FTDP-17 Mutations in Tau Alter the Regulation of Microtubule Dynamics

AN “ALTERNATIVE CORE” MODEL FOR NORMAL AND PATHOLOGICAL TAU ACTION

Received for publication, May 8, 2008, and in revised form, September 17, 2008. Published, JBC Papers in Press, October 21, 2008, DOI 10.1074/jbc.M803519200

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Mutations affecting either the structure or regulation of the microtubule-associated protein Tau cause neuronal cell death and dementia. However, the molecular mechanisms mediating these deleterious effects remain unclear. Among the most characterized activities of Tau is the ability to regulate microtubule dynamics, known to be essential for proper cell function and viability. Here we have tested the hypothesis that Tau mutations causing neurodegeneration also alter the ability of Tau to regulate the dynamic instability behaviors of microtubules. Using in vitro microtubule dynamics assays to assess average microtubule growth rates, microtubule growth rate distributions, and catastrophe frequencies, we found that all tested mutants possessing amino acid substitutions or deletions mapping to either the repeat or interrepeat regions of Tau do indeed compromise its ability to regulate microtubule dynamics. Further mutational analyses suggest a novel mechanism of Tau regulatory action based on an “alternative core” of microtubule binding and regulatory activities composed of two repeats and the interrepeat between them. In this model, the interrepeat serves as the primary regulator of microtubule dynamics, whereas the flanking repeats serve as tethers to properly position the interrepeat on the microtubule. Importantly, since there are multiple interrepeats on each Tau molecule, there are also multiple cores on each Tau molecule, each with distinct mechanistic capabilities, thereby providing significant regulatory potential. Taken together, the data are consistent with a microtubule misregulation mechanism for Tau-mediated neuronal cell death and provide a novel mechanistic model for normal and pathological Tau action.

The microtubule-associated protein Tau is necessary for the establishment of neuronal cell polarity, axonal outgrowth, and axonal transport and the maintenance of axonal morphology (1–5). Tau dysfunction has long been correlated with a variety of neurodegenerative diseases, including Alzheimer disease, fronto-temporal dementia, and Parkinsonism associated with chromosome 17 (FTDP-17), Pick disease, and progressive supranuclear palsy. Each of these diseases is characterized by extensive neuronal cell death and the presence of abnormal pathological fibers composed primarily of hyperphosphorylated Tau (6–8). In 1998, direct genetic linkages between mutations in the Tau gene and FTDP-17 were reported (9–12). These mutations all exhibit dominant phenotypes and fall into two classes: structural mutations that alter the encoded sequence of the Tau protein and regulatory mutations that alter the pattern of Tau RNA alternative splicing (13). Mutations in the latter class do not affect the primary sequence of Tau but rather alter the expression ratios of the different wild type isoforms. The fact that such alterations cause neurodegeneration indicates that different Tau isoforms must exert at least some functionally distinct effects.

Alternative splicing of Tau RNA produces six different Tau isoforms in the central nervous system; these isoforms fall into two groups known as 4-repeat Tau and 3-repeat Tau (14–16). As seen in Fig. 1A, 4-repeat Tau contains four 18-amino acid imperfect repeats near the COOH terminus separated from one another by 13–14-amino acid interrepeats. The exclusion of exon 10, which encodes the first interrepeat and second repeat, results in 3-repeat Tau. Mechanistically, both 3-repeat and 4-repeat Tau bind directly to microtubules, stimulate microtubule polymerization, and regulate microtubule dynamics (17–23). Both quantitative and qualitative mechanistic differences exist between the two isoform classes, with 4-repeat Tau generally being more potent than 3-repeat Tau (17, 20, 22, 24, 25).

A number of models have been suggested to explain the mechanistic significance of the repeat/interrepeat region of Tau. Initially, each 18-amino acid repeat was thought to serve as

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1 This work was supported, in whole or in part, by National Institutes of Health Grants NS-35010 (to S. C. F.), NS-13560 (to L. W.), and CA-57291 (to M. A. J.). This work was also supported by a National Science Foundation predoctoral fellowship (to S. F. L.), an initiation grant from IIT Kanpur (to A. B.), and National Science Foundation Grant ITR-0331697 (to A. K. S., S. C. F., and L. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 The online version of this article (available at http://www.jbc.org) contains supplemental Figs. S1 and S2.

