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Biochimica et Biophysica Acta 1739 (2005) 268–279

BIOCHIMICA ET BIOPHYSICA ACTA
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Review

# Inability of tau to properly regulate neuronal microtubule dynamics: a loss-of-function mechanism by which tau might mediate neuronal cell death

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Received 1 July 2004; accepted 20 July 2004
Available online 6 August 2004

**Abstract**

Interest in the microtubule-associated protein tau stems from its critical roles in neural development and maintenance, as well as its role in Alzheimer's, FTDP-17 and related neurodegenerative diseases. Under normal circumstances, tau performs its functions by binding to microtubules and powerfully regulating their stability and growing and shortening dynamics. On the other hand, genetic analyses have established a clear cause-and-effect relationship between tau dysfunction/mis-regulation and neuronal cell death and dementia in FTDP-17, but the molecular basis of tau's destructive action(s) remains poorly understood. One attractive model suggests that the intracellular accumulation of abnormal tau aggregates causes cell death, i.e., a gain-of-toxic function model. Here, we describe the evidence and arguments for an alternative loss-of-function model in which tau-mediated neuronal cell death is caused by the inability of affected cells to properly regulate their microtubule dynamics due to mis-regulation by tau. In support of this model, our recent data demonstrate that missense FTDP-17 mutations that alter amino acid residues near tau's microtubule binding region strikingly modify the ability of tau to modulate microtubule dynamics. Additional recent data from our labs support the notion that the same dysfunction occurs in the FTDP-17 regulatory mutations that alter tau RNA splicing patterns. Our model posits that the dynamics of microtubules in neuronal cells must be tightly regulated to enable them to carry out their diverse functions, and that microtubules that are either over-stabilized or under-stabilized, that is, outside an acceptable window of dynamic activity, lead to neurodegeneration. An especially attractive aspect of this model is that it readily accommodates both the structural and regulatory classes of FTDP-17 mutations.
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*Keywords:* Tau; Microtubule dynamics; FTDP-17; Alzheimer's disease; Cytoskeleton; Loss-of-function

## 1. Introduction

### *1.1. Abnormal tau action correlates with neuronal cell death and dementia*

Tau dysfunction has long been correlated with neurodegenerative disorders, most notably Alzheimer's disease. Indeed, the intracellular accumulation of abnormal tau fibers (neurofibrillary tangles, or "NFT"s) is one of two hallmark pathological features of Alzheimer's disease, the other being extracellular amyloid plaques. Abnormal tau fibers are also observed in a number of other neurodegenerative "tauopathies", including fronto-temporal dementia with parkinsonism linked to chromosome 17 ("FTDP-17"), progressive supranuclear palsy, Pick disease and others (for recent reviews, see Refs.[1–3]). However, the ubiquitous correlation between the presence of these fibers and Alzheimer's and other tauopathies does not address whether or not aberrant tau action might actually cause neuronal cell death and dementia.

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doi:10.1016/j.bbadis.2004.07.002

### 1.2. Abnormal tau action causes neuronal cell death and dementia

A time-tested and proven strategy to establish causation in innumerable diseases is genetics; once the gene(s) in question are identified, subsequent biochemical and cell biological investigations can determine the molecular mechanisms underlying the destructive action of the gene and its products. In the case of tau, the lack of any genetic linkage between tau and any neurodegenerative condition prior to 1998 led most investigators to believe that tau dysfunction was likely a secondary event in the progression of neurodegeneration. However, in 1998, several groups identified direct genetic linkages between mutations in the tau gene and FTDP-17, a collection of neurodegenerative dementias with diverse but overlapping clinical and pathological features, including neuronal cell death and insoluble aggregates of tau that are similar in many ways to NFTs [4–6]. Interestingly, there are no amyloid plaques in FTDP-17, and the disease phenotype is dominant. These data demonstrate clearly that errors in tau action can cause neuronal cell death and dementia.

### 1.3. Mechanistically, how might tau cause neuronal cell death and dementia?

Now that the genetic linkage is established between mutations in tau and FTDP-17, the next critical issue to address is the molecular basis of tau-mediated neuronal cell death and dementia. However, in order to understand abnormal tau action, it is important to first understand the normal role of tau in cells. Tau is essential for establishing neuronal cell polarity and axonal outgrowth during development and for maintaining axonal morphology and axonal transport in mature cells [7–11] (for reviews, see Refs. [1,12]). Tau performs these functions by binding directly to microtubules and regulating their growing and shortening dynamics [13–22]. Since (a) numerous studies have established that proper regulation of microtubule dynamics is essential for cell viability (see below for more details) and (b) tau is a major regulator of microtubule dynamics [18–22], it is not surprising that cells exert powerful regulation over tau activity. This regulation is accomplished via two main mechanisms. First, alternative RNA splicing leads to the expression of two classes of tau proteins, the more potent "4-repeat tau" isoforms and the less potent "3-repeat tau" isoforms ([14,19,22–28]; see Fig. 1 for a schematic). Second, cells also regulate tau activity via kinase and phosphatase activity upon its approximately two dozen phosphorylation sites (for review, see Ref. [1]).

