Ser202, and Ser404; furthermore, phosphorylation by DYRK1A strongly inhibited the ability of tau to promote microtubule assembly [117]. The finding that gene dosage of Dyrk1 affects tau splicing may explain the early-onset tauopathy in individuals with DS [118].

While these studies always only test a subset of tau phosphorylation sites, they still highlight a role for cdk5 and its activator p25 in Aβ pathology, and for GSK-3β, DYRK1A, and TTBK1 in tau aggregation. Whether targeting a specific kinase in the development of an AD therapy will be a fruitful approach is still a matter of debate. It may well be that in a human setting a general subtle downregulation of kinase(s) in the brain may be more beneficial.

6. Tau phosphorylation in phosphatase transgenic mice

Of the serine/threonine-specific phosphatases that are abundant in the brain, protein phosphatase 2A (PP2A) is a major phosphatase implicated in tau dephosphorylation [119], perhaps more so because PP2A can bind directly to tau [120–122]. PP2A is a trimeric holoenzyme that consists of a catalytic subunit C and a scaffolding subunit A. The A/C core enzyme associates with variable regulatory subunits of the PR55(B), PR56/61(B′), PR59/72/130(B″), and PR93/110(B‴) families to form heterotrimers [123]. Recruitment of regulatory B subunits into the holoenzyme is dependent upon the highly conserved DYFL motif in the carboxy-terminus of subunit C that undergoes methylation at Leu309, thereby affecting subunit B recruitment [124,125]. The resolution of the structure of the B subunit provides significant insight into how PP2A dephosphorylates tau [126]. Interestingly, methylation of the C subunit does not seem to be required for *in vitro* assembly of the PP2A holoenzyme involving either B or B′. The authors argue that the regulatory subunits may be sequestered in a specific cellular compartment and that the methylated carboxy-terminus of the C subunit may allow subcellular targeting for holoenzyme assembly [126].

Dephosphorylation of tau can be blocked in cells by okadaic acid (OA), an inhibitor of the two phosphatases PP1 and PP2A [127,128]. When rat brain slices were incubated with OA, tau became phosphorylated at multiple sites [129]. A role for PP2A in AD is implicated by the finding that its activity is reduced in AD brain [130]; furthermore, association analyses indicate that a CAG repeat polymorphism in one of the B subunits may confer susceptibility to AD [131].

Transgenic strategies targeting PP2A include gene knockouts, overexpression of regulatory subunits, and dominant negative mutant approaches [132]. A knockout of the major catalytic subunit Cα caused delayed embryonic lethality [133], as the highly homologous Cβ subunit failed to complement the lack of Cα in mesoderm formation [134], due to a different subcellular localization of the two catalytic subunits [135]. Under physiological conditions, the A and C subunits are ubiquitously expressed, whereas the regulatory B subunits show a tissue-specific expression; they also reveal a differential expression pattern in brain and during neuronal differentiation [136–138].

To address the role of PP2A in tau phosphorylation *in vivo*, transgenic mice were generated that express a dominant negative mutant form of the catalytic subunit Cα of PP2A, L199P, in neurons. The transgenic mice have a reduced PP2A activity resulting in a pre-NFT phenotype, with phosphorylation of endogenous murine tau at the AT8 (pSer202/pThr205) and Ser422 epitopes [139]. This effect may be directly mediated by PP2A, but could also occur indirectly, via deregulated kinases such as ERK and JNK, that are themselves substrates of PP2A [140]. The carboxy-terminal DYFL motif of the catalytic subunit has a role in the recruitment of B subunits into the PP2A complex, by methylating leucine 309 and phosphorylating tyrosine 307 [123]. To determine the role of the DYFL motif in PP2A activity *in vivo*, a second dominant negative mutant strain was established that expressed the L309A mutant form of Cα in neurons. This caused an altered subunit composition of the PP2A holoenzyme *in vivo*. In the brain, tau was hyperphosphorylated at Ser202/Thr205 (AT8/CP13); furthermore, there was an impaired dephosphorylation of the intermediate filament protein vimentin [141]. The additional expression of the L309A transgene expression in the Harderian (lacrimal) gland caused a delayed postnatal development and hypoplasia of this gland, causing enophthalmos [142].

When the PP2A L309A mutant mice were crossed with P301L tau mutant *pR5* mice, this caused a sevenfold increased number of hippocampal neurons that specifically phosphorylated the Ser422 epitope of tau. The double-transgenic mice showed eightfold increased numbers of NFTs compared to *pR5* mice, in agreement with the previous finding that NFT formation is correlated with and preceded by phosphorylation of tau at the Ser422 epitope [143]. The critical role of the DYFL motif in PP2A function, with particular regards to the cytoskeleton, is further demonstrated by cell culture experiments, which showed that expression of mutants such as L309Δ induces a loss of microtubules [144]. As mentioned above, in addition to methylation at the carboxy-terminal Leu309, phosphorylation at Tyr307 also regulates PP2A function [123]. PP2A phosphorylated at

Tyr307 associates with pre-tangles and NFTs in areas such as the entorhinal cortex and the hippocampus, brain areas where the neurofibrillary changes are initiated [145]. Together, these studies demonstrate a crucial role for PP2A in tau phosphorylation. As many kinases are themselves substrates of PP2A, the control of PP2A goes beyond the reversible phosphorylation of its non-enzymatic substrates. It would not be surprising to find that targeting of PP2A, in particular of its regulatory subunits, represents a valid means of treating AD.

7. Tau phosphorylation in mice with an amyloid pathology

Tau phosphorylation is part of the phenotypic characterization of APP mutant mouse models. In the following, we selected a few examples to demonstrate how Aβ affects endogenous tau phosphorylation *in vivo*. For example, in one of the earliest reports of a strong Aβ plaque-forming mouse model, the *APP23* mice, increased tau phosphorylation was found to parallel Aβ peptide deposition [146]. *APP23* mice have been thoroughly investigated, recently by using 3D reconstruction techniques to determine how small Aβ aggregates associated with the microvasculature lead to morphological and architectural alterations of the vasculature, thus resulting in an altered local blood flow [147]. Even more studies have been conducted with the APP mutant mouse strain *Tg2576* in which an anti-Aβ treatment provided evidence for a slow seeding mechanism preceding a rapid fibrillogenesis in determining the extent of Aβ deposition [148]. Formation of AT8 (pSer202/pThr205)-positive dystrophic neurites was found to occur simultaneously with Congo red-positive plaque development [149]. Oxidative stress seems to play an important role in the Aβ pathology. Superoxide dismutase 2 (SOD2) is an enzyme which detoxifies reactive oxygen species that are produced predominantly by mitochondria. Mice lacking SOD2 die within the first week of life, and develop a complex heterogeneous phenotype arising from mitochondrial dysfunction and oxidative stress [150]. By crossing the *Tg2576* mice onto an SOD2 heterozygous knockout background, it was found that this mitochondrial SOD2 deficiency both exacerbated Aβ plaque burden and tau phosphorylation at Ser396 [151].

Combinatorial approaches were particularly helpful in elucidating the role of Aβ in tau phosphorylation and aggregation. When P301L tau transgenic *JNPL3* mice were crossed with Aβ plaque-forming *Tg2576* mice, a sevenfold increase in NFT induction was seen [152]. In a parallel study with P301L tau transgenic *pR5* mice, we found that stereotaxic injections with $A\beta_{42}$ fibrils caused a fivefold increase in NFT formation compared to uninjected mice [153]. NFT formation was tightly correlated with phosphorylation of tau at Ser422 and AT100 (pThr212/pSer214), but not the AT8 (pSer202/pThr205) epitope. Mutagenesis of phospho-sites in a human SH-SY5Y tissue culture system revealed that the Ser422 epitope is required for the Aβ-mediated formation of tau filaments [154–156]. Aβ further exacerbates a mitochondrial dysfunction that characterizes the *pR5* mice [157,158]. In a third study, Aβ plaque and NFT pathology was combined in a single animal by a triple transgenic approach [159]. In these *3xtg-AD* mice (that express P301L mutant tau as well as mutant APP and PS1), synaptic dysfunction, including deficits in long-term potentiation, was found to precede Aβ plaque and NFT formation. The mice showed a remarkable phosphorylation pattern in that AT8 (pSer202/pThr205) staining was only evident after 6 months and PHF-1 (pSer396/pSer404) only at around 18 months of age, as shown for the hippocampus and cortex, despite a markedly high transgene expression and prominent HT7 (human tau) reactivity [160]. In a follow-up of the stereotaxic injection approach, Bolmont and colleagues diluted brain extracts from aged Aβ plaque-forming *APP23* transgenic mice and intracerebrally infused these in to young P301L tau transgenic mice. They found that 6 months after the infusion a tau pathology was induced in the injected hippocampus but also in brain regions well beyond the injection sites such as the entorhinal cortex and amygdala, areas with neuronal projection to the injection site [161]. This is similar to findings in pR5 mice where Aβ while injected into the hippocampus and somatosensory cortex induced NFT formation in the amygdala, an area with neuronal projections to the injection site [153]. Together, this demonstrates that Aβ can induce tau hyperphosphorylation and augment a pre-existing tau pathology in mice.

