

## Accelerated Publications

### Detection of GTP and $P_i$ in Wild-Type and Mutated Yeast Microtubules: Implications for the Role of the GTP/GDP- $P_i$ Cap in Microtubule Dynamics<sup>†</sup>

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**ABSTRACT:** Microtubule dynamics are believed to be controlled by a stabilizing cap of tubulin dimers at microtubule ends that contain either GTP or GDP and  $P_i$  in the exchangeable nucleotide site (E-site) of the  $\beta$ -subunit. However, it has been difficult to obtain convincing evidence to support this hypothesis because the quantity of GTP and  $P_i$  in the E-site of assembled brain tubulin (the tubulin used in most studies thus far) is extremely low. In this study, we have measured the amount of GTP and  $P_i$  in the E-site of wild-type and mutated yeast assembled tubulins. In contrast to brain microtubules, 6% of the tubulin in a wild-type yeast microtubule contains a combination of E-site GTP and  $P_i$ . This result indicates that GTP hydrolysis and  $P_i$  release are not coupled to dimer addition to the end of the microtubule and supports the hypothesis that microtubules contain a cap of tubulin dimers with GTP or  $P_i$  in their E-sites. In addition, we have measured the E-site content of GTP and  $P_i$  in microtubules assembled from two yeast tubulins that had been mutated at residues T107 and T143 in  $\beta$ -tubulin, sites thought to interact with the nucleotide bound in the E-site. Previous studies have shown that microtubules containing these mutated tubulins have modified dynamic behavior in vitro. The results from these experiments indicate that the GTP or GDP- $P_i$  cap model does not adequately explain yeast microtubule dynamic behavior.

Microtubules (MTs)<sup>1</sup> are polymers of tubulin, a heterodimeric protein that binds two molecules of GTP, one to the  $\alpha$ -subunit nonexchangeable site (N-site) and the other to the  $\beta$ -tubulin exchangeable site (E-site) (reviewed in ref 1). Only the E-site GTP is hydrolyzed during tubulin assembly into MTs, and it is believed that this reaction plays

a central role in the growing and shortening kinetics of MTs. MTs are dynamic structures; in a population of steady-state MTs, some shorten while others grow, even though the mass of the polymer remains constant. This behavior has been observed in cultured cells as well as in vitro and is thought to be a necessary property of MTs as they search for their targets, for example, kinetochores (2), and to promote chromosome movement (3–5). The importance of MT

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<sup>1</sup> Abbreviations: MT, microtubule; tubulin-GTP<sub>E</sub>, tubulin dimer containing GTP in the E-site; tubulin-(GDP- $P_i$ )<sub>E</sub>, tubulin dimer containing GDP and  $P_i$  in the E-site; PIPES, piperazine-*N,N'*-bis(2-ethanesulfonic acid); EGTA, ethylene glycol bis( $\beta$ -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; PEM buffer, 100 mM PIPES, 2 mM EGTA, and 1 mM MgSO<sub>4</sub> (pH 6.9); DMSO, dimethyl sulfoxide; MAP, microtubule-associated protein.

dynamics to the cell is evident from the fact that dynamics change drastically during the cell cycle. For example, mitotic MTs are much more dynamic than interphase microtubules (6, 7). Furthermore, low concentrations of antimitotic drugs, such as vinblastine and Taxol, appear to block mitosis by inhibiting the dynamics of mitotic MTs without causing them to depolymerize (8, 9).

MT dynamic behavior is believed to result from alternating gain or loss of a stabilizing "cap" at the end of the MT, consisting of tubulin subunits containing GTP or GDP and  $P_i$  in the E-site (10, 11; reviewed in ref 12). Consistent with this idea is the observation that MTs assembled in vitro with nonhydrolyzable GTP analogues are more stable than those assembled with GTP (13, 14), presumably because the tubulin-analogue complex mimics a stabilizing tubulin-GTP complex. GTP hydrolysis at the E-site is thought to be a requirement for the dynamic behavior of MTs; therefore, MT stability could be influenced by the rate of the hydrolytic reaction. Experiments in which mutated yeast tubulins were used demonstrated that MTs with increased GTPase activity were more dynamically unstable than wild-type MTs (15). Thus, MT dynamics could be regulated by modulation of tubulin GTPase activity, thereby influencing the size or persistence of the GTP or GDP- $P_i$  cap.