The nature of the FTDP-17 mutations provides some insights into the possible biochemical mechanisms of tau-mediated neuronal cell death. First, the mutations fall into two general classes. One class consists of amino-acid substitution mutations that map to the microtubule binding region of tau, or immediately adjacent to it in sequences known to mediate or regulate tau's microtubule binding and assembly activities [4–6,15,17,23,26,29]. The clustering of these mutations in a known functional region of the protein immediately raises the possibility of loss-of-function effects. The second class of FTDP-17 tau mutants alters the pattern of tau RNA alternative splicing [5,30]. Whereas normal adult human brain expresses roughly equal amounts of 4-repeat and 3-repeat tau, the mutations generally lead to a significant increase in the amount of 4-repeat tau at the expense of 3-repeat tau such that 4-repeat tau comprises approximately 75% of the total while 3-repeat tau comprises only approximately 25% [5,30,31]. However, it must be emphasized that these mutations do not alter the amino acid sequences of the encoded tau proteins since they generally map to intronic sequences involved in the splicing process or are silent. Thus, the splicing mutations are strictly regulatory—they alter the expression pattern of otherwise wild-type polypeptides.

A number of molecular mechanisms have been proposed that might explain tau-mediated neuronal cell death. These are schematized in Fig. 2. The most widely held model suggests that some mechanism, environmental or genetic, causes tau to interact aberrantly with microtubules, accumulate in the cytoplasm and then form aggregates. A relatively recent modification of this model suggests that abnormal tau might form tau aggregates initially right on the microtubule [32]. In either case, the neurofibrillary tangles accumulate in the cell and are proposed to be cytotoxic. An alternative "dosage effect" model suggests that there is an acceptable range of tau activity that must be maintained by neurons in order to maintain viability [22,28]. By this model, environmental or genetic effects that drive the level of tau activity above or below tolerable levels lead to cell death. In this model, the neurofibrillary tangles are a consequence of cell death rather than a cause of it. A third model is really a blend of the first two models. In this case, abnormal environmental or genetic influences lead to abnormal interactions between tau and microtubules, which in turn lead to the formation of neurofibrillary tangles as proposed in the first model. However, the net effect of neurofibrillary tangle formation is the sequestration of tau and, therefore, a significant reduction in the level of tau interacting with the cell's microtubules. In this model, cell death results by virtue of causing the level of tau activity to drop below some minimal level necessary for cell viability. In genetic terms, the molecular basis of cell death in the first model is a gain-of-function (toxicity of the tangles) whereas the second and third are loss-of-function/haplo-insufficiency models (failure to maintain tau activity levels within a tolerable range).

Our most recent work examining the effects of the wild-type and FTDP-17 tau molecules upon the ability of tau to regulate microtubule dynamics [22,28], taken together with a large body of literature investigating microtubule dynamics and regulatory mechanisms acting upon them (see below for details), has led us to focus our efforts on the two loss-of-

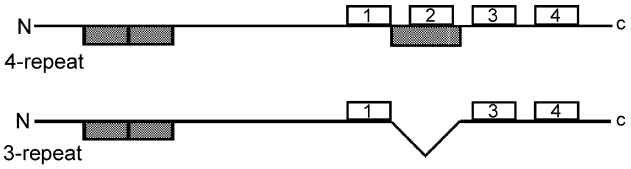


Fig. 1. Schematic diagram of 4-repeat and 3-repeat tau. Boxes above each line correspond to the 18-amino-acid imperfect repeats. Hatched boxes below each line correspond to regions encoded by alternatively spliced exons. The inclusion or excision of sequences encoded by the exon in the repeat region leads to the synthesis of 4-repeat or 3-repeat tau, respectively.

function hypotheses described above. More specifically, we have recently proposed that tau-mediated neuronal cell death is caused by the inability of affected cells to properly regulate the dynamic behavior of their microtubules [22,28]. Our model suggests that mutations leading to reduced tau activity (such as the amino acid substitution tau mutations that compromise tau's ability to regulate microtubule dynamics; see Section 3, below, and references cited therein) lead to overly active microtubules, which in turn lead to cell death. At the other extreme, our model suggests that overly suppressed microtubule dynamics, such as would be predicted for the FTDP-17 tau RNA splicing mutations in which the more potent 4-repeat tau levels are elevated and the less potent 3-repeat tau levels are reduced, will also lead to cell death. This perspective is presented schematically in Fig. 2 (pathways 2 and 3) and also in Fig. 3. An especially attractive aspect of this model is that it readily accommodates both the structural and regulatory classes of FTDP-17 mutations.

Since the regulation of microtubule dynamics is at the very heart of our model, we next present a detailed description of microtubule dynamics and regulatory mechanisms acting upon them. Subsequently, we will consider the role of tau and its regulation of microtubule dynamics in pathological tau action.

## 2. Neuronal microtubules: dynamics, regulation and importance

### *2.1. Microtubules and their dynamics in cells*

#### *2.1.1. Non-neuronal cells*

Our understanding of microtubule dynamics in cells is most advanced for non-neuronal dividing cells. Micro-

