Assembly of paired helical filaments from mouse tau: implications for the neurofibrillary pathology in transgenic mouse models for Alzheimer's disease

T. Kampers[a], M. Pangalos[b], H. Geerts[c], H. Wiech[a], E. Mandelkow[a,*]

[a] *Max-Planck-Unit for Structural Molecular Biology, Notkestrasse 85, D-22603 Hamburg, Germany*
[b] *Division of Biotechnology, Janssen Pharmaceutica, Turnhoutseweg 30, B-2340 Beerse, Belgium*
[c] *Division of Life Sciences, Janssen Pharmaceutica, Turnhoutseweg 30, B-2340 Beerse, Belgium*

Received 26 March 1999

Abstract In Alzheimer's disease and related dementias, human tau protein aggregates into paired helical filaments and neurofibrillary tangles. However, such tau aggregates have not yet been demonstrated in transgenic mouse models of the disease. One of the possible explanations would be that mouse tau has different properties which prevents it from aggregating. We have cloned several murine tau isoforms, containing three or four repeats and different combinations of inserts, expressed them in *Escherichia coli* and show here that they can all be assembled into paired helical filaments similar to those in Alzheimer's disease, using the same protocols as with human tau. Therefore, the absence of pathologically aggregated tau in transgenic mice cannot be explained by intrinsic differences in mouse tau protein and instead must be explained by other as yet unknown factors.

Key words: Alzheimer's disease; Microtubule; Neurofibrillary tangle; Paired helical filament; Self assembly; Tau protein

1. Introduction

The aggregation of the microtubule-associated protein (MAP) tau into paired helical filaments (PHFs) and neurofibrillary tangles (NFT) is one of the hallmarks of Alzheimer disease (AD). Their density and spreading from the transentorhinal region to the neocortex can be used to subdivide the progression of AD into six stages [10,3]. Besides AD, several related dementias (e.g. corticobasal degeneration, Pick's disease, prion diseases) show neurofibrillary pathology and in some cases, such as frontotemporal dementia with Parkinsonism, the disease correlates with mutations in the human tau gene located on chromosome 17 [68,55,37,64,35,17,59,29]. Pathological tau aggregates have also been observed in aged monkeys, dogs, bears, goats and sheep (e.g. [71,72,18,50,51]), however, they have not been observed in rodents such as rats or mice so far. Furthermore, transgenic animals carrying mutations in genes involved in familial AD (e.g. the mutations in amyloid precursor protein (APP) or presenilins, review [62]) have been shown to result in increased levels of the Aβ peptide and deposition of amyloid plaques in a manner similar to AD neuropathology, but no tau deposits have been seen [24,46,36,65]. Similar amyloid changes occur in transgenic mice bearing mutations in the presenilin gene with increases in the Aβ40 and Aβ42 deposition [20,67,8], but so far, no evidence of neurofibrillary pathology has been reported. Even the expression of human tau in transgenic mice did not lead to tau aggregates [30,13].

One could hypothesize that the failure of tau to aggregate in transgenic mouse models of AD may reside in the small but significant differences existing between the amino acid sequences of mouse and human. In the CNS, human tau can be expressed in six isoforms arising from alternative splicing [25,2] and a similar diversity exists in other animals. On the protein level, four isoforms have been reported in adult bovine tau [34] and three or four isoforms in adult rat or mouse tau (rat: [41,40,49,21], mouse: [30,13]). The isoforms differ by the presence or absence of one of the four pseudo-repeats in the microtubule-binding domain and one or two near N-terminal inserts (Fig. 1). In the case of humans, the smallest isoform (3R-0N) has been shown to predominate in fetal brain while adult brains contain a mixture of different isoforms with three or four repeats [25,27,12]. In rats, the smallest isoform also dominates in fetal brain, whereas the adult brain contains only the three isoforms with four repeats [41,40,27,49]. Further 'big tau' isoforms exist in peripheral nerves (mouse, human, rat [19,26,9] and non-neuronal cells may contain tau as well [44,31]).

Tau was originally cloned from mouse in the 3-repeat 'fetal' form [43]. A comparison of human tau and mouse tau shows some differences in the N-terminal domain while the C-terminal domain remains almost identical (Fig. 2). It is the C-terminal region that is responsible for promoting microtubule assembly as well as the aggregation of tau into PHFs, both in vivo [70] and in vitro [69]. The N-terminal half of the protein has been shown to inhibit PHF formation, but this inhibition can be overcome in vitro by polyanionic cofactors such as heparin, RNA or poly-Glu [54,28,39,33,22]. The similarity of the microtubule-binding domain in human and mouse tau suggests that both proteins should aggregate under similar conditions. On the other hand, the occurrence of conservative missense mutations in the microtubule-binding region of human tau, which lead to the aggregation of tau in tauopathies such as FTDP-17, indicates that even seemingly subtle changes can have gross effects on the tau function (see references above and reviews by [63,45]).

In view of the importance of transgenic mouse models for analyzing AD, these considerations make it necessary to test the assembly behavior of mouse tau isoforms directly. Here, we report that mouse brains show a transition from the small-

*Corresponding author. Fax: (49) (40) 89716822.