8. Tau phosphorylation in flies and worms

More recently, invertebrate species have gained considerable attention. Despite their reduced complexity, they offer distinct advantages compared to mice. The life span of both the nematode *Caenorhabditis elegans* and the fruitfly *Drosophila melanogaster* is short. Costs for maintenance are low, handling is relatively easy, mass production is possible, and screening for mutations is simple. Also, working with these two species does not need approval from animal ethics committees.

Several of the genes that are implicated in AD pathogenesis have been expressed in flies and worms. For example, both wild-type and FTDP-17 mutant human tau have been expressed in the fly. These reproduced key features of the human disease, including adult onset, progressive neurodegeneration, enhanced toxicity of mutant tau, accumulation of abnormal tau, and anatomical selectivity. Interestingly, immunoreactivity for phospho-epitopes such as 12E8 (pSer262/pSer356), AT100 (pThr212/pSer214), and AT180 (pThr231/pSer235) increased as the flies aged, whereas immunoreactivity using a phosphorylation-independent antibody was unaltered. Neurodegeneration, can occur without NFT formation [162], which is consistent with studies in mice [80]. When wild-type human tau was expressed in combination with Shaggy, the *Drosophila* GSK-3β homolog and *wnt* signaling pathway component, this lead to a neurofibrillary pathology with tau filaments [163].

When going through the vast literature on animal models of AD, it becomes obvious that most of the studies addressing the role of tau phosphorylation in tau aggregation and neurodegeneration are descriptive, although a few mutagenesis studies have been performed. To address the role of distinct tau phosphorylation sites in controlling tau neurotoxicity, a mutagenesis study was performed in *Drosophila* which indicates that here, no single phosphorylation residue plays a dominant role in controlling tau toxicity, but rather that tau phosphorylation sites work in concert to promote neurotoxicity *in vivo* [164].

The 12E8 epitope, located in the microtubule-binding domain of tau, is a substrate of MARK [44,165]. Work in *Drosophila* showed that the MARK homologue PAR-1 is a physiological tau kinase that plays a central role in regulating tau phosphorylation and toxicity, without promoting NFT formation [165]. Mutating the PAR-1 phosphorylation site Ser262/Ser356 (12E8) by alanine substitution abolished tau toxicity. When human tau was expressed in the *Drosophila* eye along with either GSK-3β/Shaggy or Cdk5, this enhanced toxicity of tau [166]. Lithium administration inhibited GSK-3β activity and thus reduced tau phosphorylation but did not ameliorate tau-induced toxicity, possibly reflecting high levels of tau expression [166].

Compared to *Drosophila*, the nematode is even easier to work with [167]. What is unique to *C. elegans* is that wild-type adult worms contain a constant number of 959 somatic cells. Not only is the cell number constant but also the position of each cell. Moreover, the worm is transparent and hence, it is easy to track cells and follow cell lineages. The nervous system of an adult hermaphrodite *C. elegans* consists of only 302 neurons that form approximately 7000 synapses. Transgenic animals can be generated either using microinjection or ballistic approaches [168]. Increasingly, *C. elegans* is employed for studies into diabetes and AD. Regarding the latter, research currently progresses along two lines: firstly the characterization of *C. elegans*

homologues of AD-related genes, and secondly the expression of human AD genes in *C. elegans* [167].

Expression of P301L and V337M mutant tau in *C. elegans* led to behavioral, synaptic, and pathological abnormalities, and caused an earlier and more severe phenotype than overexpression of wild-type human tau. Substantial neurodegeneration followed by loss of neurons occurred after insoluble tau began to accumulate. Tau was phosphorylated at the PHF-1 (pSer396/pSer404), AT270 (pThr181), and 12E8 (pSer262/pSer356) epitopes in both the soluble and insoluble fraction, and at Ser422 and AT8 (pSer202/pThr205) only in the soluble fraction. However, tau phosphorylation did not appear to correlate with the severity of the phenotype [169]. The fact that neurological symptoms were apparent before insoluble tau accumulated indicates that toxicity does not depend on the formation of large tau aggregates. To determine the role of tau phosphorylation in disease more directly, a short, fetal isoform of human tau was expressed, with a total of 10 serines and threonines replaced by either glutamate (a so-called pseudophosphorylation or PHP construct) or alanine [170]. Both wild-type and PHP tau induced a progressive age-dependent phenotype of uncoordinated locomotion (*unc*) in the absence of neuronal degeneration. In comparison, the alanine mutant transgenic worms displayed a reduced survival and developed an earlier *unc* phenotype, indicating that phosphorylation at these sites alone is not the cause of the observed defects [170]. However, it is difficult to draw any conclusions with regards to the role of single phospho-epitopes in pathogenesis, as in the PHP construct, 10 sites were pseudophosphorylated simultaneously.

9. Implications of animal work for pathogenesis and therapy

Which phosphorylation sites are critical in triggering the cascade of neuronal dysfunction and, eventually, neuronal loss in tauopathies? What are the implications of data obtained in animal models for the development of therapies? Obviously, the promiscuity of kinases and phosphatases and the interdependence of phosphorylation sites, along with a profile that can differ from one neuron to the next, renders it difficult at present to pinpoint specific phosphorylation sites and their regulation [171–173].

We have found *in vivo* that NFT formation is associated with the phosphorylation of the pSer422 and pThr212/pSer214 (AT100) epitope [153] (Fig. 1). This and additional studies including site-directed mutagenesis of tau phosphorylation sites suggest a role for specific phospho-epitopes and hence kinases and phosphatases in tau aggregation and neuronal dysfunction [63,154,174,175]. Other studies support the notion that a generally increased phosphorylation rather than phosphorylation of specific sites is needed [156,164]. This concept is also supported by a recent analysis of tau aggregation and the role of phosphorylation in this process in an inducible N2a cell line that expresses ΔK280 full-length tau with and without truncated forms of tau [176]. While this study is restricted to 4R tau and needs to be validated *in vivo*, interestingly, aggregation of full-length tau was found to be triggered by truncated fragments and was associated with the phosphorylation of most of the few sites analyzed. These were distributed between both soluble and aggregated tau, suggesting that none of the sites determines aggregation in an all-or-none manner [176]. Overall, whereas phosphorylation in the repeat domain tends to inhibit aggregation, other sites show a tendency to support aggregation. Together, this has obvious implications for treatment strategies as it would imply that phosphorylation in general, rather than specific tau kinases, needs to be blocked [87].

Other take home lessons are that transgenic animal models prove that expression of FTDP-17 mutant tau accelerates tau pathology, causing NFT formation within the lifetime of mice. They underscore the role of serine/threonine-specific phosphorylation in tau aggregation. Strong evidence has been provided for a role of the tau phospho-epitopes AT100 (pThr212/pSer214) and S422 in tau aggregation as indicated above, although a map of "necessary" and "sufficient" epitopes of tau is still lacking. Based on the currently available data it appears that phosphorylation of tau is closely linked to tau aggregation and fibril formation.

A tau-directed treatment strategy may target any step involved in converting tau to a toxic species [177]. Alternatively, tau levels (and hence levels of phosphorylated tau) may be altogether reduced as it has been shown that Aβ toxicity is exerted via tau and that excitotoxicity can be rescued when tau levels are reduced [178].

Several therapeutic strategies for treating AD on the basis of tau hyperphosphorylation are available as has been discussed recently [179]: these include (1) inhibition of GSK-3β, Cdk5 and other tau kinases; (2) restoration of PP2A activity; and (3) targeting O-glycosylation of tau [179].

A more recent study evaluated the concept of the promiscuity of kinases by using an orally bioavailable and blood–brain barrier (BBB)-penetrating analog of the relatively non-specific protein kinase inhibitor K252a [180]. This compound prevented motor deficits in the P301L tau transgenic mouse line *JNPL3* and reduced levels of soluble aggregated hyperphosphorylated tau. Interestingly, NFT numbers were not reduced, suggesting that the main cytotoxic effects of tau are not exerted by NFTs, but by lower molecular mass aggregates. This finding is in line with studies where a reduction in the expression of transgenic human P301L tau led to a recovery of memory function and stabilization of neuron numbers, despite the continued accumulation of NFTs [80].

In many studies the GSK-3β inhibitor lithium has been evaluated. For example, in 3xtg-AD mice that develop both plaques and tangles, lithium chloride reduced tau phosphorylation but did not significantly alter the Aβ load [181]. In a second mutant human tau transgenic mouse model, treatment with lithium chloride resulted in a significant inhibition of GSK-3 activity. After normalization to total levels of tau, lithium chloride treatment was found to result in a significantly decreased phosphorylation at putative GSK-3-directed sites, including pSer202 (CP13) and pSer396/pSer404 (PHF-1). Phosphorylation at sites not recognized by GSK-3 such as pSer422 or pSer262 appeared not to be affected. There were significantly reduced levels of aggregated, insoluble tau. Administration of another GSK-3 inhibitor also correlated with reduced insoluble tau levels, supporting the idea that lithium exerts its effect through GSK-3 inhibition. Levels of aggregated tau correlated strongly with the degree of axonal degeneration, and lithium chloride-treated mice showed less degeneration when administration was started during early stages of NFT development [182]. Very recently, a zebrafish model has been established which reproduced tau hyperphosphorylation, NFT formation, neuronal and behavioral disturbances, as well as cell death. Of the many inhibitors of GSK-3β tested in the fish model, a compound called AR-534 turned out to reduce tau hyperphosphorylation *in vivo*, without causing toxic side-effects [183].