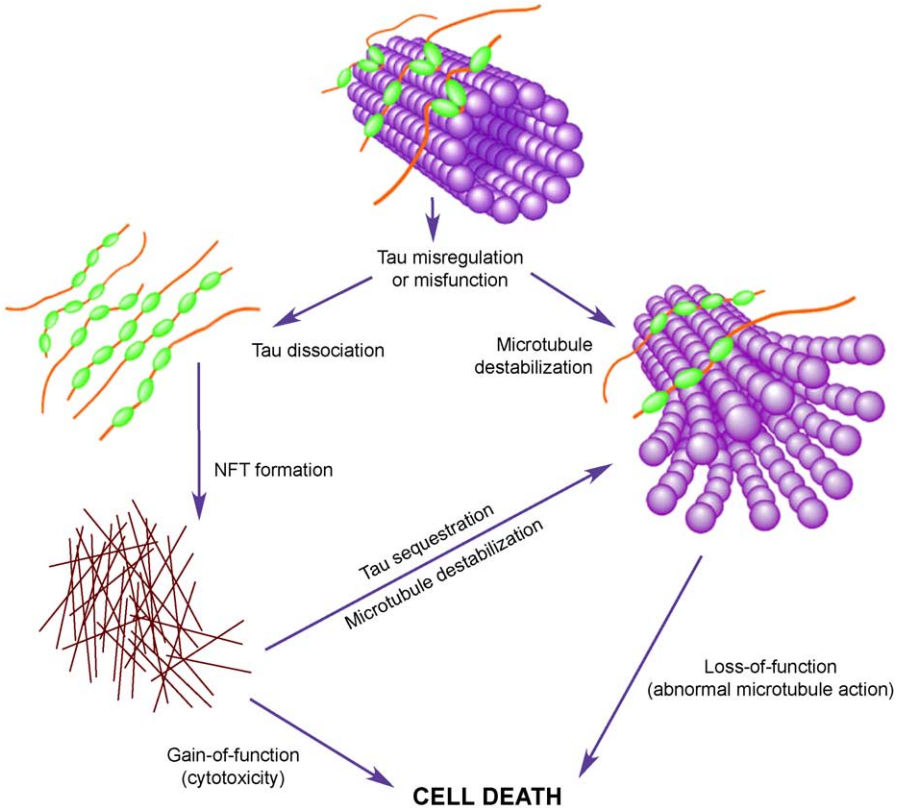


Fig. 2. Three possible pathways of tau-mediated neuronal cell death. Pathway 1—This gain-of-function model suggests that some genetic or environmental factor(s) cause tau to dissociate from microtubles and aggregate into abnormal, cytotoxic neurofibrillary tangles. Pathway 2—This dosage-effect/loss-of-function model suggests that some genetic or environmental factor(s) cause tau to improperly regulate microtubule dynamics, leading to under- or over-stabilized microtubles. As a result, these improperly regulated microtubules cannot perform their normal essential cellular functions, leading to cell death. Pathway 3—This model incorporates elements of Pathways 1 and 2. In this model, some genetic or environmental factor(s) cause tau to dissociate from microtubules and aggregate into abnormal neurofibrillary tangles. The sequestration of tau in these aggregates results in less tau to regulate microtubule dynamics, leading to over-active microtubles that cannot perform their essential functions, leading to cell death.

tubule dynamics in dividing mammalian cells are relatively rapid, especially during mitosis where extremely rapid dynamics are critical for the proper and timely construction and functioning of the mitotic spindle [33–36]. More specifically, microtubules in non-neuronal interphase cells have variable half-times for exchange of their tubulin with tubulin in the soluble pool ("turnover") in the range of 15 min to several hours while cells in the midst of mitosis have half-times of 10–15 s. In contrast, microtubules assembled in vitro with pure tubulin, such as those derived from dividing HeLa cells, exhibit remarkably slow microtubule dynamics [37]. These and other observations have led to the realization that the rapid microtubule dynamics in dividing cells are created by regulatory MAPs that function to increase the dynamics as cells progress into mitosis [38–41]. Further, microtubules located in different regions of a single cell often exhibit markedly different dynamics, leading to the conclusion that different dynamic behaviors are important determinants of different functional needs [33,35,43–48].

Interestingly, control of dynamics in dividing cells not only involves regulators that speed dynamics such as *m*itotic *c*entromere *a*ssociated *k*inesin (MCAK [49]), but appears to also involve regulatory proteins that suppress dynamics such as the high molecular weight protein known as MAP 4 [38,50]. Taken together, it is widely held that the dynamics of microtubules in dividing cells are controlled by a delicate balance between MAPs that speed dynamics and others that suppress dynamics [38–41].

### 2.1.2. Neuronal Cells

Microtubules are especially prominent in neuronal cells. In developing neurons, they are important for establishing neuronal cell polarity and process outgrowth [48,50–54]. In adult neurons, they are essential for proper structure, function and viability [46,47,53–57].