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; MAPs, microtubule-associated proteins; NFT, neurofibrillary tangle; PHF, paired helical filament; ThS, thioflavin S

PII: S0014-5793(99)00522-0

est tau isoform (3R-0N) in fetal brain (appearing around E14) to a mixture of three 4-repeat isoforms (4R-0N, -1N, -2N) in the adult brain, reminiscent of rat brain. All isoforms are capable of assembling into Alzheimer-like PHFs with a similar efficiency as human tau, using conditions similar to our earlier studies with human tau [39,22,23]. This argues that sequence differences do not prevent the aggregation of mouse tau and that other unknown mechanisms must inhibit assembly of tau to AD-like PHFs in mice.

2. Materials and methods

2.1. Cloning of tau isoforms from mouse brain

Sequence data from mouse tau (accession number M18776) were used to design primers to amplify the complete mTau encoding sequence by PCR. Primers used were mTauS1(*Eco*RI) 5′-GCGAATTCCCTCTTCTGTCCTCGCCTTCTGTC-3′ and mTauAS1(*Xho*I) 5′-GCCTCGAGGATTATTGACTGCCCTGGGAGCC-3′. PCR amplifications were performed using a Marathon-Ready mouse brain cDNA (Clontech) and the Expand High Fidelity PCR system (Boehringer Mannheim) with 0.2 μM of each oligonucleotide. All PCR reactions were pre-heated to 95°C for 5 min before the addition of enzyme and included a final step of 7 min at 72°C upon completion of the amplification cycles. Cycling conditions were 45 s at 95°C, 1 min at 56°C and 1 min and 30 s at 72°C for 25 cycles. Three PCR products corresponding in size to mTau 24, 34 and 40 (four repeats with 0, 1 or 2 inserts, terminology corresponding to human tau isoforms, [25]) were TA-cloned into pCR2.1 and screened by restriction digestion. Several clones containing inserts of the appropriate size were fully sequenced on both strands using ABI prism BigDye Terminator Cycle sequencing kits and an Applied Biosystems 377XL sequencer (Perkin Elmer, Foster City, USA). Sequencher software was used for sequence assembly and manual editing (GeneCodes, Ann Arbor, USA). Subcloning of the different mtau isoforms into the bacterial expression vector pET21a (Novagen) was performed by PCR amplification from mTau/pCR2.1 plasmid DNA using primers mTauAS2(*Xho*I) 5′-GCCTCGAGCCTGATCACAAACCCTGCTTGGC-3′ and mTauS3(*Nde*I) 5′-GCGAATTCCATATGGCTGACCCTCGGCAGG-3′. PCR fragments were gel-purified and sub-cloned into dephosphorylated pET21a that was previously digested with the appropriate restriction enzymes. The resulting expression constructs were verified by complete sequence analysis as described earlier. The shortest isoform mtau23 (3R-0N) was constructed as a chimaera from a gene fragment encoding the N-terminal half of mouse tau (lacking the N-terminal inserts) and the C-terminal half of human tau (containing only three repeats). This construct contains one conservative exchange (K257R) compared to the original mouse tau sequence ([43] note that we use the numbering for human tau even for mouse tau isoforms).

2.2. Preparation of mouse tau protein

Recombinant mouse Tau isoforms were expressed in *Escherichia coli* and purified by FPLC MonoS (Pharmacia) chromatography as described for the human Tau isoforms (e.g. [5]). For preparation of tau from mouse brain (strain C57BlackJ6), total brain from adult or embryonic mice (12–14 days) was homogenized in extraction buffer (50 mM Na-PIPES pH 6.9, 500 mM NaCl, 5 mM EGTA, 50 mM NaF, 0.5 mM PMSF, 1 mM Na_3VO_4, 5 mM DTT, 5 mM $Na_2H_2P_2O_7$, 1 μM microcystin, 10 μg/ml leupeptin, aprotinin and pepstatin A) and centrifuged for 20 min at 14 000 rpm. The supernatant was boiled for 10 min at 100°C and recentrifuged. The supernatant was acetone-precipitated, washed, dried and resuspended in 50 mM Tris, 5 mM $MgCl_2$, pH 8.3. Samples for dephosphorylation were dialyzed against 100 mM Tris-HCl pH 8.2. Dephosphorylation of tau protein was done with alkaline phosphatase (Boehringer) at a concentration of 400 U/ml for 20 h at 37°C. The purity of the proteins was analyzed by SDS-PAGE. Protein concentrations were determined by the BCA method.

2.3. PHF assembly

Varying concentrations of mouse tau isoforms (typically in the range of 50–200 μM) in volumes of 50 μl were incubated at 37°C in 100 mM Tris-HCl pH 6.8 up to 6 days. Protien solutions contained various anionic cofactors: tRNA (from bovine liver), heparin (average molecular weight (MW) of 3000), poly-L-glutamate (average MW of 1000) at approximately equimolar or indicated concentrations. All chemicals were obtained from Sigma.

2.4. Electron microscopy

600-mesh-carbon-coated copper grids were glow-discharged twice (CTA 010, Balzers Union). They were incubated on a drop of protein solution for 1.5 min and negatively stained with 2% uranyl acetate for 0.5–1 min. The specimens were examined in a Philips CM12 electron microscope at 100 kV.

2.5. Fluorescence spectroscopy

For the quantification of PHF assembly, fluorescence emission spectra of thioflavine S (ThS, Sigma) bound to tau filaments were measured with a Spex Fluoromax (Spex Instruments SA) with excitation at 400 nm and emission at 500 nm (excitation and emission bandwidths were set at 5 nm [22]). Filament formation was followed by diluting the samples to 1 μM protein in 20 mM MOPS pH 7.0 in the presence of 5 μM ThS.