The existence of a MT stabilizing cap is supported by work from many groups (reviewed in ref 12); however, several different models have been proposed for the mechanism of the gain and loss of such a cap. One model proposes that the addition of a tubulin subunit to the MT end is independent of, or uncoupled from, GTP hydrolysis (10, 16, 17). This model predicts that at high concentrations of tubulin, for example, during the initial stages of assembly, a large cap would exist because tubulin-GTP dimers would add to the growing end of the MT at a rate faster than the rate of E-site GTP hydrolysis. As steady state is approached and the concentration of unpolymerized tubulin decreases, the cap would become small because dimers would add at a rate just slightly faster than the rate of GTP hydrolysis. Another model for GTP cap formation is that dimer addition is coupled to GTP hydrolysis (18–20). According to this model, there is a fixed-size GTP cap, independent of the assembly rate or of the free tubulin concentration, which cannot exceed the 13 terminal subunits because the addition of a dimer to the end of the MT is coupled with the hydrolysis of GTP by the previously terminal subunit.

In addition to the mechanism of cap formation, there is also the question of the chemical nature of the stabilizing cap, whether it consists of tubulin-GTP<sub>E</sub> or tubulin-(GDP- $P_i$ )<sub>E</sub>. Evidence for a GDP- $P_i$  cap comes from studies with brain tubulin in which  $P_i$  was detected in MTs under rapid initial assembly conditions using high tubulin concentrations and equimolar concentrations of Taxol (21), as well as from studies showing that  $P_i$  release occurred at a slightly slower rate than MT formation, also under conditions of very rapid assembly (22). Further support for a GDP- $P_i$  cap comes from experiments which demonstrated that MTs are stabilized by  $\text{BeF}_3^-$  (23, 24), an anion that presumably binds to the  $\gamma$ -phosphoryl binding site in the nucleotide binding sites of ATP- and GTP-requiring proteins (25, 26). The stabilization of MTs by  $\text{BeF}_3^-$  suggests that tubulin-(GDP- $\text{BeF}_3^-$ )<sub>E</sub> acts as a transition-state analogue in the conversion of tubulin-GTP<sub>E</sub> to tubulin-(GDP- $P_i$ )<sub>E</sub>.

To address the mechanism of GTP hydrolysis and the role of E-site GTP and/or  $P_i$  in controlling MT dynamics, we measured the GTP and  $P_i$  content of MTs assembled from wild-type *Saccharomyces cerevisiae* tubulin and from two different mutated yeast tubulins. The mutations are at residues T107 and T143 in the  $\beta$ -subunit, residues that are thought to interact with the nucleotide on the basis of analogies with other GTP and ATP binding proteins. Indeed, the recently described electron crystallographic structure of tubulin is consistent with these residues being located near the E-site nucleotide (27). The T107-mutated tubulin forms MTs that have increased GTPase activity and decreased stability (15), while the T143-mutated tubulin forms MTs with decreased GTPase activity that are quite stable (C. A. Dougherty, C. R. Sage, A. Davis, and K. W. Farrell, unpublished results). In this work, in contrast to results obtained with vertebrate brain MTs, we found that yeast MTs contain a readily detectable amount of GTP<sub>E</sub> and  $P_{iE}$ , evidence that supports the uncoupled mechanism of GTP hydrolysis. Further, our data from experiments analyzing MTs assembled from mutated tubulins indicate that the simple cap model alone cannot explain MT dynamic behavior. A model is presented to explain our findings.

## EXPERIMENTAL PROCEDURES

**Purification of Tubulin.** MAP-free bovine brain tubulin was prepared from three-cycle purified MAP-rich microtubule protein using phosphocellulose chromatography as described by Mitchison and Kirschner (11). Yeast tubulin was prepared by the method of Barnes et al. (28) as modified by Davis et al. (29).