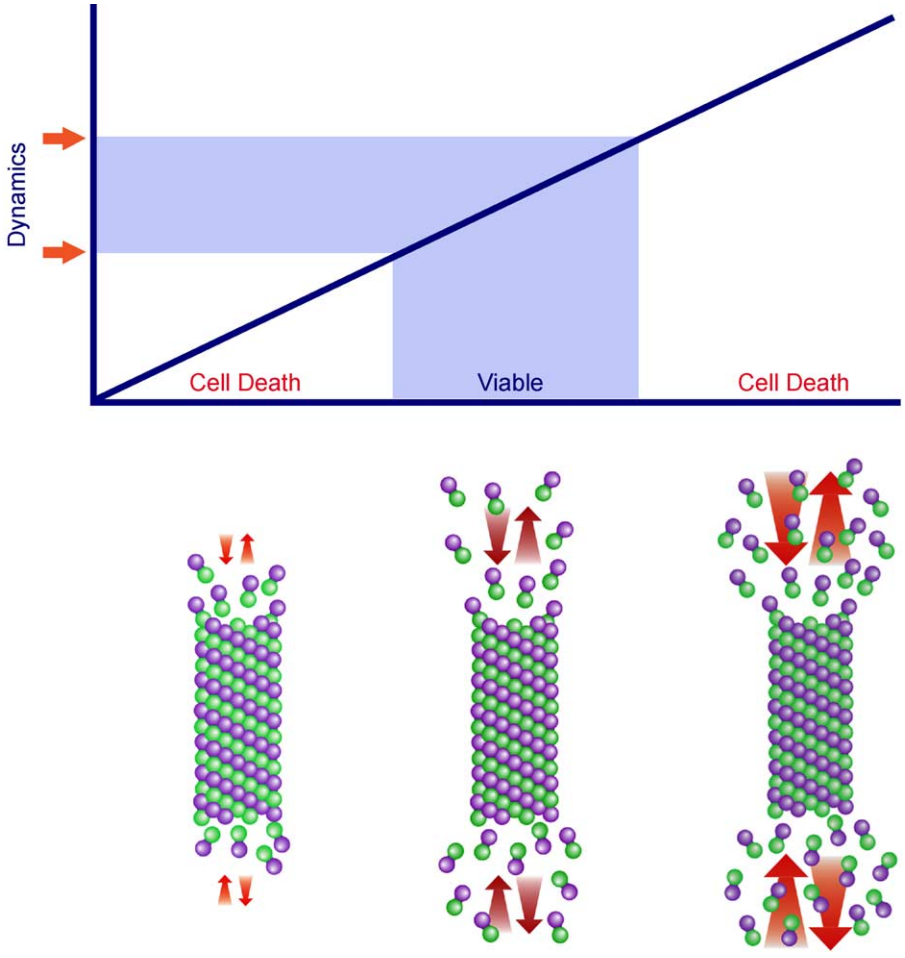


Fig. 3. Microtubule dynamics must be maintained within a tolerable window of activity to maintain viability. Cell function and viability require properly regulated microtubule dynamics. Overly suppressed dynamics, such as occurs in the presence of taxol [80] and as we propose might occur in the FTDP-17 RNA splicing mutations in which 4-repeat tau is over-expressed and 3-repeat tau is under-expressed, results in cell death. Similarly, overly dynamic microtubules, such as occurs in taxol-resistant and dependent cells in the absence of taxol [77] and as might occur in the FTDP-17 missense mutations and in cells expressing hyperphosphorylated tau, also results in cell death.

Just as in dividing cells, it is highly likely that the dynamics of the various anatomically distinct neuronal microtubule populations must be finely regulated. For example, microtubules along the length of fully differentiated axons must be relatively stable compared with mitotic cell microtubules so as to ensure that motor molecules carrying their various cargoes in both anterograde and retrograde directions have relatively stable microtubules upon which they can move. In fact, most axonal microtubules are relatively stable, but not static (half-times of 45–90 min; Ref. [58]) and their relative stability is believed to be critical for proper function. However, axonal microtubules must also retain some dynamics to enable them to move down the axon to its end under conditions in which the end of the axon is attached in a synapse. Also consistent with data from non-neuronal cells, axonal microtubules in growing axons can have different stabilities depending upon their position within the axon, with those closer to the growth cone exhibiting more dynamic behavior than those nearer to the cell body [47,48,57]. In growth cones, both stable and highly dynamic microtubules are present [48,52], and the dynamics of growth cone microtubules appear to be extremely important in growth cone function. For example, Tanaka et al. [48] found that rapid microtubule dynamics are required for the forward translocation of the growth cone and axon elongation. These workers also speculated that dynamic microtubules play a special role in controlling the orientation of the more stable microtubules, which may be a key step in growth cone turning.

Taken together, these many cellular observations highlight the importance of gaining a detailed mechanistic understanding of how microtubule dynamics are determined and controlled.

### 2.2. A mechanistic view of microtubule dynamics; "*dynamic instability*" and "*treadmilling*"

In order to gain a detailed understanding of microtubule dynamics and their regulation in cells, it is instructive to first consider the relevant in vitro biochemical investigations of microtubules and their dynamics. Microtubule polymerization in vitro occurs by a nucleation-elongation mechanism in which the relatively slow formation of a poorly defined microtubule nucleus is followed by relatively rapid elongation of the microtubule at both ends by the reversible, noncovalent, addition of α–β tubulin dimers (reviewed in Ref. [43]). The two ends of microtubules differ, both structurally and kinetically. One end, termed the plus end, grows and shortens more rapidly than the opposite, or minus end, and exhibits more robust dynamics than the minus end (see below) both in vitro and in cells. In the cell body of neurons, as occurs with most other cells, nucleation appears to be facilitated by a third type of tubulin, γ-tubulin, and occurs at the minus ends at poorly defined regions in the cell cytoplasm known as microtubule-organizing centers or centrosomes.