Furthermore, the protein kinase inhibitor rapamycin has been reported to reduce toxicity in *Drosophila* expressing either wild-type or mutant forms of tau, probably by reducing the amount of insoluble tau [184]. Rapamycin induces autophagy through inhibition of the protein kinase mammalian target of rapamycin (mTOR) [185]. It remains to be seen whether the beneficial effects of rapamycin are related to TOR-dependent abnormal cell cycle activation that has been described in *Drosophila* tauopathy models.

While these data encourage the use of kinase inhibitors, at the same time, microtubules, known to be stabilized by tau, have also emerged as a drug target, as they can be strengthened by drugs such as paclitaxel [186]. Which of the approaches will be translated into human practice remains to be seen. As selective vulnerability characterizes the AD brain, different brain areas may be differently susceptible to any of these treatments [187].

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References

[1] D.J. Selkoe, Alzheimer's disease is a synaptic failure, Science 298 (2002) 789–791.
[2] M. Goedert, R. Jakes, M.G. Spillantini, R.A. Crowther, P. Cohen, E. Vanmechelen, A. Probst, J. Gotz, K. Burki, Tau protein in Alzheimer's disease, Biochem. Soc. Trans. 23 (1995) 80–85.
[3] V.M. Lee, M. Goedert, J.Q. Trojanowski, Neurodegenerative tauopathies, Annu. Rev. Neurosci. 24 (2001) 1121–1159.
[4] M. Goedert, C.M. Wischik, R.A. Crowther, J.E. Walker, A. Klug, Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 4051–4055.
[5] J. Gotz, Tau and transgenic animal models, Brain Res. Brain Res. Rev. 35 (2001) 266–286.
[6] F. Chen, D. David, A. Ferrari, J. Gotz, Posttranslational modifications of tau—role in human tauopathies and modeling in transgenic animals, Curr. Drug Targets 5 (2004) 503–515.
[7] G.A. Jicha, E. Lane, I. Vincent, L. Otvos Jr., R. Hoffmann, P. Davies, A conformation- and phosphorylation-dependent antibody recognizing the paired helical filaments of Alzheimer's disease, J. Neurochem. 69 (1997) 2087–2095.
[8] A. Andreadis, W.M. Brown, K.S. Kosik, Structure and novel exons of the human tau gene, Biochemistry 31 (1992) 10626–10633.
[9] M. Goedert, R. Jakes, Expression of separate isoforms of human tau protein: correlation with the tau pattern in brain and effects on tubulin polymerization, Embo J. 9 (1990) 4225–4230.
[10] M. Hutton, C.L. Lendon, P. Rizzu, M. Baker, S. Froelich, H. Houlden, S. Pickering-Brown, S. Chakraverty, A. Isaacs, A. Grover, J. Hackett, J. Adamson, S. Lincoln, D. Dickson, P. Davies, R.C. Petersen, M. Stevens, E. de Graaff, E. Wauters, J. van Baren, M. Hillebrand, M. Joosse, J.M. Kwon, P. Nowotny, P. Heutink, et al., Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17, Nature 393 (1998) 702–705.
[11] P. Poorkaj, T.D. Bird, E. Wijsman, E. Nemens, R.M. Garruto, L. Anderson, A. Andreadis, W.C. Wiederholt, M. Raskind, G.D. Schellenberg, Tau is a candidate gene for chromosome 17 frontotemporal dementia, Ann. Neurol. 43 (1998) 815–825.
[12] M.G. Spillantini, J.R. Murrell, M. Goedert, M.R. Farlow, A. Klug, B. Ghetti, Mutation in the tau gene in familial multiple system tauopathy with presenile dementia, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 7737–7741.
[13] M. Baker, I.R. Mackenzie, S.M. Pickering-Brown, J. Gass, R. Rademakers, C. Lindholm, J. Snowden, J. Adamson, A.D. Sadovnick, S. Rollinson, A. Cannon, E. Dwosh, D. Neary, S. Melquist, A. Richardson, D. Dickson, Z. Berger, J. Eriksen, T. Robinson, C. Zehr, C.A. Dickey, R. Crook, E. McGowan, D. Mann, B. Boeve, H. Feldman, M. Hutton, Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17, Nature 442 (2006) 916–919.
[14] M. Cruts, I. Gijselinck, J. van der Zee, S. Engelborghs, H. Wils, D. Pirici, R. Rademakers, R. Vandenberghe, B. Dermaut, J.J. Martin, C. van Duijn, K. Peeters, R. Sciot, P. Santens, T. De Pooter, M. Mattheijssens, M. Van den Broeck, I. Cuijt, K. Vennekens, P.P. De Deyn, S. Kumar-Singh, C. Van Broeckhoven, Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21, Nature 442 (2006) 920–924.
[15] M. Neumann, D.M. Sampathu, L.K. Kwong, A.C. Truax, M.C. Micsenyi, T.T. Chou, J. Bruce, T. Schuck, M. Grossman, C.M. Clark, L.F. McCluskey, B.L. Miller, E. Masliah, I.R. Mackenzie, H. Feldman, W. Feiden, H.A. Kretzschmar, J.Q. Trojanowski, V.M. Lee, Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis, Science 314 (2006) 130–133.
[16] C. Cook, Y.J. Zhang, Y.F. Xu, D.W. Dickson, L. Petrucelli, TDP-43 in neurodegenerative disorders, Expert Opin. Biol. Ther. 8 (2008) 969–978.
[17] J. Guevara, B. Espinosa, E. Zenteno, L. Vazguez, J. Luna, G. Perry, R. Mena, Altered glycosylation pattern of proteins in Alzheimer disease, J. Neuropathol. Exp. Neurol. 57 (1998) 905–914.
[18] P.J. Yao, P.D. Coleman, Reduction of O-linked N-acetylglucosamine-modified assembly protein-3 in Alzheimer's disease, J. Neurosci. 18 (1998) 2399–2411.
[19] L.A. Robertson, K.L. Moya, K.C. Breen, The potential role of tau protein O-glycosylation in Alzheimer's disease, J. Alzheimers Dis. 6 (2004) 489–495.
[20] F. Liu, K. Iqbal, I. Grundke-Iqbal, G.W. Hart, C.X. Gong, O-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 10804–10809.
[21] E.M. Mandelkow, E. Thies, B. Trinczek, J. Biernat, E. Mandelkow, MARK/PAR1 kinase is a regulator of microtubule-dependent transport in axons, J. Cell Biol. 167 (2004) 99–110.
[22] M. Goedert, M.G. Spillantini, R. Jakes, R.A. Crowther, E. Vanmechelen, A. Probst, J. Gotz, K. Burki, P. Cohen, Molecular dissection of the paired helical filament, Neurobiol. Aging 16 (1995) 325–334.
[23] T. Tanaka, K. Iqbal, E. Trenkner, D.J. Liu, I. Grundke-Iqbal, Abnormally phosphorylated tau in SY5Y human neuroblastoma cells, FEBS Lett. 360 (1995) 5–9.
[24] J.W. Mandell, G.A. Banker, A spatial gradient of tau protein phosphorylation in nascent axons, J. Neurosci. 16 (1996) 5727–5740.
[25] I. Grundke-Iqbal, K. Iqbal, Y.C. Tung, M. Quinlan, H.M. Wisniewski, L.I. Binder, Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 4913–4917.
[26] Y. Ihara, N. Nukina, R. Miura, M. Ogawara, Phosphorylated tau protein is integrated into paired helical filaments in Alzheimer's disease, J. Biochem. (Tokyo) 99 (1986) 1807–1810.
[27] M. Hasegawa, R. Jakes, R.A. Crowther, V.M. Lee, Y. Ihara, M. Goedert, Characterization of mAb AP422, a novel phosphorylation-dependent monoclonal antibody against tau protein, FEBS Lett. 384 (1996) 25–30.
[28] R. Hoffmann, V.M. Lee, S. Leight, I. Varga, L. Otvos, Unique Alzheimer's disease paired helical filament specific epitopes involve double phosphorylation at specific sites, Biochemistry 36 (1997) 8114–8124.
[29] T. Bussiere, P.R. Hof, C. Mailliot, C.D. Brown, M.L. Caillet-Boudin, D.P. Perl, L. Buee, A. Delacourte, Phosphorylated serine422 on tau proteins is a pathological epitope found in several diseases with neurofibrillary degeneration, Acta Neuropathol. (Berl.) 97 (1999) 221–230.
[30] S. Flament, A. Delacourte, B. Hemon, A. Defossez, [Direct demonstration of abnormal phosphorylation of Tau microtubular proteins in Alzheimer's disease], C. R. Acad. Sci. III (308) (1989) 77–82.
[31] D.P. Hanger, J.P. Brion, J.M. Gallo, N.J. Cairns, P.J. Luthert, B.H. Anderton, Tau in Alzheimer's disease and Down's syndrome is insoluble and abnormally phosphorylated, Biochem. J. 275 (Pt. 1) (1991) 99–104.
[32] M. Goedert, M.G. Spillantini, N.J. Cairns, R.A. Crowther, Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms, Neuron 8 (1992) 159–168.
[33] S.G. Greenberg, P. Davies, J.D. Schein, L.I. Binder, Hydrofluoric acid-treated tau PHF proteins display the same biochemical properties as normal tau, J. Biol. Chem. 267 (1992) 564–569.
[34] T.D. Garver, K.A. Harris, R.A. Lehman, V.M. Lee, J.Q. Trojanowski, M.L. Billingsley, Tau phosphorylation in human, primate, and rat brain: evidence that a pool of tau is highly phosphorylated in vivo and is rapidly dephosphorylated in vitro, J. Neurochem. 63 (1994) 2279–2287.
[35] H. Ksiezak-Reding, L.I. Binder, S.H. Yen, Alzheimer disease proteins (A68) share epitopes with tau but show distinct biochemical properties, J. Neurosci. Res. 25 (1990) 420–430.
[36] G. Drewes, E.M. Mandelkow, K. Baumann, J. Goris, W. Merlevede, E. Mandelkow, Dephosphorylation of tau protein and Alzheimer paired helical filaments by calcineurin and phosphatase-2A, FEBS Lett. 336 (1993) 425–432.
[37] K. Iqbal, T. Zaidi, C. Bancher, I. Grundke-Iqbal, Alzheimer paired helical filaments. Restoration of the biological activity by dephosphorylation, FEBS Lett. 349 (1994) 104–108.
[38] M. Hasegawa, M. Morishima-Kawashima, K. Takio, M. Suzuki, K. Titani, Y. Ihara, Protein sequence and mass spectrometric analyses of tau in the Alzheimer's disease brain, J. Biol. Chem. 267 (1992) 17047–17054.
[39] M. Morishima-Kawashima, M. Hasegawa, K. Takio, M. Suzuki, H. Yoshida, K. Titani, Y. Ihara, Proline-directed and non-proline-directed phosphorylation of PHF-tau, J. Biol. Chem. 270 (1995) 823–829.
[40] M. Morishima-Kawashima, M. Hasegawa, K. Takio, M. Suzuki, H. Yoshida, A. Watanabe, K. Titani, Y. Ihara, Hyperphosphorylation of tau in PHF, Neurobiol. Aging 16 (1995) 365–371 discussion 371-380.
[41] D.P. Hanger, J.C. Betts, T.L. Loviny, W.P. Blackstock, B.H. Anderton, New phosphorylation sites identified in hyperphosphorylated tau (paired helical filament-tau) from Alzheimer's disease brain using nanoelectrospray mass spectrometry, J. Neurochem. 71 (1998) 2465–2476.
[42] P. Friedhoff, E.M. Mandelkow, Tau protein, in: T. Kreis, R. Vale (Eds.), Guidebook to the cytoskeletal and motor proteins, Oxford University Press, Oxford, 1999, pp. 230–236.
[43] D.P. Hanger, H.L. Byers, S. Wray, K.Y. Leung, M.J. Saxton, A. Seereeram, C.H. Reynolds, M.A. Ward, B.H. Anderton, Novel phosphorylation sites in tau from Alzheimer brain support a role for casein kinase 1 in disease pathogenesis, J. Biol. Chem. 282 (2007) 23645–23654.
[44] J.C. Augustinack, A. Schneider, E.M. Mandelkow, B.T. Hyman, Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease, Acta Neuropathol. (Berl) 103 (2002) 26–35.
[45] Q. Zheng-Fischhofer, J. Biernat, E.M. Mandelkow, S. Illenberger, R. Godemann, E. Mandelkow, Sequential phosphorylation of Tau by glycogen synthase kinase-3beta and protein kinase A at Thr212 and Ser214 generates the Alzheimer-specific epitope of antibody AT100 and requires a paired-helical-filament-like conformation, Eur. J. Biochem. 252 (1998) 542–552.
[46] P. Derkinderen, T.M. Scales, D.P. Hanger, K.Y. Leung, H.L. Byers, M.A. Ward, C. Lenz, C. Price, I.N. Bird, T. Perera, S. Kellie, R. Williamson, W. Noble, R.A. Van Etten, K. Leroy, J.P. Brion, C.H. Reynolds, B.H. Anderton, Tyrosine 394 is phosphorylated in Alzheimer's paired helical filament tau and in fetal tau with c-Abl as the candidate tyrosine kinase, J. Neurosci. 25 (2005) 6584–6593.
[47] R. Brandt, J. Leger, G. Lee, Interaction of tau with the neural plasma membrane mediated by tau's amino-terminal projection domain, J. Cell Biol. 131 (1995) 1327–1340.
[48] G. Lee, S.T. Newman, D.L. Gard, H. Band, G. Panchamoorthy, Tau interacts with src-family non-receptor tyrosine kinases, J. Cell. Sci. 111 (1998) 3167–3177.
[49] G. Lee, R. Thangavel, V.M. Sharma, J.M. Litersky, K. Bhaskar, S.M. Fang, L.H. Do, A. Andreadis, G. Van Hoesen, H. Ksiezak-Reding, Phosphorylation of tau by fyn: implications for Alzheimer's disease, J. Neurosci. 24 (2004) 2304–2312.