2.6. Other methods

SDS gel electrophoresis was done on 8% polyacrylamide gels. Immunoblotting was done by transferring the proteins to an Immobilon membrane (Millipore) and incubated with protein G affinity-purified polyclonal antibodies pAb-anti-insert1 (SA 4473), pAb-anti-insert2 (SA 4474) and pAb-anti-tau (K9JA). Non-specific binding was blocked by low-fat milk dissolved in TBS-Tween. The bound primary antibody was detected by a peroxidase-conjugated secondary antibody (anti-rabbit).

3. Results

The aim of this study was to determine whether mouse tau isoforms were intrinsically able to form Alzheimer-like PHFs in vitro, in a manner similar to human tau, or whether mouse tau was sufficiently different such that PHF formation would be prevented. We cloned the juvenile 3-repeat isoforms 3R-0N ([43] corresponding to the human fetal isoform htau23, [25]), as well as the three 4-repeat isoforms containing 0, 1 or 2 near N-terminal inserts (4R-0N, 4R-1N, 4R-2N, corresponding to the human isoforms htau24, htau34, htau40; Fig. 1). In general, the mouse isoforms are similar to their human counterparts. Changes occur almost exclusively in the N-terminal projection domain, whereas the C-terminal assembly domains are nearly identical (Fig. 2). In particular, mouse tau shows a similar pattern of alternative splicing, having either three or four pseudo-repeats (of 31 residues each) in the C-terminal microtubule-binding domain and up to two inserts (of 29 residues each) in the N-terminal domain.

During embryonic development, no tau is present until day

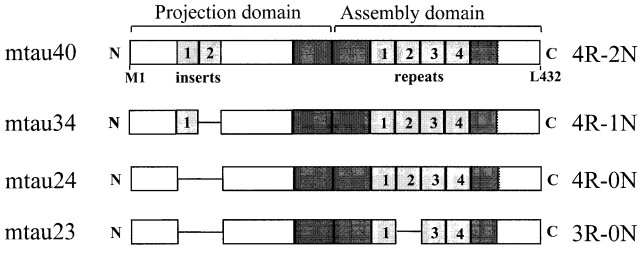

Fig. 1. Diagrams of mouse tau isoforms used in this study. Due to alternative splicing, there may be 0, 1 or 2 near N-terminal inserts of 29 residues each (I1, I2), and three or four pseudo-repeats (31 residues) in the C-terminal half (R1–R4). The C-terminal half is responsible for microtubulus stabilisation and assembly of PHFs. Fetal brain contains mainly the shortest isoform (3R-0N). Numbering of residues is in analogy with human tau40.

```
          HTAU40    - M--AEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLK     - 44
                      |  | |||||  ||||||              ||  |||||| | |||
          MTAU40    - MHMADPRQEFDTMEDHAG----------DYTLLQDQEGDMDHGLK     - 35

          HTAU40    - ESPLQTPTEDGSEEPGSETSDAKSTPTAE                      - 73
I1                    ||| | |  || ||||||||||||||||||
          MTAU40    - ESPPQPPADDGAEEPGSETSDAKSTPTAE                      - 64

          HTAU40    - DVTAPLVDEGAPGKQAAAQPHTEIPEGTT                     -102
I2                    ||||||||| || ||||||||||||||| |
          MTAU40    - DVTAPLVDERAPDKQAAAQPHTEIPEGIT                     - 93

          HTAU40    - AEEAGIGDTPSLEDEAAGHVTQARMVSKSKDGTGSDDKKAKGADGKT   -149
                      |||||||||  || |||||||||  ||  | || | ||||||||||
          MTAU40    - AEEAGIGDTPNQEDQAAGHVTQARVASK--DRTGNDEKKAKGADGKT   -138

          HTAU40    - --KIATPRGAAPPGQKGQANATRIPAKTPPAPKTPPSSGEPPKSGDRS  -195
                        |||||||| | |||  |||||||| | ||||| ||||||||  ||
          MTAU40    - GAKIATPRGAASPAQKGTSNATRIPAKTTPSPKTPPGSGEPPKSGERS  -186

          HTAU40    - GYSSPGSPGTPGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSSAKSRL  -243
                      ||||||||||||||||||||||||||||||||||||||||||  ||||
          MTAU40    - GYSSPGSPGTPGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSASKSRL  -234

          HTAU40    - QTAPVPMPDLKNVKSKIGSTENLKHQPGGGK                   -274
R1                    |||||||||||| ||||||||||||||||
          MTAU40    - QTAPVPMPDLKNVRSKIGSTENLKHQPGGGK                   -265

          HTAU40    - VQIINKKLDLSNVQSKCGSKDNIKHVPGGGS                   -305
R2                    ||||||||||||||||||||||||||||||||
          MTAU40    - VQIINKKLDLSNVQSKCGSKDNIKHVPGGGS                   -296

          HTAU40    - VQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQ                   -336
R3                    ||||||||||||||||||||||||||||||||
          MTAU40    - VQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQ                   -327

          HTAU40    - VEVKSEKLDFKDRVQSKIGSLDNITHVPGGGN                  -368
R4                    ||||||||||||||||||||||||||||||||
          MTAU40    - VEVKSEKLDFKDRVQSKIGSLDNITHVPGGGN                  -359

          HTAU40    - KKIETHKLTFRENAKAKTDHGAEIVYKSPVVSGDTSPR            -406
                      |||||||||||||||||||||||||||||||||||||||||
          MTAU40    - KKIETHKLTFRENAKAKTDHGAEIVYKSPVVSGDTSPR            -397

          HTAU40    - HLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL               -441
                      |||||||||||||||||||||||||||||||||||
          MTAU40    - HLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL               -432
```

Fig. 2. Amino acid sequence comparison of the longest tau isoforms (4R-2N) in the CNS of humans and mice. Note that the assembly domains of both proteins differ only in three amino acid residues (human to mouse, S238A, A239S, K257R).