Microtubules are not simple equilibrium polymers (e.g., see Refs. [35,42,43,59] for recent reviews). GTP binds to soluble (un-polymerized) tubulin at an exchangeable site in the β subunit and is hydrolyzed to GDP and $P_i$ as, or shortly after, the tubulin polymerizes onto growing microtubule ends. The hydrolysis of GTP creates two unique non-equilibrium dynamic behaviors. One, treadmilling [21,60–63] is characterized by net growth at the plus end of a microtubule and net shortening at the minus end. This behavior appears to be due to the difference in the concentration of free tubulin subunits in equilibrium with the microtubule ends at steady state (i.e., the critical tubulin subunit concentration). For a microtubule to treadmill, both ends of the microtubule must be able to exchange tubulin subunits with free tubulin in the soluble pool. The second behavior, dynamic instability [42,64], is characterized by switching at microtubule ends between episodes of sustained growth and relatively rapid sustained shortening. Dynamic instability is characterized by a number of well-defined and quantifiable parameters which include the rate of growth, the rate of shortening (frequently termed "shrinking"), the transition frequency from the growth or an attenuated (or paused) state to shortening (called a "catastrophe"), and the transition frequency from shortening to growth or to an attenuated state (called a "rescue"). Periods of pause or attenuation are defined operationally as times when any changes in microtubule length that may be occurring are below the resolution of the light microscope. A calculated parameter termed "dynamicity" is highly useful to describe the overall visually detectable rate of exchange of tubulin dimers at microtubule ends.

While not rigorously established, elongation and shortening of microtubules are believed to be due to the presence or absence, respectively, of a very short region of tubulin at the extreme ends of the microtubules that may consist of a conformationally stabilized form of tubulin with either GTP or GDP-Pi bound in the exchangeable site (a so-called "GTP cap") [42,65,66]. The entire remainder of the microtubule, which can consists of many thousands of tubulin dimers (there are ~1700 tubulin dimers per μm of length), appears to be in a strained conformation due to hydrolysis of the GTP and contains GDP in the exchangeable site.

Two final points are important to note. First, treadmilling and dynamic instability are compatible behaviors and a specific microtubule population may exhibit primarily treadmilling behavior, primarily dynamic instability behavior, or both [61]. The mechanisms that control the degree to which a microtubule population exhibits one or the other behavior are poorly understood, but probably involve the tubulin isotype composition of the population, the degree of posttranslational modification of the tubulin, and especially, the actions of regulatory proteins. Finally, it is important to note that both treadmilling and dynamic instability are intrinsic dynamic behaviors of microtubules composed of tubulin as the sole protein; no accessory proteins are required to create either of these dynamic behaviors and

the only energy source required is derived from hydrolysis of GTP.

### 2.3. Suppression of microtubule dynamics by antimitotic anticancer drugs (e.g., paclitaxel, colchicine) can serve as an excellent paradigm for understanding how microtubule associated proteins (MAPs such as tau) regulate neuronal microtubule dynamics

A large number of natural products having chemically diverse structures and distinct mechanisms of action are known to inhibit mitosis and are hypothesized to serve their host organisms by acting as poisons, inhibiting the growth of competing organisms by powerfully suppressing the dynamics of their mitotic spindle microtubules [59,67,68]. We suggest that these microtubule-targeted drugs can be used as paradigms for understanding the mechanisms of molecules like tau and other MAPs that regulate microtubule dynamics in neurons [69]. They can also be used as tools for assessing the importance of microtubule dynamics in neuronal cellular processes and especially for determining the importance of maintaining microtubule dynamics in a narrow range for neuronal cell viability.

The mechanisms of action of the various microtubule-targeted drugs, like the actions of many drugs that affect complex cellular processes, are themselves highly complex [59,67,68]. At relatively high concentrations, drugs such as paclitaxel, which bind to multiple tubulin binding sites along the entire surface of the microtubule, increase microtubule polymerization (such drugs are called microtubule stabilizing drugs) while others, such as colchicine and the Vinca alkaloids, which bind both to soluble tubulin and to the ends of the microtubules (and called microtubule destabilizing drugs), inhibit microtubule polymerization at high drug concentrations [68]. The increased polymerization induced by the microtubule stabilizing drugs requires the binding of relatively large numbers of drug molecules to the microtubule surfaces, e.g., as with paclitaxel. In the case of microtubule destabilizing drugs, inhibition of polymerization is caused by binding of large numbers of drug molecules to soluble tubulin, thereby reducing the soluble tubulin pool available for polymerization (e.g., colchicine) or by binding to tubulin along the length of the microtubule and actively destabilizing the microtubule lattice as with the Vinca alkaloids.

On the other hand, low concentrations of these drugs cause powerful modulation of microtubule dynamics in the absence of appreciable polymerization or depolymerization as a result of the binding of relatively small numbers of drug molecules to microtubule surfaces or ends. For example, at low concentrations, just a few vinblastine molecules bound at the plus ends of a microtubule (1–2 per microtubule) powerfully suppress in vitro dynamics [68]. The mechanism of action of low concentrations of paclitaxel, one of the best understood drugs, is described below in the context of it serving as a paradigm for the molecular mechanism of action of tau.

### 2.4. Paclitaxel as a paradigm for the mechanism of action of tau; similarities in the mechanisms of action of paclitaxel and tau

Both paclitaxel and tau bind to the surface of the microtubule along its length [70–75]. Paclitaxel binds with high affinity to the β-tubulin subunit, and the location of the binding site, which is on the inside surface of the microtubule, is known with precision because the electron crystal structure of tubulin was carried out with paclitaxel bound to the protein [73,74]. Paclitaxel is thought to gain access to its binding sites by diffusing through small openings in the microtubule or fluctuations of the microtubule lattice [72]. There is one paclitaxel-binding site on every tubulin dimer in a microtubule, and the ability of paclitaxel to strongly increase microtubule polymerization is associated with nearly 1:1 stoichiometric binding of paclitaxel to tubulin in microtubules.