**Yeast Strains.** The yeast strains used were FY41, YT143G, and YT107W. FY41 is a haploid yeast strain with a single copy of the wild-type  $\beta$ -tubulin gene, *TUB2*. YT143G is a haploid mutant strain containing a threonine to glycine substitution at position 143 in  $\beta$ -tubulin. For both strains, the  $\alpha$ -tubulin subunits were wild-type. The YT107W strain is a heterozygous diploid yeast strain containing one copy of *tub2-T107W*, which encodes a  $\beta$ -tubulin in which a tryptophan is substituted for a threonine at position 107, and one copy of *tub2-590*, an allele encoding a  $\beta$ -tubulin truncated by 12 amino acids at the C terminus. This diploid strain was used because the *tub2-T107W* mutation is a recessive lethal allele, and by using the *tub2-590* background, the expression and assembly competence of the T107W-mutated protein could be monitored with an antibody to the 12 C-terminal amino acids (15). The GTP binding activity, GTPase activity, and dynamic properties of heterozygous diploid control MTs, containing the full-length wild type and the truncated version of  $\beta$ -tubulin in equal amounts, are essentially identical to the properties of MTs containing only full-length wild-type  $\beta$ -tubulin (29).

**Nucleotide and  $P_i$  Content of Microtubules.** To measure the GTP<sub>E</sub> and  $P_{iE}$  contents of MTs, an assembly reaction mixture containing 100  $\mu\text{M}$  GTP, 8% DMSO, and 1–2 mg/mL tubulin was made in a final volume of 220  $\mu\text{L}$  of 100 mM PIPES (pH 6.9), 1 mM  $\text{MgSO}_4$ , and 2 mM EGTA (PEM buffer). From this assembly reaction, 100  $\mu\text{L}$  was used as the experimental sample, 100  $\mu\text{L}$  was used for a background control to determine the amount of radiolabel trapped in MT pellets, and 10  $\mu\text{L}$  was used to obtain MT lengths. A mixture

of [ $^3\text{H}$ ]GTP (460 Ci/mol) and [ $^{32}\text{P}$ ]GTP (2700 Ci/mol) in a 10  $\mu\text{L}$  aliquot was added to the 100  $\mu\text{L}$  experimental sample; the sample was incubated on ice for 30 min and then assembled for 40 min at 30 (yeast tubulin) or 37  $^{\circ}\text{C}$  (brain tubulin). The background control 100  $\mu\text{L}$  sample was assembled to steady state without radiolabeled nucleotides, and then 10  $\mu\text{L}$  of a solution containing 8% DMSO, [ $^3\text{H}$ ]GTP, [ $\gamma\text{-}^{32}\text{P}$ ]GTP,  $^{32}\text{P}_i$ , and 50 mM GTP was added. This made the total content of  $^3\text{H}$  and  $^{32}\text{P}$  in the control and experimental samples equivalent but reduced the specific activity in the control samples to only 2% of that in the experimental samples. Thus, the background control sample measures GTP and  $\text{P}_i$  present in the trapped volume of the MT pellet, and the value is subtracted from the experimental value. After radiolabel addition to the control sample, both samples were immediately transferred to prewarmed 250  $\mu\text{L}$  centrifugation tubes and centrifuged at 30 or 37  $^{\circ}\text{C}$  for 30 min at 30 000 rpm in a SW50.1 Beckman rotor. After sedimentation, the supernatant was underlayered with 40% sucrose in PEM buffer and then carefully removed. The 40% sucrose solution was then underlayered with 60% sucrose in PEM buffer and removed. The 60% sucrose solution was removed and the pellet rinsed five times with 60% sucrose (or until no  $^{32}\text{P}$  counts were detected in the rinse solution with a Geiger counter). The walls of the centrifuge tubes were wiped three times with Q-Tips. The pelleted MTs were resuspended in 100  $\mu\text{L}$  of PEM buffer and left at 4  $^{\circ}\text{C}$  overnight. Aliquots of the resuspended pellets were counted using Ready-Protein scintillation fluid and a Beckman scintillation counter.

To determine the nature of the  $^{32}\text{P}$  radiolabel in the various samples,  $\text{P}_i$  was extracted as a phosphomolybdate complex (30). Samples (20  $\mu\text{L}$ ) of the resuspended steady-state MTs were precipitated with equal volumes of 10% perchloric acid for 20 min on ice and centrifuged in a microfuge for 20 min. The supernatant was added to 1 mL of 0.6 mM  $\text{KH}_2\text{PO}_4$ ; then 0.5 mL of 5% ammonium molybdate in 2 M  $\text{H}_2\text{SO}_4$  was added, and the solutions were mixed briefly. The phosphomolybdate complex was then extracted into 2 mL of a 1:1 solution of cyclohexane and isobutanol. A 0.5 mL portion of the organic phase, containing the radiolabeled  $\text{P}_i$ , and 0.5 mL of the aqueous phase, containing radiolabeled GDP and GTP, were counted.