E13 (Fig. 3, lanes 1, 2). At E15, one observes two bands, a major component (~70%) representing the smallest mouse isoform containing three repeats and no inserts (mtau23, 3R-0N), followed by a minor component (~30%) containing four repeats (mtau24, 4R-0N, Fig. 3, lanes 3, 4). This mixture remains visible until at least E17. By contrast, adult mouse brain contains only the three 4-repeat isoforms with 0, 1 or 2 inserts. This switch in the splicing pattern is analogous to that

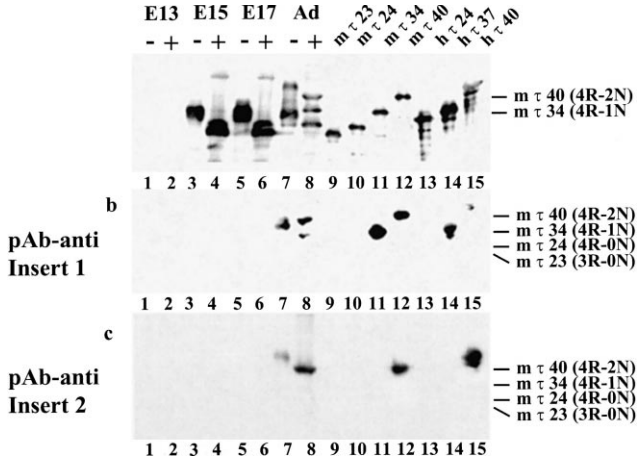

Fig. 3. Immunoblots of fetal and adult brain tau from mouse with antibody (a) pAb-anti-Tau (K9JA), (b) pAb-anti-insert 1, (c) pAb-anti-insert 2. Lanes 1 and 2: mouse tau from fetal brain day 13; lanes 3 and 4: mouse tau from fetal brain day 15; lanes 5 and 6: mouse tau from fetal brain day 17; lanes 7 and 8: mouse tau from adult brain; lanes 9–12: recombinant mouse tau23 (3R-0N), mtau24 (4R-0N), mtau34 (4R-1N), mtau40 (4R-2N); lanes 13–15; recombinant human tau, htau24, htau37, htau40. Lanes 1–8: protein tau treated with (+) or without (−) alkaline phosphatase.

in the rat or human brain [41,27,30,49], except that human brain retains 3-repeat tau isoforms even in the adult stage.

For PHF assembly experiments, we expressed the fetal isoform 3R-0N and the three 4-repeat isoforms in *E. coli* and prepared them as described previously [6]. The assembly conditions were similar to those developed earlier for human tau. In general, PHF formation is facilitated if one starts from a solution of tau dimers which can be achieved by chemical crosslinking at Cys-322 [69,61]. Secondly, the assembly proceeds most easily with tau constructs containing only the microtubule-binding domain, whereas the N-terminal domain tends to prevent PHF assembly [69]. However, this inhibition can be overcome by adding cellular polyanions, such as polysulfates (heparin, [54,28]), polyphosphates [39] or polycarboxylates (acidic peptides such as poly-Glu, [22]). Using these principles, all mouse tau isoforms could be readily induced

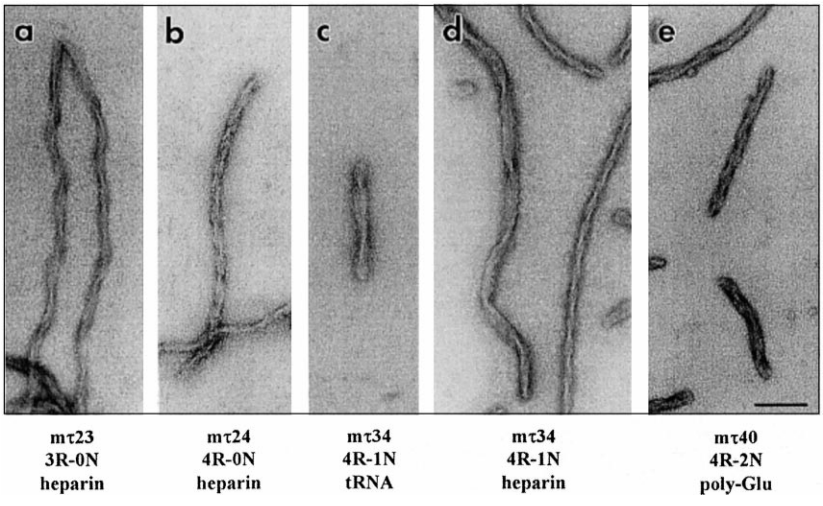

Fig. 4. Negative stain electron micrographs of PHFs assembled from mouse tau isoforms. (a) Fetal tau mtau23 (3R-0N), (b) mtau24 (4R-0N), (c, d) mtau34 (4R-1N), (e) mtau40 (4R-2N). Assembly experiments were performed using 50–200 μM mouse tau in the presence of 150–200 μM heparin (a, b, d), 0.5 mg/ml RNA (c) or 200 μM poly-L-glutamate (e). Scale bar: 100 nm.

