Three- and Four-repeat Tau Coassemble into Heterogeneous Filaments

AN IMPLICATION FOR ALZHEIMER DISEASE

Ayisha Siddiqua and Martin Margittai

From the Department of Chemistry and Biochemistry, University of Denver, Denver, Colorado 80208

Tau filaments are the pathological hallmark of numerous neurodegenerative diseases including Alzheimer disease, Pick disease, and progressive supranuclear palsy. In the adult human brain, six isoforms are expressed that differ by the presence or absence of the second of four semiconserved repeats. As a consequence, half of the tau isoforms have three repeats (3R tau), whereas the other half of the isoforms have four repeats (4R tau). Tauopathies can be characterized based on the isoform composition of their filaments. Alzheimer disease filamentous inclusions contain all isoforms. Pick disease filaments contain 3R tau. Progressive supranuclear palsy filaments contain 4R tau. Here, we used site-directed spin labeling of recombinant tau in conjunction with electron paramagnetic resonance spectroscopy to obtain structural insights into these filaments. We find that filaments of 4R tau and 3R tau share a highly ordered core structure in the third repeat with parallel, in-register arrangement of $\beta$-strands. This structure is conserved regardless of whether full-length isoforms (htau40 and htau23) or truncated constructs (K18 and K19) are used. When mixed, 3R tau and 4R tau coassemble into heterogeneous filaments. These filaments share the highly ordered core in the third repeat; however, they differ in their overall composition. Our findings indicate that at least three distinct types of filaments exist: homogeneous 3R tau, homogeneous 4R tau, and heterogeneous 3R/4R tau. These results suggest that individual filaments found in Alzheimer disease are structurally distinct from those in the 3R and 4R tauopathies.

Alzheimer disease (AD) is the most common fatal neurodegenerative disease. It is characterized by extracellular plaques of amyloid $\beta$ and intracellular inclusions of tau (1). The inclusions are composed of tau filaments bundled into neurofibrillary tangles. Tau pathology in the absence of amyloid $\beta$ deposits is the hallmark of >20 additional neurodegenerative diseases, including progressive supranuclear palsy, corticobasal degeneration, and Pick disease (2). A clear link between tau and disease has been established through the discovery of various mutations in the tau gene (3–5) resulting in a diverse group of familial forms of frontotemporal dementia and parkinsonism. In the central nervous system of the adult human brain, six tau isoforms are expressed ranging in size from 352 to 441 amino acids (6–8) (Fig. 1A). These isoforms are defined by the presence or absence of a 29– or 58-amino acid insert in the N terminus and the inclusion or exclusion of a 31-amino acid repeat segment in the C terminus. As a consequence, three of the isoforms contain four repeats, whereas the other three isoforms contain three repeats. The repeats carry out the major known biological function of tau: the binding to and stabilization of microtubules (9, 10). Upon self-aggregation into filaments, it is the repeat region that is protected from proteases (11, 12), establishing the structured core. The N- and C-terminal regions by contrast remain largely disordered, forming a fuzzy coat (13). In the monomeric proteins, these regions appear to fold back onto the repeats (14), forming transient intramolecular contacts (15). These interactions, together with the overall positive charge of the protein, greatly inhibit aggregation. As a consequence, the addition of negatively charged cofactors such as heparin (16) and removal of the N and C termini, exemplified by the 4R and 3R constructs K18 and K19 (17) (Fig. 1B), accelerate aggregation. The filaments of both full-length (18) and truncated tau (19) have a cross-$\beta$ structure in which $\beta$-strands run perpendicular to the long fiber axis and are spaced $\sim 4.7$ Å apart. This structural feature is a common denominator of fibrils belonging to the amyloid class (20). AD tau filaments are composed of all 3R and 4R isoforms (21, 22). Whether these isoforms segregate into homogeneous filaments or coassemble into heterogeneous filaments is currently unknown. The role of tau filaments in disease has been controversial (23). In particular, it has been debated whether filaments might have an altogether protective function by preventing individual tau molecules from partitioning into toxic oligomers (24). Recent findings suggest that tau filaments could play an important role in the propagation of the misfolded state from one cell to the next (25). In tissue culture, filaments transferred among neighboring cells (26). In addition, injections of insoluble tau into mouse brain resulted in the spreading of misfolding through different brain regions (27). These properties are reminiscent of those observed for prion proteins (28). Although the overall evidence for prion-type spreading mechanisms in tauopathies is scarce, the cited publications have lent new urgency and relevance to better understand the structure of tau filaments. The analysis of fila-