[50] K. Bhaskar, S.H. Yen, G. Lee, Disease-related modifications in tau affect the interaction between Fyn and Tau, J. Biol. Chem. 280 (2005) 35119–35125.
[51] L.M. Ittner, J. Gotz, Pronuclear injection for the generation of transgenic mice, Nat. Protoc. 2 (2007) 1206–1215.
[52] J. Gotz, L.M. Ittner, Animal models of Alzheimer's disease and frontotemporal dementia, Nat. Rev. Neurosci. 9 (2008) 532–544.
[53] N. Shahani, S. Subramaniam, T. Wolf, C. Tackenberg, R. Brandt, Tau aggregation and progressive neuronal degeneration in the absence of changes in spine density and morphology after targeted expression of Alzheimer's disease-relevant tau constructs in organotypic hippocampal slices, J. Neurosci. 26 (2006) 6103–6114.
[54] J. Gotz, A. Probst, M.G. Spillantini, T. Schafer, R. Jakes, K. Burki, M. Goedert, Somatodendritic localization and hyperphosphorylation of tau protein in transgenic mice expressing the longest human brain tau isoform, EMBO J. 14 (1995) 1304–1313.
[55] J.P. Brion, G. Tremp, J.N. Octave, Transgenic expression of the shortest human tau affects its compartmentalization and its phosphorylation as in the pretangle stage of Alzheimer's disease [see comments], Am. J. Pathol. 154 (1999) 255–270.
[56] T. Ishihara, M. Hong, B. Zhang, Y. Nakagawa, M.K. Lee, J.Q. Trojanowski, V.M. Lee, Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform, Neuron 24 (1999) 751–762.
[57] K. Spittaels, C. Van den Haute, J. Van Dorpe, K. Bruynseels, K. Vandezande, I. Laenen, H. Geerts, M. Mercken, R. Sciot, A. Van Lommel, R. Loos, F. Van Leuven, Prominent axonopathy in the brain and spinal cord of transgenic mice overexpressing four-repeat human tau protein, Am. J. Pathol. 155 (1999) 2153–2165.
[58] A. Probst, J. Gotz, K.H. Wiederhold, M. Tolnay, C. Mistl, A.L. Jaton, M. Hong, T. Ishihara, V.M. Lee, J.Q. Trojanowski, R. Jakes, R.A. Crowther, M.G. Spillantini, K. Burki, M. Goedert, Axonopathy and amyotrophy in mice transgenic for human four-repeat tau protein, Acta Neuropathol. (Berl.) 99 (2000) 469–481.
[59] T. Ishihara, B. Zhang, M. Higuchi, Y. Yoshiyama, J.Q. Trojanowski, V.M. Lee, Age-dependent induction of congophilic neurofibrillary tau inclusions in tau transgenic mice, Am. J. Pathol. 158 (2001) 555–562.
[60] J. Gotz, R.M. Nitsch, Compartmentalized tau hyperphosphorylation and increased levels of kinases in transgenic mice, Neuroreport 12 (2001) 2007–2016.
[61] K. Duff, H. Knight, L.M. Refolo, S. Sanders, X. Yu, M. Picciano, B. Malester, M. Hutton, J. Adamson, M. Goedert, K. Burki, P. Davies, Characterization of pathology in transgenic mice over-expressing human genomic and cDNA tau transgenes, Neurobiol. Dis. 7 (2000) 87–98.
[62] K.L. Tucker, M. Meyer, Y.A. Barde, Neurotrophins are required for nerve growth during development, Nat. Neurosci. 4 (2001) 29–37.
[63] I. Kelleher, C. Garwood, D.P. Hanger, B.H. Anderton, W. Noble, Kinase activities increase during the development of tauopathy in htau mice, J. Neurochem. 103 (2007) 2256–2267.
[64] J. Gotz, M. Tolnay, R. Barmettler, F. Chen, A. Probst, R.M. Nitsch, Oligodendroglial tau filament formation in transgenic mice expressing G272V tau, Eur. J. Neurosci. 13 (2001) 2131–2140.
[65] J. Gotz, N. Deters, A. Doldissen, L. Bokhari, Y. Ke, A. Wiesner, N. Schonrock, L.M. Ittner, A decade of tau transgenic animal models and beyond, Brain Pathol. 17 (2007) 91–103.
[66] F.J. Hoerndli, M. Toigo, A. Schild, J. Gotz, P.J. Day, Reference genes identified in SH-SY5Y cells using custom-made gene arrays with validation by quantitative polymerase chain reaction, Anal. Biochem. 335 (2004) 30–41.
[67] D. David, F. Hoerndli, J. Gotz, Functional genomics meets neurodegenerative disorders. Part I. Transcriptomic and proteomic technology, Prog. Neurobiol. 76 (2005) 153–168.
[68] F. Hoerndli, D. David, J. Gotz, Functional genomics meets neurodegenerative disorders. Part II. Application and data integration, Prog. Neurobiol. 76 (2005) 169–188.
[69] D.C. David, L.M. Ittner, P. Gehrig, D. Nergenau, C. Shepherd, G. Halliday, J. Gotz, β-Amyloid treatment of two complementary P301L tau-expressing Alzheimer's disease models reveals similar deregulated cellular processes, Proteomics 6 (2006) 6566–6577.
[70] R.A. Sowell, J.B. Owen, D.A. Butterfield, Proteomics in animal models of Alzheimer's and Parkinson's diseases, Ageing Res. Rev. 8 (2009) 1–17.
[71] L. Pennanen, H. Welzl, P. D'Adamo, R.M. Nitsch, J. Gotz, Accelerated extinction of conditioned taste aversion in P301L tau transgenic mice, Neurobiol. Dis. 15 (2004) 500–509.
[72] L. Pennanen, D.P. Wolfer, R.M. Nitsch, J. Gotz, Impaired spatial reference memory and increased exploratory behavior in P301L tau transgenic mice, Genes Brain Behav. 5 (2006) 369–379.
[73] B. Allen, E. Ingram, M. Takao, M.J. Smith, R. Jakes, K. Virdee, H. Yoshida, M. Holzer, M. Craxton, P.C. Emson, C. Atzori, A. Migheli, R.A. Crowther, B. Ghetti, M.G. Spillantini, M. Goedert, Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein, J. Neurosci. 22 (2002) 9340–9351.
[74] J. Lewis, E. McGowan, J. Rockwood, H. Melrose, P. Nacharaju, M. Van Slegtenhorst, K. Gwinn-Hardy, P.M. Murphy, M. Baker, X. Yu, K. Duff, J. Hardy, A. Corral, W.L. Lin, S.H. Yen, D.W. Dickson, P. Davies, M. Hutton, Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein, Nat. Genet. 25 (2000) 402–405.
[75] J. Gotz, F. Chen, R. Barmettler, R.M. Nitsch, Tau filament formation in transgenic mice expressing P301L tau, J. Biol. Chem. 276 (2001) 529–534.
[76] N. Deters, L.M. Ittner, J. Gotz, Divergent phosphorylation pattern of tau in P301L tau transgenic mice, Eur. J. Neurosci. 28 (2008) 137–147.
[77] K. Iqbal, A.C. Alonso, C.X. Gong, S. Khatoon, J.J. Pei, J.Z. Wang, I. Grundke-Iqbal, Mechanisms of neurofibrillary degeneration and the formation of neurofibrillary tangles, J. Neural Transm., Suppl. 53 (1998) 169–180.