3 The abbreviations used are: FTDP-17, fronto-temporal dementia and Parkinsonism linked to chromosome 17; HPLC, high pressure liquid chromatography; Pipes, 1,4-piperazinediethanesulfonic acid; MES, 4-morpholineethanesulfonic acid; WT, wild type.
The underlying molecular mechanism(s) leading to neuronal cell death due to Tau dysfunction/misregulation are not understood. A widely held, gain-of-toxic function model suggests that Tau mutations lead to the formation and accumulation of abnormal intracellular Tau fibers, which in turn cause cell death by virtue of their proposed cytotoxicity (10, 42, 43). This model is consistent with the ubiquitous presence of abnormal Tau fibers in numerous neurodegenerative diseases (7). These fibers are all the more remarkable given the extreme solubility of normal Tau, even at high temperatures or low pH. However, recent work shows that Tau-mediated neuronal cell death occurs in several animal models in the absence of abnormal Tau fibers (44–47). An alternative, "microtubule misregulation" model suggests that Tau mutations interfere with the normal ability of Tau to regulate microtubule dynamics, which in turn leads to cell death (17, 20, 22, 48, 49). This model is consistent with a variety of pharmacological and somatic cell genetics investigations demonstrating that tight regulation of microtubule dynamics is essential for proper cell function and viability (50–53).

To assess further the molecular basis of normal and pathological Tau action, we have used video microscopy to analyze the dynamic instability behavior of individual microtubules regulated by wild type and various FTDP-17 mutant Tau proteins. We show that amino acid substitution and deletion mutations that map to either the repeats or interrepeats in Tau and that cause neuronal cell death and dementia alter the ability of Tau to regulate microtubule dynamic instability in vitro. Alternatively, mutations mapping outside the region of repeats and interrepeats do not affect the regulation of microtubule dynamics in this reconstituted in vitro system. These findings provide strong support for the view that abnormal Tau action might lead to neuronal cell death and dementia through aberrant regulation of microtubule dynamics. Additionally, our data lead us to propose a novel model for Tau-mediated regulation of microtubule dynamic instability based on an "alternative core" mechanism.

**EXPERIMENTAL PROCEDURES**

**Generation of FTDP-17 Constructs**—Expression vectors containing the human cDNA sequences for 3-repeat Tau (pET vector, 352aa; lacking either amino-terminal alternatively spliced exon) and 4-repeat Tau (pRK vector, 383aa; lacking either amino-terminal alternatively spliced exon) were kind gifts from Dr. Gloria Lee (University of Iowa) and Dr. Kenneth Kosik (University of California, Santa Barbara). 4-Repeat G272V, 4-repeat P301L, 4-repeat S305N, 4-repeat R406W, 3-repeat P270L, 3-repeat G271V, and 3-repeat G273V were generated from the corresponding wild type construct by QuikChange site-directed mutagenesis (Stratagene). All mutations were verified by DNA sequence analysis.

**Purification of Tau and Tubulin**—Tau was expressed and purified, as described (17). Briefly, Tau was expressed in Rosetta (DE3) pLacI cells (Novagen, Madison, WI). Bacteria were lysed by sonication and boiled for 20 min. Heat-stable proteins were isolated by centrifugation, bound to a phos-
Tau Mutations Alter Regulation of Microtubule Dynamics

phocellulose column, and eluted with a salt gradient (0.2-1.0 M NaCl). Tau-containing fractions were pooled and further purified using reverse-phase HPLC (DeltaPak-C18; Millipore, Billerica, MA). HPLC fractions containing Tau were pooled, lyophilized, and resuspended in BRB-80 buffer (80 mM Pipes, pH 6.8, 1 mM EGTA, 1 mM MgSO4) with 0.1% β-mercaptoethanol. The concentration of each Tau sample was determined by an SDS-PAGE comparison with a Tau mass standard, the concentration of which was established by amino acid analysis (22).

Tubulin was purified, as described (54). Briefly, microtubule-associated protein-rich bovine brain microtubule protein was prepared by two cycles of assembly and disassembly. Tubulin was purified from other microtubule proteins by elution through a Whatman P-11 phosphocellulose column equili-

brated in PEM50 (50 mM Pipes, 1 mM MgSO4, 1 mM EGTA, 0.1 mM GTP). Purified tubulin (>99% pure) was drop-frozen in liquid nitrogen and stored at −70 °C.