---

1 To whom correspondence should be addressed: Dept. of Chemistry and Biochemistry, University of Denver, 2190 East Iliff Ave., Denver, CO 80208. Tel.: 303-871-4135; Fax: 303-871-2254; E-mail: martin.margittai@du.edu.

2 The abbreviations used are: AD, Alzheimer disease; 3R, three-repeat; 4R, four-repeat.

3 This work was supported by grants from CurePSP – Society for Progressive Supranuclear Palsy (to M. M.).

4 The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3.
Plasmid Constructs—In a first step, two PCR reactions generated the DNA fragments of the large isoform of tau (htau40) and fetal tau (htau23) with NcoI and XhoI cleavage sites at their 5’ and 3’ ends. In these reactions, htau40 in pRK172 (provided by Dr. Goedert at the Medical Research Council Laboratory, Cambridge, UK) and htau23 in pBluescript (provided by Dr. Matt Farrer at the Mayo Clinic, Jacksonville, FL) served as templates. The fragments were subsequently cloned into the NcoI/XhoI cleavage sites of pET-28b. The two cysteines in htau40 and the single cysteine in htau23 were replaced by serines. In a second set of PCR reactions, fragments K18 and K19 (36) were generated, which then were cloned into the NcoI/XhoI cleavage sites of pET-28b. In a next step, single cysteines were introduced by site-directed mutagenesis using the QuikChange method from Stratagene/Agilent Technologies. The correctness of all sequences was verified by DNA sequencing.

Protein Expression and Purification—All proteins were expressed and purified according to previously published protocols that involved htau40 (37). In brief, proteins were expressed in the Escherichia coli strain BL21 (DE3). The bacteria were pelleted and taken up in resuspension buffer (20 mM Pipes, pH 6.5, 500 mM NaCl, 1 mM EDTA, 50 mM 2-mercaptoethanol). The cells were ruptured through consecutive steps of heating (20 min at 80 °C) and sonification. Centrifugation for 20 min at 15,000 × g separated soluble tau from cellular debris. The addition of ammonium sulfate to the supernatant (60% mass per volume) precipitated tau. After 1 h, the precipitate was spun down for 15 min at 15,000 × g. The protein was resuspended (H2O, 2 mM DTT), passed through a syringe filter, and loaded onto a Mono S column (GE Healthcare). Elution occurred through the application of a NaCl gradient. After SDS-PAGE analysis, correct protein fractions were pooled and stored at −80 °C. Further purification was achieved by applying the samples onto a preparative Superdex 200 column. Peak fractions were combined and precipitated overnight by an equal volume of methanol containing 5 mM DTT. In the cases of K18 and K19, a 3-fold excess of acetone (5 mM DTT) was used for precipitation instead. The samples were pelleted and redistributed into equal aliquots. After centrifugation and a final washing of pellets with methanol (2 mM DTT), proteins were stored at −80 °C.