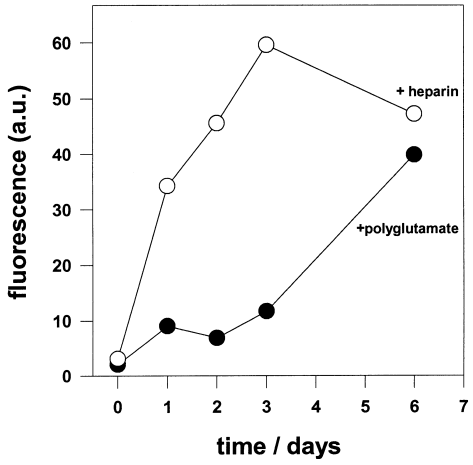

Fig. 5. Assembly kinetics of mtau24 (4R-0N) (50 μM) using the ThS fluoresence assay [22]. (a) In the presence of poly-L-glutamate (200 μM) or (b) in the presence of heparin (50 μM).

to form PHF-like filaments. In the electron microscope, they showed the typical two-stranded appearance, with crossover repeats around 80 nm and widths varying between 10 and 20 nm (Fig. 4).

The assembly process could be monitored in real time using an assay based on the fluorescence enhancement of ThS [22]. This shows that PHF assembly from mouse tau can be induced by all three types of polyanions mentioned above, but with different efficiencies, in the order heparin > RNA > poly-Glu (Fig. 5). This behavior is similar to that of human tau. We therefore conclude that mouse tau is as able as human tau to form PHFs in vitro.

4. Discussion

A pathological cascade has been proposed for AD beginning with an altered metabolism of APP and leading to the increased production of the β-amyloid peptide, subsequent amyloid deposition, followed by the formation of NFTs and finally leading to neuronal cell death [42,62]. In support of this hypothesis, the link between APP, β-amyloid formation and deposition has been demonstrated in transgenic mouse models carrying the APP gene bearing different familial AD mutations [16,36,46,20,24,58]. Thus far, these mouse models have all failed to develop PHFs and NFTs, even though this neuropathological hallmark is required to be present (as well as amyloid plaques), by definintion, in order for a positive diagnosis of AD to be given post-mortem [10,3]. To address this problem directly, transgenic mice carrying the human tau gene have been developed [30,13], but despite the elevated levels of tau (human and total tau), these mouse models have also failed to develop any neurofibrillary pathology. The only sign of tau alteration in APP or tau transgenic mice has been a somewhat elevated level of tau phosphorylation at the sites recognized by antibodies sensitive to phosphorylated Ser-Pro or Thr-Pro motifs [30,13] or an altered conformation recognized by the antibody Alz-50 [65,13]. However, altered phosphorylation can be induced by several conditions such as the fetal stage, hormone levels, phosphatase activity post-mortem, Aβ toxicity [53,12,47,14,7]. Moreover, even when 'aberrant' levels of phosphorylation are detected, there is no indication of incipient PHF assembly. A similar lack of correlation between tau phosphorylation and PHF assembly is observed in cell models as well (e.g. [52,1,56]).

The most natural explanation for the absence of PHFs in mice would be the inability of mouse tau to form PHFs. The sequence alignment between human and mouse tau shows that they are very similar, especially in the C-terminal half which is important for PHF assembly (Fig. 1 and Fig. 2). Nevertheless, even seemingly minor differences could have large effects on self-assembly, as is impressively demonstrated by the point mutations leading to the assembly of hemoglobin in sickle cell anemia, transthyretin in systemic amyloidosis, prion protein in Creutzfeld-Jacob disease and others (reviews, [66,15,32,57]). It was therefore necessary to test the in vitro characteristics of mouse tau isoforms directly. The results show that the differences in the sequence between mouse and human tau have no noticeable influence on the aggregation properties of the proteins. Thus, there must be different explanations for the failure of mouse (or human) tau to aggregate in transgenic mouse models. For example, it is possible that the factors that nucleate PHF formation in neurons are different between mouse and human brains (in vitro they are mimicked by polyanions such as heparin, RNA or poly-L-glutamate). It is also conceivable that the isoform composition matters for PHF aggregation in vivo. It has been shown that rat and human brains have a transient stage where only the smallest tau isoform with three repeats and no inserts occurs [41,27] and the same is true for the mouse brain, as shown here. By contrast, human brains differ from rodents in the adult splicing pattern. Human tau is a mixture of six splicing isoforms with three or four repeats, whereas only 4-repeat forms occur in adult rodent tau. This difference may be related to different stabilities of an mRNA stem loop at exon 10. However, this in itself cannot explain the failure of mouse brains to develop PHFs since certain human Alzheimer-related tauopathies also show a preponderance of 4-repeat tau isoforms [37,64].