Microtubule Co-sedimentation Assays—Tubulin (15 μM tubulin dimer) was mixed with wild type or FTDP-17 mutant 3-repeat or 4-repeat Tau at 0.27 μM (1:55 Tau/tubulin) in PMEM (87 mM Pipes, 36 mM MES, 1.4 mM MgCl2, 1 mM EDTA, pH 6.8) with 2 mM GTP. Microtubules were assembled at 35 °C until steady state was achieved (2 h), layered over an 80-μl sucrose cushion (50% sucrose in PMEM, 2 mM GTP) in 5 × 20-mm ultraclear centrifuge tubes (Beckman Instruments, Palo Alto, CA), and centrifuged in a Beckman AH650 swinging bucket rotor for 12 min at 35,000 rpm (150,000 × g) at 35 °C. Supernatants and pellets were harvested and solubilized in SDS-PAGE sample buffer. Relative amounts of Tau in the supernatants and pellets were determined by SDS-PAGE and immunoblotting with the monoclonal antibody Tau-1 (55).

Dynamic Instability Analyses—Purified tubulin (15 μM tubulin dimer) was polymerized on sea urchin (Strongylocentrotus purpuratus) axonemal seeds at 37 °C in the presence or absence of purified wild type or FTDP-17 mutant Tau protein in PMEM buffer (87 mM Pipes, 36 mM MES, 1.4 mM MgCl2, 1 mM EDTA, pH 6.8) and 2 mM GTP. The dynamics of individual microtubules were recorded at 37 °C using video-enhanced differential interference contrast microscopy. The ends were designated as plus or minus on the basis of the growth rate, the number of microtubules that grew at opposite ends of the seeds, and the relative lengths of the microtubules (21, 56). Plus ends were analyzed during the early elongation phase of polymerization (2–10 min after initiation of polymerization). Life histories of individual microtubules were collected as described in Ref. 21 with modifications. Data points were collected at 1–3-s intervals. A microtubule was considered to be growing or shortening if it increased or decreased in length at a rate >0.5 μm/min. Microtubules exhibiting growth rates of ≤0.5 μm/min over a period greater than 30 s were considered to be in an attenuated state. Average growth rates are the average of independent growth events. The catastrophe frequency was calculated by dividing the number of shortening events by the total time tracked. Each condition was filmed over multiple days using 2–5 distinct Tau/tubulin/GTP mixes (3–4 slides each). No gross variation in microtubule dynamics was observed between mixes or slides of a given condition. Microtubules were tracked by two independent investigators, each of whom obtained approximately equal average growth rates for each condition.

RESULTS

In order to compare the abilities of wild type and mutant Tau proteins to regulate microtubule dynamics, we first wanted to establish experimental conditions in which all Tau proteins used in the study are bound to microtubules to similar extents. Using a co-sedimentation assay, we compared the ability of each Tau molecule to bind to microtubules when assembled at a 1:55 Tau/tubulin dimer molar ratio, the standard ratio for our in vitro microtubule dynamic instability assays. Both 4-repeat and 3-repeat wild type Tau and seven different FTDP-17 mutant Tau proteins (4-repeat R406W, 4-repeat Δ280K, 4-repeat S305N, 4-repeat P301L, 4-repeat G272V, 3-repeat G272V, and 3-repeat R406W) were >90% microtubule-associated under these conditions (data not shown). Thus, any differences in the abilities of wild type and mutant Tau isoforms to regulate microtubule dynamics can be attributed to inherent mechanistic differences in the response of the microtubules to microtubule-bound Tau isoforms.