Spin Labeling and Filament Assembly—Protein pellets (4–6 mg) were solubilized in 200 μl of guanidine hydrochloride and reacted with an ~10-fold molar excess of either paramagnetic label [1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-methyl]methanethiosulfonate (38) or nonparamagnetic label [1-acetyl-2,2,5,5-tetramethyl-3-pyrroline-3-methyl]methanethiosulfonate (39), both purchased from Toronto Research Chemicals, Downsview, Canada. The labels were attached to single cysteines forming disulfide linkages. After a 1-h incubation at 25 °C, the proteins were passed over PD-10 desalting columns from GE Healthcare (elution buffer: 10 mM Hepes, pH 7.4, 100 mM NaCl) to remove all denaturant and unreacted label. Protein concentrations of K18 and K19 were determined by the BCA method (Pierce); concentrations of htau23 and htau40 (in 6 M guanidine hydrochloride) were determined by UV absorption at 276 nm. 20–30 μM tau was mixed with heparin (average molecular mass of 5,000 Da, Celsus, Cincinnati, OH) at a protein-heparin molar ratio of 4:1. htau40 and htau23 were allowed to aggregate for 12 days. K18 and K19, which have faster assembly kinetics, were incubated for 3 days. All reactions occurred at 25 °C under agitating conditions. For all mixing experiments, final protein concentrations were 50 – 60 μM. As for the smaller constructs, the protein-heparin molar ratio was 4:1. Filaments were pelleted for 30 min at 100,000 × g and washed with elution buffer.

EPR Data Collection and Analysis—All protein pellets were transferred into borosilicate capillaries (0.6-mm inner diameter × 0.84-mm outer diameter, VitroCom, Mountain Lakes, NJ). Measurements were carried out in a Bruker EMX spectrometer fitted with an ER 4119HS resonator. All spectra had a scan width of 150 G and an incident microwave power of 12 milliwatts. Spectra were normalized to the same number of spins using double integration. A minor background originating from <0.5% soluble tau was subtracted from all spectra.

Negative Stain Electron Microscopy—250-mesh carbon-coated copper grids were placed for 1 min on 10-μl drops of tau filaments (3–4 μm) and for 30 s on 10-μl drops of 2% uranyl acetate and subsequently air-dried on filter paper. All images were taken with a Philips/FEI Tecnai-12 electron trans-
mission microscope operating at 80 keV and equipped with a Gatan CCD camera.

RESULTS

Defining the Stable Ordered Core in Filaments of htau40—Previous investigations on the conformation of tau filaments have revealed that residues in the third repeat of tau comprise a highly ordered core with parallel, in-register arrangement of β-strands (33). As these studies only included residues up to position 320, the first half of repeat 3, it remained unclear how far the crystal-like core structure would extend. In a first step toward addressing this question, we labeled the naturally occurring cysteine at position 322 with the paramagnetic label (1-oxy-2,2,5,5-tetramethyl-Δ3-methyl]-methanethiosulfonate (38). Cross-linking resulted in the new side chain R1 (Fig. 2A). The monomeric protein produced an EPR spectrum with three sharp lines characteristic for intrinsically disordered regions (Fig. 2B, left panel). After induction with heparin and 12 days of incubation under agitation, a single-line EPR spectrum was obtained (Fig. 2B, right panel). This spectrum is a signature feature for parallel, in-register arrangement of β-strands (35), indicating that the ordered core extends beyond the previously published region. Negative stain electron microscopy confirmed the presence of filaments (Fig. 2C). Next, we spin-labeled tau mutants at single cysteines ranging from position 321 to position 358. All monomeric proteins revealed EPR spectra with three sharp lines (supplemental Fig. S1), indicating a predominance of random structure. These findings are in good agreement with previous studies concluding that tau is an intrinsically disordered protein (40, 41). The formation of filaments resulted in significant changes in line shape. Close inspection of all spectra (Fig. 3A) reveals two distinct regions. One region (residues 321–336), which extends to the end of repeat 3, gives rise to single-line spectra. Consequently, this region is part of the highly ordered core. The other region (residues 337–358) starting with repeat 4 produces spectra with single lines (e.g. 348), three lines (e.g. 346), or mixtures thereof (e.g. 357). Such spectral heterogeneity was previously observed for repeat 2 (34) and revealed a less stable, independent domain. Single amino acid substitutions in repeat 2 caused a conformational switch between stacked and disordered conformations (34). In line with the parallel, in-register arrangement of β-strands in that region, introduction of charged residues prevented folding, whereas introduction of β-branched residues promoted folding.