Alzheimer tau differs from normal tau not only in terms of aggregation, but also in terms of post-translational modifications, notably phosphorylation. It is possible that the modifications of mouse tau are different from those of human tau which could subsequently affect aggregation. On the basis of current knowledge however, this is not likely, since the assembly of PHFs in vitro can be achieved independently of phosphorylation [69]. A debate on whether abnormal phosphorylation promotes PHF aggregation has continued for some time and changes in the phosphorylation are often regarded as hallmarks of incipient aggregation. Indeed, phosphorylation appears to precede tangle formation in degenerating neurons [4,11]. In several mouse lines, increased levels of phosphorylation have been observed, e.g. cells from mice carrying human APP mutations [24,65], cells overexpressing the kinase mos [38] or overexpressing human tau [30,13]. However, the fact that none of these models have developed tangles would argue that phosphorylation does not necessarily predispose tau for aggregation. This is consistent with our recent findings that phosphorylation protects tau against aggregation, rather than promoting it [60]. Nevertheless, it is still possible that other modifications such as glycation or transglutamination may cause PHF aggregation in humans but not in mice [73,74,48]. These modifications have yet to be characterized for mouse brain tau.

Acknowledgements: We thank Dr Irm Hermans-Borgmeyer (Hamburg) for the help with mouse brain preparations, Anja Konopatzki (Hamburg) and Leisbet van der Helm (Beerse) for expert technical assistance, Dr Marc Mercken (Beerse) and Dr Eva-Maria Mandelkow (Hamburg) for advice and discussions and the Deutsche Forschungsgemeinschaft for financial support. This work represents part of the doctoral thesis of T.K.