Most FTDP-17 Tau Mutations Alter the Ability of Tau to Regulate the Microtubule Growth Rate and Catastrophe Frequency—To test the hypothesis that amino acid substitution or deletion mutations in Tau that cause FTDP-17 cause alterations in the ability of Tau to regulate microtubule dynamics, we analyzed the dynamic instability behavior during the early elongation phase of assembly at plus ends of individual microtubules in vitro. Microtubules were grown in the absence or presence of wild type or mutant 3-repeat or 4-repeat Tau. A schematic illustrating the growing and shortening dynamics of a representative individual microtubule is shown in Fig. S1. Because our studies were performed during the early elongation phase of microtubule polymerization, the microtubules predominantly grew and displayed relatively few catastrophes or periods of attenuation (20). Consistent with our earlier work...
wild type 4-repeat Tau significantly increased and wild type 3-repeat Tau significantly decreased \( p < 0.01 \) the average growth rate relative to non-Tau-treated control microtubules (Table 1 and Fig. 2A).

Both 4-repeat and 3-repeat Tau reduced the catastrophe frequency relative to control microtubules, with 4-repeat Tau being more potent than 3-repeat Tau (Table 1 and Fig. 2B).

Amino acid substitution or deletion mutations that cause FTDP-17 generally resulted in an alteration of wild type Tau regulatory activity on microtubule dynamic instability (Table 1 and Fig. 2). Notably, the two mutations located in the interrepeats (i.e. 4-repeat AK280 located in the first interrepeat and 4-repeat S305N located in the second interrepeat; see Fig. 1A) yielded similar loss of Tau regulatory activity, markedly affecting both average microtubule growth rate and the catastrophe frequency parameters. Mutations mapping within the repeat regions exhibited complex phenotypes, to be described below in more detail. Finally, both 3-repeat R406W and 4-repeat R406W mutants, located downstream of the overall repeat/interrepeat region, did not significantly alter either the growth rate or the catastrophe frequency.

G272V and P301L Mutations Exhibit Unpredicted and Complex Phenotypes—As shown in Fig. 3A, the G272V and P301L mutations are located within the highly con-
Tau Mutations Alter Regulation of Microtubule Dynamics

![Diagram](image-url)

FIGURE 3. A, the amino acid sequence of the four repeats of the repeat/interrepeat region of Tau. At the end of each repeat is a highly conserved PGGG sequence. The positions of the G272V and P301L mutations, located in repeats 1 and 2, respectively, are noted. B, alternative core model for Tau mediated regulation of microtubule dynamics. The boxes above each line correspond to the 18-amino acid imperfect repeats, separated from one another by 13–14-amino acid interrepeats, designated as A, B, or C. In the alternative core model, the most NH2-terminal contiguous repeat-interrepeat-repeat region is proposed to be the functional unit regulating microtubule dynamics. In such a functional core unit, the interrepeat serves as the primary regulator of microtubule dynamics, whereas the adjacent flanking repeats serve to bind to the microtubule and thereby tether the interrepeat into position. Mutations that affect the ability of a repeat to interact properly with the microtubule result in utilization of the next contiguous repeat-interrepeat-repeat unit in the COOH-terminal direction. Thus, 4-repeat (4R) and 3-repeat (3R) wild type Tau are predicted to utilize repeat 1/interrepeat A/repeat 2 and repeat 1/interrepeat B/repeat 3 as their core regulator of microtubule dynamics, respectively. Because repeat 1 is disrupted by the G272V mutation, 4-repeat and 3-repeat G272V are predicted to utilize repeat 2/interrepeat B/repeat 3 and repeat 3/interrepeat C/repeat 4, respectively. The 4-repeat P301L mutation is predicted to utilize repeat 3/interrepeat C/repeat 4.

erved Pro-Gly-Gly-Gly motif located at the COOH-terminal end of each imperfect repeat. These PGGG sequences, also present in other microtubule-associated proteins, namely MAP2 and MAP4, are believed to form tight turns upon association with microtubules (60, 61). Replacement of one of the glycine residues with a more bulky amino acid, such as valine, as occurs in G272V, could disrupt such a tight turn by steric hindrance.

Surprisingly, microtubules with bound 4-repeat G272V Tau exhibited not only lower growth rates than microtubules with bound 4-repeat wild type Tau but also significantly lower growth rates than control microtubules assembling in the absence of any Tau at all (Fig. 2 and Table 1). Indeed, 4-repeat G272V Tau regulates the microtubule growth rate and catastrophe frequency nearly identically as does 3-repeat wild type Tau (Fig. 2, A and B, and Table 1). To ensure that these data were not due to an idiosyncratic property of a given Tau or tubulin preparation, these analyses were repeated with completely independent tubulin, wild type 4-repeat, wild type 3-repeat, and 4-repeat G272V Tau preparations and yielded similar results (data not shown).