Here, we observe a similar trend for repeat 4. The substitution of Asp-348, for example, results in a single-line spectrum (representing ordered structure), whereas substitution of Val-350 produces a three-line spectrum (representing disordered structure). Importantly, single amino acid substitutions in repeat 3 (Fig. 3A) (33) produce single-line spectra, regardless of which amino acid is replaced. The signal amplitude increases linearly with increasing amounts of mobile component (34). An amplitude plot (Fig. 3B) illustrates low mobility for residues in repeat 3 and varied mobility for residues in repeat 4. Combined with our previous observations (33), these data reveal a stable, highly ordered core structure of the third repeat that is flanked by marginally stable regions that can switch between conformationally distinct states.

Preservation of Core Structure in htau23 and Smaller Tau Fragments—Our investigations thus far have focused solely on the largest isoform (htau40) containing four microtubule binding repeats. Here, we asked whether fetal tau (htau23), which lacks the second repeat, contains a similarly ordered core. To address this question, we spin-labeled six different single cysteine mutants in the third repeat (positions 309, 310, 311, 317, 322, and 326). All monomeric proteins produced EPR spectra with three sharp lines (supplemental Fig. S2). This observation is again in good agreement with the current notion that soluble tau is intrinsically disordered. Next, we induced filament formation by the addition of heparin. Similar to htau40, assembly was allowed to proceed for 12 days while agitating at room temperature. Filaments were harvested by centrifugation and placed into EPR capillaries for spectroscopic measurement. All positions investigated revealed single-line EPR spectra (Fig. 4, left column), indicating parallel, in-register arrangement of β-strands. Next, we asked whether smaller fragments of tau that have been used in the past as valuable models for tau aggregation (see for example Ref. 17) would reveal a similar structural arrangement. For that purpose, we produced an identical set of single cysteine mutants of the 4R and 3R constructs K18 and K19 (Fig. 1). As expected, the spin-labeled proteins in their soluble form produced spectra with three sharp lines (supplemental Fig. S2).
indicating random structure. All filaments that were subsequently formed gave single-line spectra (Fig. 4, center and right columns) similar to those of their full-length counterparts. These results suggest that the parallel, in-register arrangement of β-strands is an intrinsic feature of the repeat region and may be a common theme in all filaments of isoforms and constructs containing the stable third repeat.

**3R and 4R Tau Combine to Form Heterogeneous Filaments**

Because of the structural similarities of filaments formed from 3R and 4R tau (see also Refs. 33 and 42), the question arose whether these constructs could assemble into mixed filaments. In a first step, we attached a paramagnetic label at position 310 of K18 and a nonparamagnetic label (which produces no EPR signal) at position 310 of K19. This resulted in the side chains R1 (Fig. 2A) and R1" (39), respectively. Both proteins were subsequently mixed at different molar ratios. After 3 days of incubation, all filaments were sedimented at 100,000 × g and transferred into EPR capillaries. At low spin concentration (10% R1), a three-line EPR spectrum was obtained (Fig. 5A, red trace) with outer peak separation of 68 G. Such a spectrum is typical for an immobilized site with little interaction between spin labels (37). At intermediate spin concentration (60% R1), the spectrum broadened and the amplitude of the central peak decreased (Fig. 5A, green trace). These changes are due to dipolar interactions between labels, which are effective at distances between 8 and 25 Å (31). At high spin concentration (100% R1), a single-line spectrum with increased amplitude was observed (Fig. 5A, black trace). This spectrum is due to spin exchange between contacting labels (35). The observation of a three-line EPR spectrum at low spin concentration (10% R1) suggests that K18 has coassembled with K19. Segregation into separate filaments can be excluded as this would have retained the contacts between individual spin labels resulting in a single-line spectrum. To obtain a more comprehensive picture of the coassembly between K18 and K19, we performed a titration series in which the concentration of K18R1 was successively increased from 5 to 100%. The spectra of the filaments were taken and amplitudes of the central lines were plotted as a function of the mole percent of K18R1 (Fig. 5B). The amplitude changes are similar to those that had been previously observed in other