Radiolabel found in the control pellet, as described above, was due to trapping of supernatant in the pellet. The fraction of the radioactivity found in this pellet was used to correct the amount of label in the experimental pellets. The radioactivity in the experimental pellets above this background was used with the protein concentration to determine the stoichiometry of GXP ( $^3\text{H}$ /dimer) and GTP or  $\text{P}_i$  ( $^{32}\text{P}$ /dimer). The results from the phosphomolybdate extraction were used to distinguish the stoichiometry of  $\text{P}_i$ /dimer from the stoichiometry of GTP/dimer. The  $\text{P}_i$  and GTP per MT were calculated using the average length of the MTs, obtained by examination with the electron microscope after fixing and staining aliquots at the time of centrifugation (31) using a value of 1690 dimers/ $\mu\text{m}$  of MT.

Protein concentrations were determined using the Bradford method (32) with bovine serum albumin as a standard.

Table 1: GTP<sub>E</sub> and P<sub>iE</sub> Content of Bovine Brain and Wild-Type Yeast Microtubules<sup>a</sup>

tubulin source	% $^{32}\text{P}$ as $\text{P}_i$ <sup>b</sup>	$\text{P}_i/\text{MT}$ <sup>c</sup>	GTP/MT <sup>c</sup>
bovine brain	44	10 $\pm$ 6	12 $\pm$ 7
yeast	58 $\pm$ 8	248 $\pm$ 104	173 $\pm$ 50

<sup>a</sup> The results are means of five experiments for wild-type yeast and two for bovine brain tubulin. <sup>b</sup> The percent of  $^{32}\text{P}$  as  $\text{P}_i$  was calculated from the amount of label that partitioned into the organic phase in the phosphomolybdate procedure as described in Experimental Procedures. <sup>c</sup>  $\text{P}_i/\text{MT}$  and GTP/MT values are the products of the fraction of the  $^{32}\text{P}$  as  $\text{P}_i$  or GTP, the  $^{32}\text{P}/^3\text{H}$  stoichiometry ratio, the MT average length, and the number of dimers per micromole of MT (1690).

## RESULTS AND DISCUSSION

*Dimer Addition, GTP Hydrolysis, and  $\text{P}_i$  Release Are Uncoupled in Yeast MT Assembly.* To distinguish between the coupled and uncoupled models for dimer addition to the end of a MT and to determine whether the cap contains GTP or GDP and  $\text{P}_i$ , we designed an experimental strategy for detecting the amount of tubulin–GTP<sub>E</sub> and tubulin–(GDP– $\text{P}_i$ )<sub>E</sub> in steady-state MTs. MTs were assembled to steady state in the presence of [ $^3\text{H}$ ]GTP and [ $\gamma\text{-}^{32}\text{P}$ ]GTP, and the stoichiometry of  $^3\text{H}$  and  $^{32}\text{P}$  incorporation into the polymer was determined by a sedimentation assay (Experimental Procedures). To correct for non-E-site radioactivity, a duplicate sample was assembled in the absence of radiolabeled GTP and just prior to centrifugation, an equal amount of label, in the form of GTP and  $\text{P}_i$ , together with a large excess of unlabeled GTP was added. The unlabeled GTP reduced the specific activity of the radiolabel in the control sample 50-fold compared to that in the experimental sample. As a result, radiolabel incorporated into control MT samples by treadmilling and dynamic instability was no more than 2% of that in the experimental samples. Since the total amount of radiolabel was the same in the background control and experimental samples, the amount of radiolabel in the MT pellets due to trapped supernatant could be determined from the background samples and subtracted from the experimental sample values. The results were standardized by expressing them as a ratio of  $^{32}\text{P}$  stoichiometry/ $^3\text{H}$  stoichiometry to account for different amounts of radiolabeled nucleotide incorporated into the MT.