[78] P. Delobel, I. Lavenir, G. Fraser, E. Ingram, M. Holzer, B. Ghetti, M.G. Spillantini, R.A. Crowther, M. Goedert, Analysis of tau phosphorylation and truncation in a mouse model of human tauopathy, Am. J. Pathol. 172 (2008) 123–131.
[79] Z. Berger, H. Roder, A. Hanna, A. Carlson, V. Rangachari, M. Yue, Z. Wszolek, K. Ashe, J. Knight, D. Dickson, C. Andorfer, T.L. Rosenberry, J. Lewis, M. Hutton, C. Janus, Accumulation of pathological tau species and memory loss in a conditional model of tauopathy, J. Neurosci. 27 (2007) 3650–3662.
[80] K. Santacruz, J. Lewis, T. Spires, J. Paulson, L. Kotilinek, M. Ingelsson, A. Guimaraes, M. DeTure, M. Ramsden, E. McGowan, C. Forster, M. Yue, J. Orne, C. Janus, A. Mariash, M. Kuskowski, B. Hyman, M. Hutton, K.H. Ashe, Tau suppression in a neurodegenerative mouse model improves memory function, Science 309 (2005) 476–481.
[81] M. Ramsden, L. Kotilinek, C. Forster, J. Paulson, E. McGowan, K. SantaCruz, A. Guimaraes, M. Yue, J. Lewis, G. Carlson, M. Hutton, K.H. Ashe, Age-dependent neurofibrillary tangle formation, neuron loss, and memory impairment in a mouse model of human tauopathy (P301L), J. Neurosci. 25 (2005) 10637–10647.
[82] L.M. Ittner, T. Fath, Y.D. Ke, M. Bi, J. van Eersel, K.M. Li, P. Gunning, J. Gotz, Parkinsonism and impaired axonal transport in a mouse model of frontotemporal dementia, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 15997–16002.
[83] M. Neumann, W. Schulz-Schaeffer, R.A. Crowther, M.J. Smith, M.G. Spillantini, M. Goedert, H.A. Kretzschmar, Pick's disease associated with the novel Tau gene mutation K369I, Ann. Neurol. 50 (2001) 503–513.
[84] J. van Eersel, M. Bi, Y.D. Ke, J.R. Hodges, J.H. Xuereb, G.C. Gregory, G.M. Halliday, J. Gotz, J.J. Kril, L.M. Ittner, Phosphorylation of soluble tau differs in Pick's disease and Alzheimer's disease brains, J Neural Transm 116 (2009) 1243–1251.
[85] L.M. Ittner, Y.D. Ke, J. Gotz, Phosphorylated tau interacts with c-Jun N-terminal kinase-interacting protein 1 (JIP1) in Alzheimer disease, J. Biol. Chem. 284 (2009) 20909–20916.
[86] F. Clavaguera, T. Bolmont, R.A. Crowther, D. Abramowski, S. Frank, A. Probst, G. Fraser, A.K. Stalder, M. Beibel, M. Staufenbiel, M. Jucker, M. Goedert, M. Tolnay, Transmission and spreading of tauopathy in transgenic mouse brain, Nat. Cell Biol. 11 (2009) 909–913.
[87] J. Gotz, J.R. Streffer, D. David, A. Schild, F. Hoerndli, L. Pennanen, P. Kurosinski, F. Chen, Transgenic animal models of Alzheimer's disease and related disorders: histopathology, behavior and therapy, Mol. Psychiatry 9 (2004) 664–683.
[88] P. Kurosinski, J. Gotz, Glial cells under physiologic and pathological conditions, Arch. Neurol. 59 (2002) 1524–1528.
[89] D.P. Hanger, K. Hughes, J.R. Woodgett, J.P. Brion, B.H. Anderton, Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase, Neurosci. Lett. 147 (1992) 58–62.
[90] E.M. Mandelkow, G. Drewes, J. Biernat, N. Gustke, J. Van Lint, J.R. Vandenheede, E. Mandelkow, Glycogen synthase kinase-3 and the Alzheimer-like state of microtubule-associated protein tau, FEBS Lett. 314 (1992) 315–321.
[91] K. Baumann, E.M. Mandelkow, J. Biernat, H. Piwnica-Worms, E. Mandelkow, Abnormal Alzheimer-like phosphorylation of tau-protein by cyclin-dependent kinases cdk2 and cdk5, FEBS Lett. 336 (1993) 417–424.
[92] B.T. Hyman, T.E. Elvhage, J. Reiter, Extracellular signal regulated kinases. Localization of protein and mRNA in the human hippocampal formation in Alzheimer's disease, Am. J. Pathol. 144 (1994) 565–572.
[93] B. Trinczek, J. Biernat, K. Baumann, E.M. Mandelkow, E. Mandelkow, Domains of tau protein, differential phosphorylation, and dynamic instability of microtubules, Mol. Biol. Cell 6 (1995) 1887–1902.
[94] C.H. Reynolds, M.A. Utton, G.M. Gibb, A. Yates, B.H. Anderton, Stress-activated protein kinase/c-jun N-terminal kinase phosphorylates tau protein, J. Neurochem. 68 (1997) 1736–1744.
[95] C.H. Reynolds, A.R. Nebreda, G.M. Gibb, M.A. Utton, B.H. Anderton, Reactivating kinase/p38 phosphorylates tau protein in vitro, J. Neurochem. 69 (1997) 191–198.
[96] J. Guimera, C. Casas, X. Estivill, M. Pritchard, Human minibrain homologue (MNBH/DYRK1): characterization, alternative splicing, differential tissue expression, and overexpression in Down syndrome, Genomics 57 (1999) 407–418.
[97] I. Ferrer, T. Gomez-Isla, B. Puig, M. Freixes, E. Ribe, E. Dalfo, J. Avila, Current advances on different kinases involved in tau phosphorylation, and implications in Alzheimer's disease and tauopathies, Curr. Alzheimer Res. 2 (2005) 3–18.
[98] J.J. Pei, W.L. An, X.W. Zhou, T. Nishimura, J. Norberg, E. Benedikz, J. Gotz, B. Winblad, P70 S6 kinase mediates tau phosphorylation and synthesis, FEBS Lett. 580 (2006) 107–114.
[99] S. Sato, R.L. Cerny, J.L. Buescher, T. Ikezu, Tau-tubulin kinase 1 (TTBK1), a neuron-specific tau kinase candidate, is involved in tau phosphorylation and aggregation, J. Neurochem. 98 (2006) 1573–1584.
[100] T. Li, H.K. Paudel, Glycogen synthase kinase 3beta phosphorylates Alzheimer's disease-specific Ser396 of microtubule-associated protein tau by a sequential mechanism, Biochemistry 45 (2006) 3125–3133.
[101] T. Li, C. Hawkes, H.Y. Qureshi, S. Kar, H.K. Paudel, Cyclin-dependent protein kinase 5 primes microtubule-associated protein tau site-specifically for glycogen synthase kinase 3beta, Biochemistry 45 (2006) 3134–3145.
[102] K. Spittaels, C. Van Den Haute, J. Van Dorpe, H. Geerts, M. Mercken, K. Bruynseels, R. Lasrado, K. Vandezande, I. Laenen, T. Boon, J. Van Lint, J. Vandenheede, D. Moechars, R. Loos, F. Van Leuven, Glycogen synthase kinase-3beta phosphorylates protein tau and rescues the axonopathy in the central nervous system of human four-repeat tau transgenic mice, J. Biol. Chem. 275 (2000) 41340–41349.