References

[1] Anderton, B.H., Brion, J.P., Couck, A.M., Davis, D.R., Gallo, J.M., Hanger, D.P., Ladhani, K., Latimer, D.A., Lewis, C. and Lovestone, S. (1995) Neurobiol. Aging 16, 389–402.
[2] Andreadis, A., Brown, W.M. and Kosik, K.S. (1992) Biochemistry 31, 10626–10633.
[3] Ball, M., Braak, H., Coleman, P., Dickson, D., Duyckaerts, C., Gambetti, P., Hansen, L., Hyman, B., Jellinger, K., Markesbery, W., Perl, D. and Powers, J. (1997) Neurobiol. Aging 18, 1–2.
[4] Bancher, C., Brunner, C., Lassmann, H., Budka, H., Jellinger, K., Wiche, G., Seitelberger, F., Grundke-Iqbal, I., Iqbal, K. and Wisniewski, H.M. (1989) Brain Res. 477, 90–99.
[5] Biernat, J., Gustke, N., Drewes, G., Mandelkow, E.-M. and Mandelkow, E. (1993) Neuron 11, 153–163.
[6] Biernat, J., Mandelkow, E.M., Schröter, C., Lichtenberg-Kraag, B., Steiner, B., Berling, B., Meyer, H.E., Mercken, M., Vandermeeren, A., Goedert, M. and Mandelkow, E. (1992) EMBO J. 11, 1593–1597.
[7] Biernat, J. and Mandelkow, E.-M. (1999) Mol. Biol. Cell 10, 727–740.
[8] Borchelt, D.R., Thinakaran, G., Eckman, C.B., Lee, M.K., Davenport, F., Ratovitsky, T., Prada, C.M., Kim, G., Seekins, S., Yager, D., Slunt, H.H., Wang, R., Seeger, M., Levey, A.I., Gandy, S.E., Copeland, N.G., Jenkins, N.A., Price, D.L., Younkin, S.G. and Sisodia, S.S. (1996) Neuron 17, 1005–1013.
[9] Boyne, L.J., Tessler, A., Murray, M. and Fischer, I. (1995) J. Comp. Neurol. 358, 279–293.
[10] Braak, H. and Braak, E. (1991) Acta Neuropathol. 82, 239–259.
[11] Braak, H., Braak, E. and Mandelkow, E.-M. (1994) Acta Neuropathol. 87, 554–567.
[12] Bramblett, G.T., Goedert, M., Jakes, R., Merrick, S.E., Trojanowski, J.Q. and Lee, V.M. (1993) Neuron 10, 1089–1099.
[13] Brion, J.P., Tremp, G. and Octave, J.N. (1999) Am. J. Pathol. 154, 255–270.
[14] Busciglio, J., Lorenzo, A., Yeh, J. and Yankner, B.A. (1995) Neuron 14, 879–888.
[15] Castano, E.M. and Frangione, B. (1995) Curr. Opin. Neurol. 8, 279–285.
[16] Citron, M., Oltersdorf, T., Haass, C., McConlogue, L., Hung, A.Y., Seubert, P., Vigo-Pelfrey, C., Lieberburg, I. and Selkoe, D.J. (1992) Nature 360, 672–674.
[17] Clark, L.N., Poorkaj, P., Wszolek, Z., Geschwind, D.H., Nasreddine, Z.S., Miller, B., Li, D., Payami, H., Awert, F., Markopoulou, K., Andreadis, A., D'Souza, I., Lee, V.M., Reed, L., Trojanowski, J.Q., Zhukareva, V., Bird, T., Schellenberg, G. and Wilhelmsen, K.C. (1998) Proc. Natl. Acad. Sci. USA 95, 13103–13107.
[18] Cork, L.C., Powers, R.E., Selkoe, D.J., Davies, P., Geyer, J.J. and Price, D.L. (1988) J. Neuropathol. Exp. Neurol. 47, 629–641.
[19] Couchie, D., Mavilia, C., Georgieff, I., Liem, R., Shelanski, M. and Nunez, J. (1992) Proc. Natl. Acad. Sci. USA 89, 4378–4381.
[20] Duff, K., Eckman, C., Zehr, C., Yu, X., Prada, C.M., Perez-tur, J., Hutton, M., Buee, L., Harigaya, Y., Yager, D., Morgan, D., Gordon, M.N., Holcomb, L., Refolo, L., Zenk, B., Hardy, J. and Younkin, S. (1996) Nature 383, 710–713.
[21] Ferreira, A., Lu, Q., Orecchio, L. and Kosik, K. (1997) Mol. Cell Neurosci. 9, 220–234.
[22] Friedhoff, P., Schneider, A., Mandelkow, E.-M. and Mandelkow, E. (1998) Biochemistry 37, 10223–10230.
[23] Friedhoff, P., von Bergen, M., Mandelkow, E.-M., Davies, P. and Mandelkow, E. (1998) Proc. Natl. Acad. Sci. USA 95, 15712–15717.
[24] Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., Blackwell, C., Carr, T., Clemens, J., Donaldson, T., Gillespie, F., Guido, T. and Hagopian, S. (1995) Nature 373, 523–527.
[25] Goedert, M., Spillantini, M., Jakes, R., Rutherford, D. and Crowther, R.A. (1989) Neuron 3, 519–526.
[26] Goedert, M., Spillantini, M.G. and Crowther, R.A. (1992) Proc. Nat. Acad. Sci. USA 89, 1983–1987.
[27] Goedert, M., Jakes, R., Crowther, R.A., Six, J., Lubke, U., Vandermeeren, M., Cras, P., Trojanowski, J.Q. and Lee, V.M.Y. (1993) Proc. Natl. Acad. Sci. USA 90, 5066–5070.
[28] Goedert, M., Jakes, R., Spillantini, M.G., Hasegawa, M., Smith, M.J. and Crowther, R.A. (1996) Nature 383, 550–553.
[29] Goedert, M., Crowther, R.A. and Spillantini, M.G. (1998) Neuron 21, 955–958.
[30] Götz, J., Probst, A., Spillantini, M.G., Schafer, T., Jakes, R., Burki, K. and Goedert, M. (1995) EMBO J. 14, 1304–1313.
[31] Gu, Y.J., Oyama, F. and Ihara, Y. (1996) J. Neurochem. 67, 1235–1244.
[32] Harper, J. and Lansbury, P.J. (1997) Ann. Rev. Biochem. 66, 385–407.
[33] Hasegawa, M., Crowther, R.A., Jakes, R. and Goedert, M. (1997) J. Biol. Chem. 272, 33118–33124.
[34] Himmler, A. (1989) Mol. Cell. Biol. 9, 1389–1396.
[35] Hong, M., Zhukareva, V., Vogelsberg-Ragaglia, V., Wszolek, Z., Reed, L., Miller, B.I., Geschwind, D.H., Bird, T.D., McKeel, D., Goate, A., Morris, J.C., Wilhelmsen, K.C., Schellenberg, G.D., Trojanowski, J.Q. and Lee, V.M. (1998) Science 282, 1914–1917.
[36] Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F. and Cole, G. (1996) Science 274, 99–102.
[37] Hutton, M., Lendon, C.L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, A., Hackett, J., Adamson, J., Lincoln, S., Dickson, D., Davies, P., Petersen, R.C., Stevens, M., de Graaff, E., Wauters, E., van Baren, J., Hillebrand, M., Joosse, M., Kwon, J.M., Nowotny, P. and Heutink, P. (1998) Nature 393, 702–705.
[38] James, N.D., Davis, D.R., Sindon, J., Hanger, D.P., Brion, J.P., Miller, C.C., Rosenberg, M.P., Anderton, B.H. and Propst, F. (1996) Neurobiol. Aging 17, 235–241.
[39] Kampers, T., Friedhoff, P., Biernat, J., Mandelkow, E.-M. and Mandelkow, E. (1996) FEBS Lett. 399, 344–349.
[40] Kanai, Y., Takemura, R., Oshima, T., Mori, H., Ihara, Y., Yanagisawa, M., Masaki, T. and Hirokawa, N. (1989) J. Cell Biol. 109, 1173–1184.
[41] Kosik, K.S., Orcchio, L.D., Bakalis, S. and Neve, R.L. (1989) Neuron 2, 1389–1397.
[42] Lannfelt, L., Folkesson, R., Mohammed, A.H., Winblad, B., Hellgren, D., Duff, K. and Hardy, J. (1993) Behav. Brain Res. 57, 207–213.
[43] Lee, G., Cowan, N. and Kirschner, M. (1988) Science 239, 285–288.
[44] Lopresti, P., Szuchet, S., Papasozomenos, S., Zinkowski, R. and Binder, L. (1995) Proc. Natl. Acad. Sci. USA 92, 10369–10373.
[45] Mandelkow, E. and Mandelkow, E.-M. (1998) Trends Cell Biol. 8, 425–427.
[46] Masliah, E., Sisk, A., Mallory, M., Mucke, L., Schenk, D. and Games, D. (1996) J. Neurosci. 16, 5795–5811.
[47] Matsuo, E.S., Shin, R.-W., Billingsley, M.L., Van deVoorde, A., O'Connor, M., Trojanowski, J.Q. and Lee, V.M.-Y. (1994) Neuron 13, 989–1002.
[48] Miller, M.L. and Johnson, G.V. (1995) J. Neurochem. 65, 1760–1770.
[49] Müller, R., Heinrich, M., Heck, S., Blohm, D. and Richter-Landsberg, C. (1997) Cell Tissue Res. 288, 239–249.
[50] Nelson, P.T., Greenberg, S.G. and Saper, C.B. (1994) Neurosci. Lett. 170, 187–190.
[51] Nelson, P.T., Stefansson, K., Gulcher, J. and Saper, C.B. (1996) J. Neurochem. 67, 1622–1632.
[52] Nuydens, R., Heers, C., Chadarevian, A., Dejong, M., Nuyens, R., Cornelissen, F. and Geerts, H. (1995) Brain Res. 688, 86–94.
[53] Papasozomenos, S.C. (1997) Proc. Natl. Acad. Sci. USA 94, 6612–6617.
[54] Perez, M., Valpuesta, J.M., Medina, M., Degarcini, E.M. and Avila, J. (1996) J. Neurochem. 67, 1183–1190.