We previously proposed that Tau interacts with microtubules via a "core microtubule-binding unit" composed of the first two repeats and the interrepeat between them (25). In this model, downstream repeats and interrepeats contribute only minimally to microtubule binding affinity. More specifically, as schematized in the top half of Fig. 3B, the proposed core microtubule binding unit in wild type 4-repeat Tau is composed of repeat 1-interrepeat A-repeat 2, whereas it is repeat 1-interrepeat B-repeat 3 for wild type 3-repeat Tau.

Integrating the data in Table 1 and Fig. 2 with the core microtubule binding domain model led us to consider a novel and testable hypothesis for how Tau might associate with microtubules and regulate their dynamics. The hypothesis is based upon the core microtubule binding unit (Fig. 3B) and builds upon it by proposing that 1) the interrepeat of the most NH2-terminal intact repeat/interrepeat/repeat unit in wild type Tau is the primary regulator of microtubule dynamics; 2) the two repeats residing on either side of the regulator interrepeat serve as tethers, binding to the microtubule and positioning their interrepeat in an appropriate position on the microtubule to regulate its dynamics; and 3) structural alterations (such as mutations or perhaps post-translational modifications) that interfere with the ability of the two flanking repeats to bind to the microtubule and thereby tether their interrepeat cause utilization of the adjacent (more COOH-terminal) repeat/interrepeat/repeat unit. According to this model, mutations mapping to interrepeats should not affect which interrepeat is positioned to regulate microtubule dynamics (since the flanking repeats are normal), but the mutation could affect the ability of the interrepeat to regulate microtubule dynamics.

By this model, the 4-repeat G272V mutation in repeat 1 results in the inactivation of the core unit using interrepeat A, which in turn forces utilization of the core unit using interrepeat B. Thus, 4-repeat G272V Tau would use the same primary regulator utilized by wild type 3-repeat Tau (interrepeat B; Fig. 3B). The prediction of the model is that 4-repeat G272V should exhibit the properties of 3-repeat wild type Tau, which is supported by the data in Table 1 and Fig. 2, A and B.

The model also suggests that both the 3-repeat G272V mutation and the 4-repeat P301L mutations should disrupt their original repeat/interrepeat/repeat units by inactivating repeat 1 and 2, respectively. Both of these disruptions lead to the utilization of the repeat 3/interrepeat C/repeat 4 unit (Fig. 3B). Both Tau molecules are therefore predicted to regulate dynamic instability similarly to one another and differently from their wild type Tau counterparts. The data in Fig. 2 and Table 1 show that these predictions are met both for growth rate and catastrophe frequency. Although both 3-repeat G272V and 4-repeat P301L Tau induced microtubule growth rates somewhat similar to those of microtubules grown in the absence of Tau, the complete lack of catastrophes observed with both of these mutant conditions (Fig. 2B) indicates that these mutated proteins retain active regulatory capabilities. To ensure that these data were not due to an idiosyncratic property of a given Tau or tubulin preparation, these analyses were repeated with independent tubulin, wild type 4-repeat, wild type 3-repeat, and 3-repeat G272V, and 4-repeat P301L Tau preparations and yielded similar results (data not shown).

To test the predictions of our model more rigorously, we next assembled microtubules in the presence of 4-repeat wild type,
Tau Mutations Alter Regulation of Microtubule Dynamics

Microtubule Growth Rate Distributions Are Consistent with the Alternative Core Model—We previously reported that microtubules exist in two subpopulations with respect to growth rate distribution, one more abundant and slower growing and a second less abundant but faster growing (20). Importantly, Tau exerted a significant effect upon these distributions. Specifically, although both 3-repeat and 4-repeat Tau increased the abundance of the faster growing subpopulation at the expense of the slower growing subpopulation, 4-repeat Tau did so at much lower Tau/tubulin molar ratios than did 3-repeat Tau. Here, to more mechanistically compare WT and FTDP-17 mutant Tau molecules as well as to perform another assessment of the alternative core model, we sought to compare the effects of WT and FTDP-17 Tau on the microtubule growth rate distribution. Histograms depicting the distribution of individual microtubule growth rates observed for each Tau isoform tested are presented in Fig. S2.