[103] T. Engel, J.J. Lucas, P. Gomez-Ramos, M.A. Moran, J. Avila, F. Hernandez, Cooexpression of FTDP-17 tau and GSK-3beta in transgenic mice induce tau polymerization and neurodegeneration, Neurobiol. Aging 97 (2005) 1259–1268.
[104] T. Engel, F. Hernandez, J. Avila, J.J. Lucas, Full reversal of Alzheimer's disease-like phenotype in a mouse model with conditional overexpression of glycogen synthase kinase-3, J. Neurosci. 26 (2006) 5083–5090.
[105] R. Gomez-Sintes, F. Hernandez, A. Bortolozzi, F. Artigas, J. Avila, P. Zaratin, J.P. Gotteland, J.J. Lucas, Neuronal apoptosis and reversible motor deficit in dominant-negative GSK-3 conditional transgenic mice, EMBO J. 26 (2007) 2743–2754.
[106] W. Noble, V. Olm, K. Takata, E. Casey, O. Mary, J. Meyerson, K. Gaynor, J. LaFrancois, L. Wang, T. Kondo, P. Davies, M. Burns, Veeranna, R. Nixon, D. Dickson, Y. Matsuoka, M. Ahlijanian, L.F. Lau, K. Duff, Cdk5 is a key factor in tau aggregation and tangle formation in vivo, Neuron 38 (2003) 555–565.
[107] F. Plattner, M. Angelo, K.P. Giese, The roles of cyclin-dependent kinase 5 and glycogen synthase kinase 3 in tau hyperphosphorylation, J. Biol. Chem. 281 (2006) 25457–25465.
[108] Y. Wen, E. Planel, M. Herman, H.Y. Figueroa, L. Wang, L. Liu, L.F. Lau, W.H. Yu, K.E. Duff, Interplay between cyclin-dependent kinase 5 and glycogen synthase kinase 3 beta mediated by neuregulin signaling leads to differential effects on tau phosphorylation and amyloid precursor protein processing, J. Neurosci. 28 (2008) 2624–2632.
[109] J.C. Cruz, H.C. Tseng, J.A. Goldman, H. Shih, L.H. Tsai, Aberrant Cdk5 activation by p25 triggers pathological events leading to neurodegeneration and neurofibrillary tangles, Neuron 40 (2003) 471–483.
[110] D. Muyllaert, D. Terwel, A. Kremer, K. Sennvik, P. Borghgraef, H. Devijver, I. Dewachter, F. Van Leuven, Neurodegeneration and neuroinflammation in cdk5/p25-inducible mice: a model for hippocampal sclerosis and neocortical degeneration, Am. J. Pathol. 172 (2008) 470–485.
[111] S. Sato, J. Xu, S. Okuyama, L.B. Martinez, S.M. Walsh, M.T. Jacobsen, R.J. Swan, J.D. Schlautman, P. Ciborowski, T. Ikezu, Spatial learning impairment, enhanced CDK5/p35 activity, and downregulation of NMDA receptor expression in transgenic mice expressing tau-tubulin kinase 1, J. Neurosci. 28 (2008) 14511–14521.
[112] W.B. Zigman, I.T. Lott, Alzheimer's disease in Down syndrome: neurobiology and risk, Ment. Retard. Dev. Disabil. Res. Rev. 13 (2007) 237–246.
[113] M. Martinez de Lagran, X. Altafaj, X. Gallego, E. Marti, X. Estivill, I. Sahun, C. Fillat, M. Dierssen, Motor phenotypic alterations in TgDyrk1a transgenic mice implicate DYRK1A in Down syndrome motor dysfunction, Neurobiol. Dis. 15 (2004) 132–142.
[114] X. Altafaj, J. Ortiz-Abalia, M. Fernandez, M.C. Potier, J. Laffaire, N. Andreu, M. Dierssen, C. Gonzalez-Garcia, V. Cena, E. Marti, C. Fillat, Increased NR2A expression and prolonged decay of NMDA-induced calcium transient in cerebellum of TgDyrk1A mice, a mouse model of Down syndrome, Neurobiol. Dis. 32 (2008) 377–384.
[115] K.J. Ahn, H.K. Jeong, H.S. Choi, S.R. Ryoo, Y.J. Kim, J.S. Goo, S.Y. Choi, J.S. Han, I. Ha, W.J. Song, DYRK1A BAC transgenic mice show altered synaptic plasticity with learning and memory defects, Neurobiol. Dis. 22 (2006) 463–472.
[116] G. Arque, V. Fotaki, D. Fernandez, M. Martinez de Lagran, M.L. Arbones, M. Dierssen, Impaired spatial learning strategies and novel object recognition in mice haploinsufficient for the dual specificity tyrosine-regulated kinase-1A (Dyrk1A), PLoS ONE 3 (2008) e2575.
[117] S.R. Ryoo, H.K. Jeong, C. Radnaabazar, J.J. Yoo, H.J. Cho, H.W. Lee, I.S. Kim, Y.H. Cheon, Y.S. Ahn, S.H. Chung, W.J. Song, DYRK1A-mediated hyperphosphorylation of Tau. A functional link between Down syndrome and Alzheimer disease, J. Biol. Chem. 282 (2007) 34850–34857.
[118] J. Shi, T. Zhang, C. Zhou, M.O. Chohan, X. Gu, J. Wegiel, J. Zhou, Y.W. Hwang, K. Iqbal, I. Grundke-Iqbal, C.X. Gong, F. Liu, Increased dosage of Dyrk1A alters alternative splicing factor (ASF)-regulated alternative splicing of tau in Down syndrome, J. Biol. Chem. 283 (2008) 28660–28669.
[119] M. Goedert, R. Jakes, Z. Qi, J.H. Wang, P. Cohen, Protein phosphatase 2A is the major enzyme in brain that dephosphorylates tau protein phosphorylated by proline-directed protein kinases or cyclic AMP-dependent protein kinase, J. Neurochem. 65 (1995) 2804–2807.
[120] E. Sontag, V. Nunbhakdi-Craig, G. Lee, G.S. Bloom, M.C. Mumby, Regulation of the phosphorylation state and microtubule-binding activity of Tau by protein phosphatase 2A, Neuron 17 (1996) 1201–1207.
[121] E. Sontag, V. Nunbhakdi-Craig, G. Lee, R. Brandt, C. Kamibayashi, J. Kuret, C.L. White 3rd, M.C. Mumby, G.S. Bloom, Molecular interactions among protein phosphatase 2A, tau, and microtubules. Implications for the regulation of tau phosphorylation and the development of tauopathies, J. Biol. Chem. 274 (1999) 25490–25498.
[122] F. Liu, I. Grundke-Iqbal, K. Iqbal, C.X. Gong, Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation, Eur. J. Neurosci. 22 (2005) 1942–1950.
[123] V. Janssens, J. Goris, Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling, Biochem. J. 353 (2001) 417–439.
[124] H. Xie, S. Clarke, Protein phosphatase 2A is reversibly modified by methyl esterification at its C-terminal leucine residue in bovine brain, J. Biol. Chem. 269 (1994) 1981–1984.
[125] J.C. Bryant, R.S. Westphal, B.E. Wadzinski, Methylated C-terminal leucine residue of PP2A catalytic subunit is important for binding of regulatory Balpha subunit, Biochem. J. 339 (Pt. 2) (1999) 241–246.
[126] Y. Xu, Y. Chen, P. Zhang, P.D. Jeffrey, Y. Shi, Structure of a protein phosphatase 2A holoenzyme: insights into B55-mediated Tau dephosphorylation, Mol. Cell 31 (2008) 873–885.
[127] T. Arendt, M. Holzer, R. Fruth, M.K. Bruckner, U. Gartner, Paired helical filament-like phosphorylation of tau, deposition of beta/A4-amyloid and memory impairment in rat induced by chronic inhibition of phosphatase 1 and 2A, Neuroscience 69 (1995) 691–698.