In contrast with the large numbers of taxane molecules required to increase microtubule polymerization, the binding of only a very small number of paclitaxel molecules to tubulin in a microtubule is sufficient to powerfully stabilize microtubule dynamic instability and treadmilling. For example, at steady state when the concentration of soluble tubulin and assembled microtubules remain constant, paclitaxel, at levels as low as 0.01 mol of bound paclitaxel per mole of tubulin dimer in microtubules, potently and selectively suppresses the rate and extent of shortening at microtubule plus ends in association with only a minimal increase in the mass of assembled polymer, as compared with drug-free control microtubules [70].

The mechanism of action of tau is remarkably similar to that of paclitaxel in vitro [70]. Similar to the effects of low paclitaxel concentrations on microtubule dynamics at steady state, low concentrations of 4-repeat adult tau also inhibit microtubule treadmilling and powerfully suppress the rate and extent of microtubule shortening [21,22,76]. For example, at a ratio of 4-repeat tau to tubulin of 1:16, tau reduced the shortening rate and the length shortened during a shortening event by 54% and 44%, respectively [22]. Similar but weaker effects were observed at tau/tubulin molar ratios as low as 1:332. Interestingly, although tau is known to bind to the C-termini of tubulin (on the outer surface of the microtubule), more recent data suggest that tau may also bind at or near the taxol site [75].

### 2.5. The importance of maintaining microtubule dynamics in a narrow range to support cell viability

Recent evidence suggests that microtubule dynamics during mitosis must be maintained in a narrow range for proper mitotic progression and cell viability, that is, microtubule with overly suppressed dynamics or overly robust dynamics cannot function properly and cells die. For example, at the transition from interphase to mitosis in animal cells, populations of relatively stable interphase

microtubules are replaced by populations of highly dynamic mitotic spindle microtubules (~20–50 times more dynamic than the microtubules in interphase cells). The extremely rapid dynamics of mitotic spindle microtubules are essential for the proper function of the mitotic spindle, which in a remarkably short time must properly assemble and accurately engage the duplicated chromosomes, move them to the metaphase plate, and then accurately and rapidly segregate them to the forming daughter cells at the transition from metaphase to anaphase. It is well established that suppression of spindle microtubule dynamics by drugs such as paclitaxel and the Vinca alkaloids leads to a slowing or complete block of mitosis at the metaphase to anaphase transition, a critical cell cycle checkpoint. This in turn leads to apoptotic cell death [67,68]. At the other extreme, excessively robust dynamics during mitosis also cause spindle misfunction and lead to cell death. Specifically, in lung tumor A549 cells which are both resistant to paclitaxel and dependent upon the presence of paclitaxel for viability, removal of the paclitaxel results in a significant increase in microtubule dynamics and the cells become blocked in mitosis and die as do their wild-type counterparts treated with normal paclitaxel concentrations [77]. Thus, during mitosis, there appears to be a narrow window of tolerated microtubule dynamics, and excessively rapid dynamics as well as suppressed dynamics lead to mitotic inhibition and cell death.

## 3. Tau and the regulation of microtubule dynamics: implications for neurodegeneration

Despite the enormous amount of effort focused upon tau over the past two decades, relatively little work has addressed the most mechanistic aspect of its action, that is, its ability to regulate microtubule dynamics. Most structure–function studies have focused upon the ability of tau to bind to microtubules, and a number have also assessed tau's ability to assemble microtubules from tubulin subunits (for example, see Refs. [14,15,17,19,20,23,29]). However, binding to microtubules is a prerequisite for function, but is not itself a function. The ability to assemble microtubules is surely a functional assay, but it views only one aspect of a very complex set of dynamic features. Given the importance of properly regulated microtubule dynamics to the function and viability of neurons, understanding the ability and mechanisms by which tau regulates microtubule dynamics is a fundamentally important goal.

Initially, Drechsel et al. [18], using non-steady state conditions of net microtubule assembly, found that 4-repeat tau at saturating tau/tubulin ratios strongly promotes the rate and extent of polymerization, decreases the transition frequency from the growing to shortening state, and inhibits the depolymerization rate. Trinczek et al. [19] next compared the effects of high concentrations of 3-repeat and 4-repeat tau on various dynamic instability parameters, also during the initial stages of microtubule polymerization, and observed similar but not identical effects. Especially notable was their observation that 4-repeat tau exerted a markedly stronger ability to slow the tubulin dissociation rate at microtubule ends than did 3-repeat tau, again, at very high tau/tubulin ratios. Shortly thereafter, Panda et al. [76] conducted the first examination of tau action on microtubule dynamics under steady state conditions, i.e., no net change in microtubule mass. Under these conditions, they found that 4-repeat tau reduced the rate and extent of shortening and, in contrast to the earlier work carried out under non-steady state conditions, tau also suppressed the rate and extent of growing. Most importantly, tau exerted these effects on microtubule dynamics at extraordinarily low molar ratios of tau/tubulin (as low as 1 tau per 332 tubulin dimers).