![FIGURE 3. EPR analysis of spin-labeled htau40 filaments. A, residues 321–358 were individually replaced by the side chain R1. All spectra were taken at 150 G and normalized to the same number of spins. Note that for completeness, spectrum C322R1 is reproduced from Fig. 2. The dotted line between the left and middle columns marks the boundary between repeats 3 and 4. B, the amplitudes of all EPR spectra are plotted against respective residue numbers. All amplitudes are normalized to spectrum F346R1, which has the highest amplitude.](image)

![FIGURE 4. EPR spectra of filaments from htau23, K18, and K19. Residues in the third repeat of htau23, K18, and K19 were replaced by the paramagnetic side chain R1. Spectra of all filaments were recorded at 150 G scan width and normalized to the same number of spins.](image)
spin dilution experiments (33, 43, 44). Depending on what type of spin interaction dominates at a particular dilution, different amplitudes are obtained: 1) large amplitudes for no interaction, 2) small amplitudes for dipolar coupling, and 3) slightly elevated amplitudes for spin exchange (see above). These changes occur gradually as the concentration of paramagnetic label increases and the distances between individual labels decrease. The various spacings between labels in differently composed filaments (Fig. 5B, insets I–III) can explain these amplitude changes. A very similar amplitude plot as depicted here is obtained when instead of K18, K19 is attached to the paramagnetic label (supplemental Fig. S3). This suggests that K18 and K19 have similar probabilities of integrating into the filaments. To clearly identify the core structure of heterogeneous filaments, we next mixed equimolar ratios of paramagnetically labeled K18 and K19. The resulting filaments produced a single-line EPR spectrum (Fig. 5C), suggesting crystal-type order with parallel, in-register arrangement of β-strands. Electron micrographs confirmed the presence of twisted and bundled filaments (Fig. 5D). As expected for a sample with an EPR single-line signature (33), amorphous aggregates were not observed.

Next, we asked whether full-length versions of 3R and 4R tau would also be able to coassemble. For this purpose, we used the same labeling strategy as for the K18/K19 constructs. Paramagnetic label was attached to htau40; nonparamagnetic label was attached to htau23. The assembled filaments produced similar spectra to those of the smaller constructs (Fig. 5E, compare with 5A). Accordingly, the amplitude plot obtained from a titration series of these isoforms revealed the same overall features (Fig. 5F) as the one obtained for K18 and K19 (Fig. 5B and supplemental Fig. S3). Furthermore, filaments formed from an equimolar mixture of paramagnetically labeled htau40 and htau23 produced a single-line spectrum (Fig. 5G). The presence of filaments and absence of amorphous aggregates was confirmed by negative stain electron microscopy (Fig. 5H). Combined, these results demonstrate that regardless of construct size, 3R and 4R tau coassemble into heterogeneous filaments with a core of parallel, in-register arranged β-strands.

**DISCUSSION**

Tau filaments are the pathological hallmark of numerous tauopathies. Although in most diseases, filaments are composed of either 3R or 4R tau, in AD, filaments contain all isoforms. Although the isoform composition of tau filaments had been determined nearly 20 years ago (21, 22), until now, it was not clear whether the 3R and 4R isoforms in AD might form
homogeneous or heterogeneous filaments (45). Such information, however, is important as either aggregate would imply fundamentally different mechanisms of fibrillization. In this study, we set out to obtain molecular insights into the structure of different tau filaments and to address the question of homogeneous or heterogeneous filaments. Conformational plasticity is a common property of amyloid proteins resulting in structurally distinct fibrils of homogeneously composed proteins (see for example Refs. 48–52). It is likely that similar structural polymorphisms exist for each type of tau filament. Compositional diversity and conformational plasticity allow for a plethora of tau filament structures that may exceed those of other amyloid fibrils.