[128] E. Planel, K. Yasutake, S.C. Fujita, K. Ishiguro, Inhibition of protein phosphatase 2A overrides tau protein kinase I/glycogen synthase kinase 3 beta and cyclin-dependent kinase 5 inhibition and results in tau hyperphosphorylation in the hippocampus of starved mouse, J. Biol. Chem. 276 (2001) 34298–34306.
[129] J.J. Pei, C.X. Gong, W.L. An, B. Winblad, R.F. Cowburn, I. Grundke-Iqbal, K. Iqbal, Okadaic-acid-induced inhibition of protein phosphatase 2A produces activation of mitogen-activated protein kinases ERK1/2, MEK1/2, and p70 S6, similar to that in Alzheimer's disease, Am. J. Pathol. 163 (2003) 845–858.
[130] C.X. Gong, T.J. Singh, I. Grundke-Iqbal, K. Iqbal, Phosphoprotein phosphatase activities in Alzheimer disease brain, J. Neurochem. 61 (1993) 921–927.
[131] C.M. Chen, Y.T. Hou, J.Y. Liu, Y.R. Wu, C.H. Lin, H.C. Fung, W.C. Hsu, Y. Hsu, S.H. Lee, H.M. Hsieh-Li, M.T. Su, S.T. Chen, H.Y. Lane, G.J. Lee-Chen, PPP2R2B CAG repeat length in the Han Chinese in Taiwan: association analyses in neurological and psychiatric disorders and potential functional implications, Am. J. Med. Genet. B Neuropsychiatr. Genet. 150B (2009) 124–129.
[132] J. Gotz, A. Schild, Transgenic and knockout models of PP2A, Methods Enzymol. 366 (2003) 390–403.
[133] J. Gotz, A. Probst, E. Ehler, B. Hemmings, W. Kues, Delayed embryonic lethality in mice lacking protein phosphatase 2A catalytic subunit Calpha, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 12370–12375.
[134] J. Gotz, W. Kues, The role of protein phosphatase 2A catalytic subunit Calpha in embryogenesis: evidence from sequence analysis and localization studies, Biol. Chem. 380 (1999) 1117–1120.
[135] J. Gotz, A. Probst, C. Mistl, R.M. Nitsch, E. Ehler, Distinct role of protein phosphatase 2A subunit calpha in the regulation of E-cadherin and beta-catenin during development, Mech. Dev. 93 (2000) 83–93.
[136] K. Schmidt, S. Kins, A. Schild, R.M. Nitsch, B.A. Hemmings, J. Gotz, Diversity, developmental regulation and distribution of murine PR55/B subunits of protein phosphatase 2A, Eur. J. Neurosci. 16 (2002) 2039–2048.
[137] E. Martens, S. Stevens, J. Janssens, J. Gotz, J. Goris, C. van Hoof, Genomic organisation, chromosomal localisation, tissue distribution and developmental regulation of the PR61/B' regulatory subunits of protein phosphatase 2A in mice, J. Mol. Biol. 336 (2004) 971–986.
[138] A. Schild, K. Schmidt, Y.A. Lim, Y. Ke, L.M. Ittner, B.A. Hemmings, J. Gotz, Altered levels of PP2A regulatory B/PR55 isoforms indicate role in neuronal differentiation, Int. J. Dev. Neurosci. 24 (2006) 437–443.
[139] S. Kins, A. Crameri, D.R. Evans, B.A. Hemmings, R.M. Nitsch, J. Gotz, Reduced PP2A activity induces hyperphosphorylation and altered compartmentalization of tau in transgenic mice, J. Biol. Chem. 276 (2001) 38193–38200.
[140] S. Kins, P. Kurosinski, R.M. Nitsch, J. Gotz, Activation of the ERK and JNK signaling pathways caused by neuron specific inhibition of PP2A in transgenic mice, Am. J. Pathol. 163 (2003) 833–843.
[141] A. Schild, L.M. Ittner, J. Gotz, Altered phosphorylation of cytoskeletal proteins in mutant protein phosphatase 2A transgenic mice, Biochem. Biophys. Res. Commun. 343 (2006) 1171–1178.
[142] A. Schild, S. Isenmann, N. Tanimoto, F. Tonagel, M.W. Seeliger, L.M. Ittner, A. Kretz, E. Ogris, J. Gotz, Impaired development of the Harderian gland in mutant protein phosphatase 2A transgenic mice, Mech. Dev. 123 (2006) 362–371.
[143] N. Deters, L.M. Ittner, J. Gotz, Substrate-specific reduction of PP2A activity exaggerates tau pathology, Biochem. Biophys. Res. Commun. 379 (2009) 400–405.
[144] V. Nunbhakdi-Craig, S. Schuechner, J.M. Sontag, L. Montgomery, D.C. Pallas, C. Juno, I. Mudrak, E. Ogris, E. Sontag, Expression of protein phosphatase 2A mutants and silencing of the regulatory B alpha subunit induce a selective loss of acetylated and detyrosinated microtubules, J. Neurochem. 101 (2007) 959–971.
[145] R. Liu, X.W. Zhou, H. Tanila, C. Bjorkdahl, J.Z. Wang, Z.Z. Guan, Y. Cao, J.A. Gustafsson, B. Winblad, J.J. Pei, Phosphorylated PP2A (tyrosine 307) is associated with Alzheimer neurofibrillary pathology, J. Cell. Mol. Med. 12 (2008) 241–257.
[146] C. Sturchler-Pierrat, D. Abramowski, M. Duke, K.H. Wiederhold, C. Mistl, S. Rothacher, B. Ledermann, K. Burki, P. Frey, P.A. Paganetti, C. Waridel, M.E. Calhoun, M. Jucker, A. Probst, M. Staufenbiel, B. Sommer, Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 13287–13292.
[147] E.P. Meyer, A. Ulmann-Schuler, M. Staufenbiel, T. Krucker, Altered morphology and 3D architecture of brain vasculature in a mouse model for Alzheimer's disease, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 3587–3592.
[148] R.A. Karlnoski, A. Rosenthal, D. Kobayashi, J. Pons, J. Alamed, M. Mercer, Q. Li, M. N. Gordon, P.E. Gottschall, D. Morgan, Suppression of amyloid deposition leads to long-term reductions in Alzheimer's pathologies in Tg2576 mice, J. Neurosci. 29 (2009) 4964–4971.
[149] K. Noda-Saita, K. Terai, A. Iwai, M. Tsukamoto, Y. Shitaka, S. Kawabata, M. Okada, T. Yamaguchi, Exclusive association and simultaneous appearance of congophilic plaques and AT8-positive dystrophic neurites in Tg2576 mice suggest a mechanism of senile plaque formation and progression of neuritic dystrophy in Alzheimer's disease, Acta Neuropathol. 108 (2004) 435–442.
[150] S. Melov, P. Coskun, M. Patel, R. Tuinstra, B. Cottrell, A.S. Jun, T.H. Zastawny, M. Dizdaroglu, S.I. Goodman, T.T. Huang, H. Miziorko, C.J. Epstein, D.C. Wallace, Mitochondrial disease in superoxide dismutase 2 mutant mice, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 846–851.
[151] S. Melov, P.A. Adlard, K. Morten, F. Johnson, T.R. Golden, D. Hinerfeld, B. Schilling, C. Mavros, C.L. Masters, I. Volitakis, Q.X. Li, K. Laughton, A. Hubbard, R.A. Cherny, B. Gibson, A.I. Bush, Mitochondrial oxidative stress causes hyperphosphorylation of tau, PLoS ONE 2 (2007) e536.