In the past several years, we have sought to extend these initial studies, examining the regulatory effects of both wild-type and FTDP-17 tau polypeptides upon microtubule dynamics, both in vitro [22] and in living cells [28]. These studies have provided a number of important insights into normal and pathological tau action. First, in direct comparisons of 4-repeat and 3-repeat tau, we found that both isoforms suppress microtubule dynamics, though to different extents and with at least one major qualitative difference. Specifically, 4-repeat tau reduced the rate and extent of both growing and shortening events. Three-repeat tau stabilized most dynamic parameters similarly, albeit severalfold less potently than 4-repeat tau. However, whereas 4-repeat tau was a strong stabilizer of microtubule shortening events, 3-repeat tau exhibited a minimal to negligible effect on these parameters of dynamics [22,28]. An especially clear example of these mechanistic differences is presented in Fig. 4. Rapid depolymerization clearly occurs when the microtubules are diluted into 3-repeat tau but not into 4-repeat tau. Thus, there are both quantitative and qualitative mechanistic differences between 4-repeat and 3-repeat tau, consistent with earlier indications [17,19,27]. These data predict that the FTDP-17 RNA splicing mutations are likely to cause significant and perhaps deleterious alterations in neuronal microtubule dynamics. Secondly, we compared the ability of various FTDP-17 tau variants to regulate microtubule dynamics with their wild-type counterparts, in both 4-repeat and 3-repeat tau contexts [78]. We found that all of the FTDP-17 amino acid substitution mutations that we tested significantly reduced tau's stabilizing effects on microtubule dynamics, with the exception of R406W. This latter mutation lies outside the microtubule binding region and likely causes disease by virtue of altering the ability of nearby ser/thr sites to be phosphorylated [78,79]. Thus, from this biochemical point of view, the FTDP-17 mutations exhibit loss-of-function effects.

Based upon these and pharmacological data described in the previous section, we have proposed a "dosage effect" model to explain the FTDP-17 mutant phenotypes [22,28]; this model corresponds to pathways 2 and 3 in Fig. 2. We suggest that the properly regulated activities of both tau alleles are necessary to produce appropriate amounts of each

tau isoform to maintain microtubule dynamics within the narrow range required for cell function and viability. That both tau alleles are required accounts for the dominance of the FTDP-17 phenotype. If microtubule dynamics in neurons migrate outside this range, becoming too rapid or too suppressed, cell functions dependent upon the dynamics become impaired and, in time, cell death occurs (Fig. 3). For frame-of-reference, previous work in non-neuronal cells has demonstrated that relatively subtle changes in microtubule dynamics, on the order of 30%, are sufficient to cause arrested mitosis followed by cell death [80].

This dosage effect model can readily account for both the RNA splicing and amino acid substitution classes of FTDP-17 mutations. With the RNA splicing mutations, our data suggest that increasing the ratio of 4-repeat tau to 3-repeat tau will lead to less microtubule shortening and increased microtubule stabilization [22,28]. With the mutations causing amino acid substitutions, our data suggest that the mutant tau molecules will possess defects in dynamics regulatory capabilities, resulting in overly dynamic microtubules. Our model predicts that cell death should occur in both classes of mutations, since the pattern of microtubule dynamics would migrate outside the window of viability, one way or the other (Fig. 4).

A dosage effect model has additional important features. First, it can readily accommodate the long-standing correlation between tau hyperphosphorylation and neuronal cell death. Since most tau phosphorylation events reduce tau's binding affinity for microtubules, they are also likely to reduce the ability of tau to regulate microtubule dynamics.

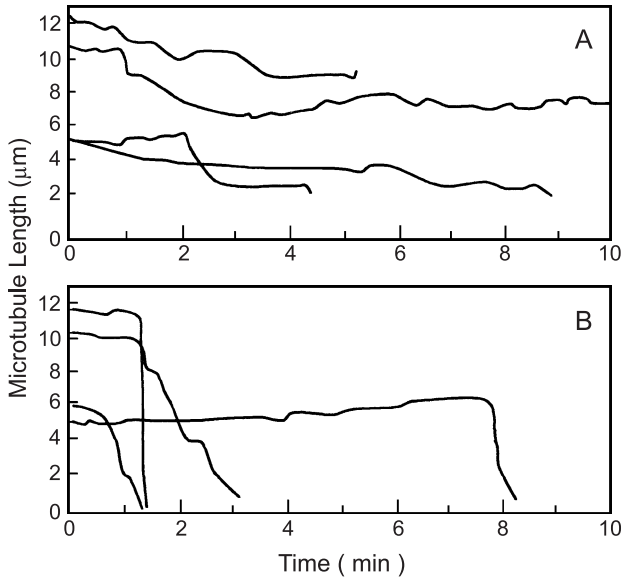


Fig. 4. The effects of 3-repeat- and 4-repeat tau on the stability of individual microtubules after dilution-induced disassembly. Microtubules were polymerized at 37 °C (12 μM tubulin) and diluted fourfold into buffer containing 0.74 μM 4-repeat tau (A) or 3-repeat tau (B). The diluted microtubule suspensions were analyzed by video microscopy between 2 and 10 min after dilution. Each trace represents a single microtubule. Reprinted by permission of PNAS.