[55] Poorkaj, P., Bird, T.D., Wijsman, E., Nemens, E., Garruto, R.M., Anderson, L., Andreadis, A., Wiederholt, W.C., Raskind, M. and Schellenberg, G.D. (1998) Ann. Neurol. 43, 815–825.
[56] Preuss, U., Doring, F., Illenberger, S. and Mandelkow, E.-M. (1995) Mol. Biol. Cell 6, 1397–1410.
[57] Prusiner, S.B., Scott, M.R., DeArmond, S.J. and Cohen, F.E. (1998) Cell 93, 337–348.
[58] Quon, D., Wang, Y., Catalano, R., Scardina, J.M., Murakami, K. and Cordell, B. (1991) Nature 352, 239–241.
[59] Reed, L.A., Schmidt, M.L., Wzsolek, Z.K., Balin, B.J., Soontornniyomkij, V., Lee, V.M., Trojanowski, J.Q. and Schelper, R.L. (1998) J. Neuropathol. Exp. Neurol. 57, 588–601.
[60] Schneider, A., Biernat, J., von Bergen, M., Mandelkow, E. and Mandelkow, E.-M. (1999) 38, 3549–3558.
[61] Schweers, O., Mandelkow, E.M., Biernat, J. and Mandelkow, E. (1995) Proc. Natl. Acad. Sci. USA 92, 8463–8467.
[62] Selkoe, D.J. (1998) Trends Cell Biol. 8, 447–453.
[63] Spillantini, M.G. and Goedert, M. (1998) Trends Neurosci. 21, 428–433.
[64] Spillantini, M.G., Murrell, J.R., Goedert, M., Farlow, M.R., Klug, A. and Ghetti, B. (1998) Proc. Natl. Acad. Sci. USA 95, 7737–7741.
[65] Sturchler-Pierrat, C., Abramowski, D., Duke, M., Wiederhold, K.H., Mistl, C., Rothacher, S., Ledermann, B.K.B., Frey, P., Paganetti, P.A., Waridel, C., Calhoun, M.E., Jucker, M., Probst, A., Staufenbiel, M. and Sommer, B. (1997) Proc. Natl. Acad. Sci. USA 94, 13287–13292.
[66] Terry, C.J., Damas, A.M., Oliveira, P., Saraiva, M.J., Alves, I.L., Costa, P.P., Matias, P.M., Sakaki, A.U. and Blake, C.C. (1993) EMBO J. 12, 735–741.
[67] Thinakaran, G., Borchelt, D.R., Lee, M.K., Slunt, H.H., Spitzer, L., Kim, G., Ratovitsky, T., Davenport, F., Nordstedt, C., Seeger, M., Hardy, J., Levey, A.I., Gandy, S.E., Jenkins, N.A., Copeland, N.G., Price, D.L. and Sisodia, S. (1996) Neuron 17, 181–190.
[68] Wilhelmsen, K.C. (1998) Cell Mol. Life Sci. 54, 920–924.
[69] Wille, H., Drewes, G., Biernat, J., Mandelkow, E.-M. and Mandelkow, E. (1992) J. Cell Biol. 118, 573–584.
[70] Wischik, C.M., Novak, M., Thogersen, H.C., Edwards, P.C., Runswick, M.J., Jakes, R., Walker, J.E., Milstein, C., Roth, M. and Klug, A. (1988) Proc. Natl. Acad. Sci. USA 85, 4506–4510.
[71] Wisniewski, H.M., Johnson, A.B., Raine, C.S., Kay, W.J. and Terry, R.D. (1970) Lab. Invest. 23, 287–296.
[72] Wisniewski, H.M., Ghetti, B. and Terry, R.D. (1973) J. Neuropathol. Exp. Neurol. 32, 566–584.
[73] Yan, S.D., Chen, X., Schmidt, A.M., Brett, J., Godman, G., Zou, Y.S., Scott, C.W., Caputo, C., Frappier, T. and Smith, M.A. (1994) Proc. Natl. Acad. Sci. USA 91, 7787–7791.
[74] Yan, S.D., Schmidt, A.M., Anderson, G.M., Zhang, J., Brett, J., Zou, Y.S., Pinsky, D. and Stern, D. (1994) J. Biol. Chem. 269, 9889–9897.