To facilitate analysis of this large group of complex histograms, we first generated a distance matrix, which statistically defines the relative distances between the growth rate distributions of each Tau isoform being tested (see "Experimental Procedures" and Table 2). We visualized this distance matrix using a heat map (Fig. 5). Both rows and columns were hierarchically clustered, and the resulting clustering dendrogram is shown for the rows. If the alternative core model is correct, Tau isoforms proposed to be using the same interrepeat regulator should cluster together. More specifically, 4-repeat wild type Tau (4R-WT) and 4-repeat R406W (both predicted to use interrepeat regulator A) should cluster together; 3-repeat R406W, 3-repeat wild type (3R-WT), and 4-repeat G272V (all predicted to use inter-repeat regulator B) should cluster together; and 4-repeat P301L and 3-repeat G272V (both predicted to use interrepeat regulator C) should cluster together. As shown in the heat map in Fig. 5, the predicted three clusters do indeed emerge.

We recognize that these sorts of statistical analyses only point to trends rather than prove assertions, but they do nonetheless suggest that the growth rate distribution data are consistent with the alternative core model.

Further Characterization of the Pro-Gly-Gly-Gly Sequence—Given the relatively high frequency of pathogenic mutations in the highly conserved PGGG regions of repeats 1 and 2 (24), we next sought to further examine one of these regions in greater detail. Therefore, we introduced mutations at each residue of the repeat 1 PGGG sequence (3-repeat P270L, 3-repeat G271V, 3-repeat G273V, 4-repeat P270L, 4-repeat G271V, and 4-repeat G273V) and assayed their effects on dynamic instability.

Mutagenesis of individual amino acids within the repeat 1 PGGG sequence in 3-repeat Tau consistently altered wild type function in a manner comparable with the FTDP-17 3-repeat G272V mutation; in each case, the mutation increased the microtubule growth rate above that observed in the absence of Tau but not up to the level of 4-repeat wild type Tau (Fig. 6). These observations indicate that each amino acid in the repeat 1 PGGG in 3-repeat Tau consistently altered wild type function in a manner comparable with the FTDP-17 3-repeat G272V.

| TABLE 2 |
| Distance matrix of relative distances between the growth rate distributions of each Tau isoform tested |
| Histogram modeling and distance computations were performed as described by Bhattacharya et al. (58). 3R, 3-repeat; 4R, 4-repeat. |
| No Tau | 3R-GV | 3R-RW | 3R-WT | 4R-GV | 4R-PL | 4R-RW | 4R-WT |
| 3R-GV | 0.00 | 0.33 | 0.49 | 0.60 | 0.23 | 0.28 | 0.48 | 0.18 |
| 3R-RW | 0.33 | 0.00 | 0.17 | 0.38 | 0.51 | 0.60 | 0.81 | 0.41 |
| 3R-WT | 0.49 | 0.17 | 0.00 | 0.26 | 0.64 | 0.77 | 0.95 | 0.53 |
| 4R-GV | 0.60 | 0.38 | 0.26 | 0.00 | 0.67 | 0.88 | 1.00 | 0.56 |
| 4R-PL | 0.23 | 0.51 | 0.64 | 0.67 | 0.00 | 0.29 | 0.33 | 0.11 |
| 4R-RW | 0.28 | 0.60 | 0.77 | 0.88 | 0.29 | 0.00 | 0.28 | 0.36 |
| 4R-WT | 0.48 | 0.81 | 0.95 | 1.00 | 0.33 | 0.28 | 0.00 | 0.44 |