Indeed, phosphorylation of tau by MAP2 kinase [81], MARK, or cdk5 [19] reduces tau's ability to influence microtubule dynamics. If the reduction of dynamics-regulating activity is sufficient to move the cell outside the dynamics window required for viability, the model predicts cell death. A second feature of the model is that neurofibrillary tangle formation may be relegated to a downstream consequence of the primary cause of cell death, which is abnormal tau-mediated regulatory effects on microtubule dynamics. Consistent with this notion, *Drosophila* overexpressing human tau exhibit many features of Alzheimer's disease, including early onset and progressive neurodegeneration but without any abnormal tau fiber formation [82,83]. The same is true in some transgenic mouse lines [84,85]. Thus, abnormal tau fiber formation is not an obligatory feature of tau-induced neuronal cell death, at least in these cases.

Finally, the dosage effect model and the gain-of-toxic-function model are not mutually exclusive. In addition to the possibility that the fibers may themselves be toxic under some circumstances, fiber formation would be expected to contribute to de-regulation of microtubule dynamics by virtue of sequestering the pool of tau, which should in turn lead to overly dynamic microtubules and subsequent cell death.

### *3.1. How might altered microtubule dynamics cause cell death?*

In dividing cells, drugs that alter microtubule dynamics can lead to aberrant cell morphology, chromosome segregation and intracellular transport mechanisms. Although chromosome segregation is not highly relevant for post-mitotic neurons, neurons should be very sensitive to perturbations affecting axonal transport or maintenance of cell morphology. Hence, if altered neuronal microtubule dynamics underlie tau-mediated neuronal cell death in FTDP-17 disease, the actual cause of cell death might be the inability to properly transport cargo along the axon and/or to maintain the elongated cell morphology necessary to acquire target derived trophic factors (for review, see Ref. [86]). Indeed, overexpression of tau in cultured neuronal cells leads to aberrant axonal transport [87]. Further, compromising axonal transport via overexpression of dynactin in mice causes late-onset death in motor neurons [88].

## 4. Final thoughts

Understanding the precise molecular mechanism(s) underlying tau-mediated neuronal cell death is of enormous importance. Unfortunately, there are strengths and weaknesses associated with each of the possible models presented in Fig. 2. Strengths of model 1 are (a) the ubiquitous nature of neurofibrillary tangles in Alzheimer's and the other tauopathies, and (b) the fact that many tau

proteins harboring one of the FTDP-17 point mutations assemble into tau fibers more efficiently than do wild-type tau isoforms [89–93] (for review, see Ref. [94]). On the other hand, a weakness is the lack of evidence supporting the notion that neurofibrillary tangles are indeed directly cytotoxic. This is admittedly a difficult question to address experimentally, in large part because the tangles are intracellular rather than extracellular structures. However, there are now numerous examples of tau-mediated neuronal cell death without abnormal tau fiber formation in various transgenic models [82–85]. Additionally, while this model easily accommodates the FTDP-17 amino acid substitutions, it is less obvious how it applies to the RNA splicing mutations in which only wild-type tau isoforms are expressed, but in altered molar ratios. Why should altered ratios of otherwise normal proteins have an increased probability of forming abnormal fibers? Major strengths of the second, "dosage-effect" model are that (a) a large body of literature demonstrates that proper regulation of microtubule dynamics is essential for cell function and viability, (b) tau is a potent regulator of microtubule dynamics [18–22,28,76] and (c) recent mechanistic analyses of both wild-type and FTDP-17 tau strongly suggest that both the amino acid substitution mutations as well as the regulatory splicing mutations should alter cellular microtubule dynamics sufficiently to cause neuronal cell death (compare Refs. [22,28] with Ref. [80]). A weakness of this model is that expression of tau molecules harboring FTDP-17 amino acid substitutions in transgenic mice often cause a more severe phenotype than does expression of comparable levels of wild-type tau [95,96]; this is inconsistent with the general loss of function nature of the amino acid substitution mutants. However, the transgenic mouse data are significantly complicated by differing genetic backgrounds, the difficulty inherent in controlling levels of expression and conflicting observations. The third model, in which tangles form and contribute to cell death by sequestering tau and thereby causing improper regulation of microtubule dynamics, may be the strongest of the three models. It does not require that the tangles be inherently cytotoxic, but only that they form. Further, the model easily accommodates the observation that FTDP-17 mutated tau appears to be more cytotoxic than wild-type tau, since the mutated tau often forms abnormal tau fibers in vitro more readily than does wild-type tau [89–93] (for review, see Ref. [94]). However, just as is true for pathway 1, this model accommodates the amino acid substitution mutations more easily than the RNA splicing mutations. Again, why should altered ratios of otherwise normal tau proteins readily form tangles? In the final analysis, although it has its obvious attraction, it may not be the case that a single mechanism is responsible for neuronal cell death in both the amino acid substitution and RNA splicing mutations.

In closing, the importance of understanding the molecular basis of tau-mediated neuronal cell death goes far beyond just FTDP-17. Although no mutations in tau have yet been linked genetically to Alzheimer's disease, it is well established that Aβ-mediated neuronal cell death requires tau [97]. This is consistent with the fact that Alzheimer's patients possess both amyloid plaques and neurofibrillary tangles while FTDP-17 patients exhibit only neurofibrillary tangles. One might therefore be able to interfere with Aβ-mediated neuronal cell death by properly controlling tau action and its downstream events. We need to learn what those events are, and how to control them.

## Acknowledgements

We are extremely grateful to the members of our labs for many stimulating discussions. We also thank Margie Jess, Janis Bunker and Michelle Massie for assistance with figures and Michelle Massie for assistance with the manuscript. Our work is supported by National Institutes of Health grants NS35010 (SCF) and NS13560 (LW).

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