[152] J. Lewis, D.W. Dickson, W.-L. Lin, L. Chisholm, A. Corral, G. Jones, S.-H. Yen, N. Sahara, L. Skipper, D. Yager, C. Eckman, J. Hardy, M. Hutton, E. McGowan, Enhanced neurofibrillary degeneration in transgenic mice expressing mutant Tau and APP, Science 293 (2001) 1487–1491.
[153] J. Gotz, F. Chen, J. van Dorpe, R.M. Nitsch, Formation of neurofibrillary tangles in P301L tau transgenic mice induced by Abeta 42 fibrils, Science 293 (2001) 1491–1495.
[154] A. Ferrari, F. Hoerndli, T. Baechi, R.M. Nitsch, J. Gotz, Beta-amyloid induces PHF-like tau filaments in tissue culture, J. Biol. Chem. 278 (2003) 40162–40168.
[155] J. Gotz, A. Schild, F. Hoerndli, L. Pennanen, Amyloid-induced neurofibrillary tangle formation in Alzheimer's disease: insight from transgenic mouse and tissue-culture models, Int. J. Dev. Neurosci. 22 (2004) 453–465.
[156] L. Pennanen, J. Gotz, Different tau epitopes define Abeta(42)-mediated tau insolubility, Biochem. Biophys. Res. Commun. 337 (2005) 1097–1101.
[157] D.C. David, S. Hauptmann, I. Scherping, K. Schuessel, U. Keil, P. Rizzu, R. Ravid, S. Dröse, U. Brandt, W.E. Müller, E. Eckert, J. Gotz, Proteomic and functional analysis reveal a mitochondrial dysfunction in P301L tau transgenic mice, J. Biol. Chem. 280 (2005) 23802–23814.
[158] A. Eckert, S. Hauptmann, I. Scherping, J. Meinhardt, V. Rhein, S. Drose, U. Brandt, M. Fandrich, W.E. Muller, J. Gotz, Oligomeric and fibrillar species of β-amyloid (Aβ42) both impair mitochondrial function in P301L tau transgenic mice, J. Mol. Med. 86 (2008) 1255–1267.
[159] S. Oddo, A. Caccamo, J.D. Shepherd, M.P. Murphy, T.E. Golde, R. Kayed, R. Metherate, M.P. Mattson, Y. Akbari, F.M. LaFerla, Triple-transgenic model of Alzheimer's disease with plaques and tangles. Intracellular abeta and synaptic dysfunction, Neuron 39 (2003) 409–421.
[160] S. Oddo, A. Caccamo, M. Kitazawa, B.P. Tseng, F.M. LaFerla, Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease, Neurobiol. Aging 24 (2003) 1063–1070.
[161] T. Bolmont, F. Clavaguera, M. Meyer-Luehmann, M.C. Herzig, R. Radde, M. Staufenbiel, J. Lewis, M. Hutton, M. Tolnay, M. Jucker, Induction of tau pathology by intracerebral infusion of amyloid-beta-containing brain extract and by amyloid-beta deposition in APP x Tau transgenic mice, Am. J. Pathol. 171 (2007) 2012–2020.
[162] C.W. Wittmann, M.F. Wszolek, J.M. Shulman, P.M. Salvaterra, J. Lewis, M. Hutton, M.B. Feany, Tauopathy in *Drosophila*: neurodegeneration without neurofibrillary tangles, Science 293 (2001) 711–714.
[163] G.R. Jackson, M. Wiedau-Pazos, T.-K. Sang, N. Wagle, C.A. Brown, S. Massachi, D.H. Geschwind, Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in *Drosophila*, Neuron 34 (2002) 509–519.
[164] M.L. Steinhilb, D. Dias-Santagata, T.A. Fulga, D.L. Felch, M.B. Feany, Tau phosphorylation sites work in concert to promote neurotoxicity in vivo, Mol. Biol. Cell 18 (2007) 5060–5068.
[165] I. Nishimura, Y. Yang, B. Lu, PAR-1 kinase plays an initiator role in a temporally ordered phosphorylation process that confers tau toxicity in *Drosophila*, Cell 116 (2004) 671–682.
[166] K.W. Chau, W.Y. Chan, P.C. Shaw, H.Y. Chan, Biochemical investigation of Tau protein phosphorylation status and its solubility properties in *Drosophila*, Biochem. Biophys. Res. Commun. 346 (2006) 150–159.
[167] M. Morcos, H. Hutter, The model *Caenorhabditis elegans* in diabetes mellitus and Alzheimer's disease, J. Alzheimers Dis. 16 (2009) 897–908.
[168] R.A. Green, A. Audhya, A. Pozniakovsky, A. Dammermann, H. Pemble, J. Monen, N. Portier, A. Hyman, A. Desai, K. Oegema, Expression and imaging of fluorescent proteins in the C. elegans gonad and early embryo, Methods Cell Biol. 85 (2008) 179–218.
[169] B.C. Kraemer, B. Zhang, J.B. Leverenz, J.H. Thomas, J.Q. Trojanowski, G.D. Schellenberg, From the Cover: Neurodegeneration and defective neurotransmission in a *Caenorhabditis elegans* model of tauopathy, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 9980–9985.
[170] R. Brandt, A. Gergou, I. Wacker, T. Fath, H. Hutter, A *Caenorhabditis elegans* model of tau hyperphosphorylation: induction of developmental defects by transgenic overexpression of Alzheimer's disease-like modified tau, Neurobiol. Aging 30 (2009) 22–33.
[171] F. Hernandez, F. Lim, J.J. Lucas, C. Perez-Martin, F. Moreno, J. Avila, Transgenic mouse models with tau pathology to test therapeutic agents for Alzheimer's disease, Mini Rev. Med. Chem. 2 (2002) 51–58.
[172] G. Li, A. Faibushevich, B.J. Turunen, S.O. Yoon, G. Georg, M.L. Michaelis, R.T. Dobrowsky, Stabilization of the cyclin-dependent kinase 5 activator, p35, by paclitaxel decreases beta-amyloid toxicity in cortical neurons, J. Neurochem. 84 (2003) 347–362.
[173] C.J. Phiel, C.A. Wilson, V.M. Lee, P.S. Klein, GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides, Nature 423 (2003) 435–439.
[174] T. Bussiere, P.R. Hof, C. Mailliot, C.D. Brown, M.L. Caillet-Boudin, D.P. Perl, L. Buee, A. Delacourte, Phosphorylated serine422 on tau proteins is a pathological epitope found in several diseases with neurofibrillary degeneration, Acta Neuropathol. (Berl.) 97 (1999) 221–230.
[175] A. Schneider, J. Biernat, M. von Bergen, E. Mandelkow, E.M. Mandelkow, Phosphorylation that detaches tau protein from microtubules (Ser262, Ser214) also protects it against aggregation into Alzheimer paired helical filaments, Biochemistry 38 (1999) 3549–3558.
[176] Y.P. Wang, J. Biernat, M. Pickhardt, E. Mandelkow, E.M. Mandelkow, Stepwise proteolysis liberates tau fragments that nucleate the Alzheimer-like aggregation of full-length tau in a neuronal cell model, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 10252–10257.
[177] C. Ballatore, V.M. Lee, J.Q. Trojanowski, Tau-mediated neurodegeneration in Alzheimer's disease and related disorders, Nat. Rev. Neurosci. 8 (2007) 663–672.
[178] E.D. Roberson, K. Scearce-Levie, J.J. Palop, F. Yan, I.H. Cheng, T. Wu, H. Gerstein, G. Q. Yu, L. Mucke, Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model, Science 316 (2007) 750–754.
[179] C.X. Gong, K. Iqbal, Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease, Curr. Med. Chem. 15 (2008) 2321–2328.
[180] S. Le Corre, H.W. Klafki, N. Plesnila, G. Hubinger, A. Obermeier, H. Sahagun, B. Monse, P. Seneci, J. Lewis, J. Eriksen, C. Zehr, M. Yue, E. McGowan, D.W. Dickson, M. Hutton, H.M. Roder, An inhibitor of tau hyperphosphorylation prevents severe motor impairments in tau transgenic mice, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 9673–9678.
[181] A. Caccamo, S. Oddo, L.X. Tran, F.M. LaFerla, Lithium reduces tau phosphorylation but not A beta or working memory deficits in a transgenic model with both plaques and tangles, Am. J. Pathol. 170 (2007) 1669–1675.
[182] W. Noble, E. Planel, C. Zehr, V. Olm, J. Meyerson, F. Suleman, K. Gaynor, L. Wang, J. LaFrancois, B. Feinstein, M. Burns, P. Krishnamurthy, Y. Wen, R. Bhat, J. Lewis, D. Dickson, K. Duff, Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 6990–6995.
[183] D. Paquet, R. Bhat, A. Sydow, E.M. Mandelkow, S. Berg, S. Hellberg, J. Falting, M. Distel, R.W. Koster, B. Schmid, C. Haass, A zebrafish model of tauopathy allows in vivo imaging of neuronal cell death and drug evaluation, J. Clin. Invest. 119 (2009) 1382–1395.
[184] Z. Berger, B. Ravikumar, F.M. Menzies, L.G. Oroz, B.R. Underwood, M.N. Pangalos, I. Schmitt, U. Wullner, B.O. Evert, C.J. O'Kane, D.C. Rubinsztein, Rapamycin alleviates toxicity of different aggregate-prone proteins, Hum. Mol. Genet. 15 (2006) 433–442.
[185] T. Noda, Y. Ohsumi, Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast, J. Biol. Chem. 273 (1998) 3963–3966.
[186] B. Zhang, A. Maiti, S. Shively, F. Lakhani, G. McDonald-Jones, J. Bruce, E.B. Lee, S.X. Xie, S. Joyce, C. Li, P.M. Toleikis, V.M. Lee, J.Q. Trojanowski, Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 227–231.
[187] J. Gotz, N. Schonrock, B. Vissel, L.M. Ittner, Alzheimer's disease selective vulnerability and modelling in transgenic mice, J. Alzheimers Dis. (2009), Jul 7 [Electronic publication ahead of print].