Ample experimental evidence suggests that phenotypic diversity in prion diseases is encoded in different misfolded conformations of the same protein (48, 53–56). The particular fragmentation and growth properties of different fibrils have provided a physical basis of how protein conformation could determine phenotype (51, 57). As pointed out above, the filaments in different tauopathies have characteristic compositions of 3R and 4R tau. Filaments in Pick disease contain 3R tau. Filaments in progressive supranuclear palsy and cortico-basal degeneration contain 4R tau. Filaments in AD incorporate both 3R and 4R tau. Because we have demonstrated that 3R and 4R tau coassemble into heterogeneous filaments, filaments in AD could be distinct from those in the 3R and 4R tauopathies. It was previously shown that filaments in AD contain roughly equimolar ratios of 3R and 4R tau (21). This ratio reflects the expression pattern of tau in the adult human brain (7). Hence, the majority of individual filaments in AD brain may be composed of evenly mixed 3R and 4R isoforms. However, because the expression patterns of tau isoforms can vary between different neuronal subpopulations (6), the specific composition of individual tau filaments could vary in a cell-specific manner. The free interchangeability of 3R and 4R tau would allow for a broad spectrum of heterogeneous filaments with diverse physical and biological properties.

Although the repeat region in tau may determine the stability of filaments (58), the N-terminal inserts could influence the interactions with cellular components. Here, we demonstrate that 3R and 4R tau can coassemble into heterogeneous filaments, expanding the number of possible filament structures. If future experiments corroborate interneuronal spreading of tau misfolding in a prion-like fashion (59), the large compositional and conformational diversity of tau filaments could be an important contributor to the large phenotypic diversity of tauopathies.

REFERENCES

1. Goedert, M., and Spillantini, M. G. (2006) Science 314, 777–781
2. Lee, V. M., Goedert, M., and Trojanowski, J. Q. (2001) Annu. Rev. Neurosci. 24, 1121–1159
3. 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., Hillenbrand, M., Joosse, M., Kwon, J. M., Heutink, P., Che, L. K., Norton, J., Morris, J. C., Reed, L. A., Trojanowski, J., Basun, H., Lannfelt, L., Neustat, M., Fahn, S., Dark, F., Tannenberg, T., Dodd, P. R., Hayward, N., Kwok, J. B., Schofield, P. R., Andreadis, A., Snowden, J., Craufurd, D., Neary, D., Owen, F., Oostra, B. A., Hardy, J., Goate, A., van Swieten, J., Mann, D., Lynch, T., and Heutink, P. (1998) Nature 393, 702–705
4. Spillantini, M. G., Murrell, J. R., Goedert, M., Farlow, M. R., Klug, A.,

3R and 4R Tau Coassemble into Heterogeneous Filaments

3R and 4R Tau Coassemble into Heterogeneous Filaments

FIGURE 6. Types of tau filaments. 3R tau (red) and 4R tau (green) assemble into at least three different types of filaments: 1) homogeneous filaments of 3R tau, 2) homogeneous filaments of 4R tau, and 3) heterogeneous filaments of 3R and 4R tau. All filaments have in common a parallel, in-register arrangement of β-strands in repeat 3.

| 3R | 3R/4R | 4R |
|----|-------|----|
| ![Image](103x612 to 247x734) | ![Image](313x200) | ![Image](313x200) |
3R and 4R Tau Coassemble into Heterogeneous Filaments

and Ghetti, B. (1998) *Proc. Natl. Acad. Sci. U.S.A.* 95, 7737–7741

5. Poorkaj, P., Bird, T. D., Wijsman, E., Nemens, E., Garruto, R. M., Anderson, L., Andreadis, A., Wiederhold, W. C., Raskind, M., and Schellenberg, G. D. (1998) *Ann. Neurol.* 43, 815–819