No Tau | 0.18 | 0.41 | 0.53 | 0.56 | 0.11 | 0.36 | 0.44 | 0.00 |

Figure 4. Increasing concentrations of wild type and mutant Tau exhibit the predicted effects on microtubule growth rates. 15 μM tubulin was grown in the presence of 0.27 μM (1:55 Tau/tubulin), 0.33 μM (1:45 Tau/tubulin), or 0.39 μM (1:38 Tau/tubulin) 3-repeat (3R) wild type Tau, 4-repeat (4R) wild type Tau, 3-repeat G272V, 4-repeat G272V, or 4-repeat P301L Tau. The horizontal dashed line indicates the average growth rate of microtubules in the absence of Tau.
and 4-repeat G271V and 4-repeat G273V exhibited only modest decreases in growth rate relative to wild type Tau. The lack of major mechanistic effects of these mutations in 4-repeat Tau is in marked contrast to the effects of the same mutations in the 3-repeat Tau context. These data do not conflict with the alternative core model; rather, despite the facts that the PGGG sequences are highly conserved and that mutations in it frequently generate dramatic phenotypes, not all PGGG positions need to be essential. The data indicate that the essential nature of any particular PGGG site or lack thereof can be isoform-specific, revealing important isoform specific structure-function differences between 3-repeat Tau and 4-repeat Tau structure and function. Thus, mutations at some PGGG sites have significant structural effects in 3-repeat Tau, whereas the same mutation in 4-repeat Tau is relatively silent.

DISCUSSION

Previous work has demonstrated that Tau is a potent regulator of microtubule dynamic instability, both in vitro and in cells. Initially, Drechsel et al. (18) observed that an adult bovine Tau preparation containing multiple Tau isoforms promoted the microtubule growth rate, inhibited the shortening rate, and decreased the catastrophe frequency in vitro. Trinczek et al. (23) assessed recombinant human 4-repeat and 3-repeat Tau and made similar observations, noting that 4-repeat Tau was more potent than 3-repeat Tau. Recent work from our laboratories has shown that both quantitative and qualitative differences exist between the abilities of 4-repeat and 3-repeat Tau to regulate dynamic instability, both in vitro and in cells (17, 22, 59). On the other hand, little is known about the ability of FTDP-17 mutant Tau isoforms to regulate the dynamic instability behavior of individual microtubules. Recent work from our laboratories has shown that most FTDP-17 Tau mutants are compromised in their abilities to regulate dynamic instability when microinjected into cells (48). Although this study has the advantage of assessing the action of wild type and mutant Tau in a cellular context, it carries with it the complication of ill defined and uncontrolled post-translational modifications, such as phosphorylation, which can potently affect Tau action (30). Here, using an in vitro reconstituted system composed solely of purified microtubules and Tau, we show that 1) all tested FTDP-17 Tau mutations that map to the repeat/interrepeat region alter the ability of Tau to regulate dynamic instability, and 2) all tested mutations mapping in the carboxyl-terminal tail of Tau, downstream of the repeat/inter-
repeat region, have negligible effects upon the ability of Tau to regulate dynamic instability. These observations are consistent with recently advanced notions suggesting that mutations in the repeat/interrepeat region cause neurodegeneration and disease by directly causing the misregulation of microtubule dynamics, whereas mutations downstream of the repeat/interrepeat region cause neurodegeneration and dementia indirectly, perhaps via aberrant phosphorylation of nearby sites on Tau, which in turn leads to misregulation of microtubule dynamics (48, 62).

Critical Role of Interrepeats and Repeats in Tau Function—We previously demonstrated that the interrepeat between repeats 1 and 2, present only in 4-repeat Tau, possessed potent microtubule binding and assembly activities and also identified the lysine at position 280 (position 272 in the rat sequence used in that earlier work) as being most critical for those activities (21, 28). This was the first indication that Tau interrepeats could have inherent microtubule regulatory activity rather than serving as simple linkers or spacers between microtubule binding repeats, as had been previously suggested (26–28). Subsequent work demonstrated that a peptide corresponding to the same interrepeat could regulate microtubule dynamics (20). Consistent with playing an essential role in Tau action, the amino acid sequence of the interrepeats is just as extraordinarily conserved across species lines as are the imperfect repeats.

In the present work, the two interrepeat mutations examined (ΔK280 and S305N) were both strongly compromised in their abilities to regulate the microtubule growth rate and the catastrophe frequency, despite the fact that they bound to microtubules with equal efficacy. This is consistent with our previous work indicating that Tau containing the ΔK280 or S305N mutation causes strong alterations in the ability to regulate microtubule dynamic instability relative to other FTDP-17 mutations when microinjected into cells (48).