6. Goedert, M., Spillantini, M. G., Potier, M. C., Ulrich, J., and Crowther, R. A. (1998) *EMBO J.* 8, 396–399

7. Goedert, M., Spillantini, M. G., Lakes, R., Rutherford, D., and Crowther, R. A. (1998) *Neuron* 3, 519–526

8. Goedert, M., and Lakes, R. (1990) *EMBO J.* 9, 4225–4230

9. Butner, K. A., and Kirschner, M. W. (1991) *J. Cell Biol.* 115, 717–730

10. Goode, B. L., and Feinstein, S. C. (1994) *Science* 264, 269–272

11. Goode, B. L., and Feinstein, S. C. (1994) *Proc. Natl. Acad. Sci. U.S.A.* 101, 10278–10283

12. Margittai, M., and Langen, R. (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101, 10278–10283

13. Margittai, M., and Langen, R. (2006) *J. Biol. Chem.* 281, 37820–37827

14. Margittai, M., and Langen, R. (2008) *Q. Rev. Biophys.* 41, 265–297

15. Gustke, N., Trinczek, B., Biernat, J., Mandelkow, E., and Mandelkow, E. (1994) *Biochemistry* 33, 9511–9522

16. Margittai, M., and Langen, R. (2006) *Methods Enzymol.* 413, 122–139

17. Berliner, L. J., Gruenwald, J., Hankovszky, H. O., and Hideg, K. (1982) *Anal. Biochem.* 119, 450–455

18. Gross, A., Columbus, L., Hideg, K., Altenbach, C., and Hubbell, W. L. (1999) *Biochemistry* 38, 10324–10335

19. Cleveland, D. W., Hwo, S. Y., and Kirschner, M. W. (1977) *J. Mol. Biol.* 116, 227–247

20. Goedert, M., Clavaguera, F., and Tolnay, M. (2010) *J. Biol. Chem.* 285, 37820–37827

21. Margittai, M., and Chen, J., and Langen, R. (2007) *J. Biol. Chem.* 282, 24970–24979

22. Cobb, N. I., Sönntig, L. D., McHarry, H., and Suresh, W. K. (2007) *Proc. Natl. Acad. Sci. U.S.A.* 104, 18946–18951

23. Adams, S. J., DeTure, M. A., McBride, M., Dickson, D. W., and Petruscelli, L. (2010) *PLoS One* 5, e10810

24. Adams, S. J., DeTure, M. A., McBride, M., Dickson, D. W., and Petruscelli, L. (2010) *PLoS One* 5, e10810

25. Li, L., von Bergen, M., Mandelkow, E., and Mandelkow, E. (2002) *J. Biol. Chem.* 277, 41390–41400

26. Frost, B., Ollesch, J., Wille, H., and Diamond, M. I. (2009) *J. Biol. Chem.* 284, 3546–3551

27. Tanaka, M., Chien, P., Naber, N., Cooke, R., and Weissman, J. S. (2004) *Nature* 428, 323–328

28. Peterson, A. T., Leapman, R. D., Guo, Z., Yau, W. M., Mattson, M. P., and Tycko, R. (2005) *Science* 307, 262–265

29. Paravastu, A. K., Leapman, R. D., Yau, W. M., and Tycko, R. (2008) *Proc. Natl. Acad. Sci. U.S.A.* 105, 18349–18354

30. Colby, D. W., Giles, K., Legname, G., Wille, H., Baskakov, I. V., DeArmond, S. J., and Prusiner, S. B. (2009) *Proc. Natl. Acad. Sci. U.S.A.* 106, 20417–20422

31. Kodali, R., Williams, A. D., Chemuru, S., and Wetzel, R. (2010) *J. Mol. Biol.* 401, 503–517

32. Bessen, R. A., and Marsh, R. F. (1994) *J. Virol.* 68, 7859–7868

33. Telling, C. G., Parchi, P., DeArmond, S. J., Cortelli, P., Montagna, P., Gabizon, R., Mastriniari, J., Lugaresi, E., Gambeti, P., and Prusiner, S. B. (1996) *Science* 274, 2079–2082

34. Collinge, J., Siddle, K. C., Meads, J., Ironside, J., and Hill, A. F. (1996) *Nature* 383, 685–690

35. King, C. Y., and Diaz-Avalos, R. (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101, 10526–10539

36. Kodali, R., von Bergen, M., Mandelkow, E., and Mandelkow, E. (2008) *Biochemistry* 47, 10526–10539

37. Goedert, M., Clavaguera, F., and Tolnay, M. (2010) *Trends Neurosci.* 33, 317–325