The critical importance of the interrepeats as well as the imperfect repeats for proper Tau function is also apparent by assessing the localization of all known mutations in Tau that cause FTDP-17 or related dementias (24). Indeed, mutations are scattered throughout the entire region of repeats and interrepeats. Amino acid alterations at eight different positions within interrepeats lead to disease (20% of all interrepeat sites), whereas alterations at 12 different positions within repeats lead to disease (17% of all repeat sites). Of the 12 repeat sites at which mutations lead to disease, five of them map to the PGGG region believed to serve as a tight turn or hinge. Taken together, it is clear that the entire region of repeats and interrepeats is essential for proper Tau function.

Alternative Core Model and Normal Tau Action—As would be expected of a regulator of a cytoskeleton network central to numerous cellular processes, Tau itself is a highly regulated protein whose activity is modulated by both alternative splicing and extensive phosphorylation. The alternative core activity model of Tau (see Fig. 3B) provides a mechanism that could be involved in both forms of this regulation. With respect to alternative RNA splicing, wild type 4-repeat and 3-repeat Tau exhibit qualitative and quantitative differences in their abilities to bind microtubules and to regulate their dynamics, and they also differ in their core repeat/interrepeat/repeat unit. Additionally, the fact that the ratio of 3-repeat to 4-repeat Tau changes as a function of development suggests that cells are normally regulating Tau activity in a prescribed manner to modulate microtubule behavior during neural development.

Regulation of the Tau core may also be occurring on a much faster time scale via Tau phosphorylation. Tau has over 2 dozen known phosphorylation sites in vivo and is known to be regulated by a number of different kinases (30). Drechsel et al. (18) demonstrated that phosphorylation of Tau by MAP2 kinase alters the ability of Tau to regulate microtubule dynamics; more recently, we have demonstrated that singly and multiply pseudophosphorylated Tau molecules are altered in their dynamics regulatory activities relative to control, nonpseudophosphorylated Tau. If phosphorylation of Tau, either of individual sites or of particular combinations of sites, affects which Tau core unit is active, this would allow for precise temporal and spatial regulation of Tau activity. Indeed, microtubule dynamics are known to vary significantly in different subcellular locations within neurons (63, 64), and differential patterns of Tau phosphorylation could contribute to these effects. Finally, the alternative core mechanism might be more broadly utilized by other microtubule-associated proteins possessing repeat/interrepeat domains similar to those of Tau, such as MAP2 and MAP4.

Implications for Neurodegenerative Disease—The underlying molecular mechanism(s) leading to Tau-mediated neuronal cell death in FTDP-17 are not clear. It has been widely suggested that the abundant and abnormal Tau aggregates found in many neurodegenerative disorders are inherently cytotoxic (10, 42). However, this model has come under increased scrutiny, since a variety of recent studies suggest that Tau aggregates are not necessary for Tau-mediated neuronal cell death (44–46, 65). Additionally, because all of the Tau present in FTDP-17 Tau RNA splicing mutants is wild type, it is unclear in these cases what changes in the Tau protein cause aggregation, unless the aggregation were a downstream consequence of abnormal Tau activity (i.e. misregulation of microtubule dynamics).

An alternative model suggests that alterations in Tau structure-function or isoform expression ratios result in misregulation of microtubule dynamics, which in turn leads to cell death. This model is supported by a large and growing body of literature demonstrating that tight regulation of microtubule dynamics is essential for proper cell function and viability (50–53). It is also supported by demonstrated differences in the abilities of 4-repeat and 3-repeat wild type Tau to regulate microtubule dynamics (providing a mechanistic rationale to explain cell death and dementia caused by mutations altering Tau isoform expression ratios (22, 59)). Most relevant to the work presented here, the misregulation of microtubule dynamics model can readily accommodate the FTDP-17 amino acid substitution and deletion mutations in Tau. More specifically, all mutations mapping to the

4 E. Kiris, M. R. Gaylord, D. Ventimiglia, M. A. Jordan, L. Wilson, and S. C. Fein-stein, manuscript in preparation.
microtubule binding region of Tau exhibit altered abilities to regulate microtubule dynamics, both in vitro (this study) and in cells (48). Taken together, these data provide strong support for the hypothesis that misregulation of microtubule dynamics plays a fundamental role in FTDP-17 and related dementias.

Acknowledgment—We thank Herb Miller for generously providing abundant and high quality purified bovine tubulin.

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Tau Mutations Alter Regulation of Microtubule Dynamics