Consistent with the results of others (19, 20, 33, 34), we found very little GTP<sub>E</sub> or P<sub>iE</sub> in brain MTs (Table 1). The  $^{32}\text{P}$  content of the experimental sample was only 15% higher than that of the background control and is equivalent to 22 dimers per MT, 10 of which were  $\text{P}_i$ . Thus, using bovine brain MTs, we could not clearly determine whether dimer addition is coupled or uncoupled from GTP hydrolysis. In contrast, the  $^{32}\text{P}$  content of experimental samples of yeast MTs was 10-fold higher than that of the background control samples. MTs assembled from yeast tubulin contained an amount of tubulin–GTP<sub>E</sub> equivalent to 2.5% of the dimers in the MT, demonstrating that dimer addition and GTP hydrolysis are uncoupled in yeast MT assembly. This represents the first finding of a substantial amount of GTP<sub>E</sub> in steady-state MTs.

We also found evidence for the presence of P<sub>iE</sub> in steady-state MTs. Of the total  $^{32}\text{P}$  content in the yeast MTs, 58% was due to  $\text{P}_i$ . The MTs contained about 170 dimers with GTP<sub>E</sub> and 240 with (GDP– $\text{P}_i$ )<sub>E</sub> (Table 1). The large amount



Table 2: GTP<sub>E</sub> and P<sub>IE</sub> Content of MTs Assembled from Mutated Yeast Tubulin<sup>a</sup>

mutated $\beta$ -tubulin	% <sup>32</sup> P as P <sub>i</sub> <sup>b</sup>	P <sub>i</sub> /MT <sup>c</sup>	GTP/MT <sup>c</sup>	GTPase activity <sup>d</sup>
$\beta$ -T107W	60 $\pm$ 19	413 $\pm$ 290	187 $\pm$ 11	15.7
$\beta$ -T143G	81 $\pm$ 5	3924 $\pm$ 1211	819 $\pm$ 53	0.07

<sup>a</sup> The results are from two experiments for T107W and three for T143G  $\beta$ -tubulin. <sup>b</sup> See Table 1. <sup>c</sup> See Table 1. <sup>d</sup> The values are relative to the value for wild-type yeast tubulin. Wild-type MT GTPase activity is 1500 P<sub>i</sub> min<sup>-1</sup> MT<sup>-1</sup> (15; C. A. Dougherty, C. R. Sage, A. S. Davis, and K. W. Farrell, unpublished results). These figures were determined in a solution lacking DMSO.

of P<sub>i</sub> in yeast MTs (in 3.5% of the dimers) demonstrates that tubulin-(GDP-P<sub>i</sub>)<sub>E</sub> is an intermediate in MT formation and that GTP hydrolysis and P<sub>i</sub> release occur as two discrete steps in MT assembly. The data also lend support to the GDP-P<sub>i</sub> cap model.

*Microtubules Assembled from Mutated Yeast Tubulins Have Different GTP and P<sub>i</sub> Contents.* The ability to detect GTP and P<sub>i</sub> in yeast MTs allowed us to investigate the relationship between steady-state GTP hydrolysis rates, MT dynamics, and the E-site nucleotide content of the MTs. We previously generated mutations in yeast  $\beta$ -tubulin at sites thought to be involved in nucleotide binding and hydrolysis on the basis of sequence homology with nucleotide binding pockets of GTP and ATP binding proteins. The recently reported structure of the tubulin dimer (27) reveals that both the sites at which mutations were made,  $\beta$ -T107 and  $\beta$ -143, are within regions of the protein that interact with the bound nucleotide. MTs containing  $\beta$ -tubulin with a T107W mutation have a 15-fold faster steady-state GTP hydrolysis rate and increased dynamic instability compared to MTs assembled from wild-type tubulin (15) (Table 2). In contrast, MTs containing the T143G mutation in  $\beta$ -tubulin have a rate of GTP hydrolysis that is only 7% of that of wild-type derived MTs and also show suppressed dynamic instability (C. A. Dougherty, C. R. Sage, A. Davis, and K. W. Farrell, unpublished data). Thus, the in vitro properties of MTs assembled from the two mutated tubulins are an indication of a correlation between the GTPase activity of the MT and the level of dynamic instability. This suggested that, in accordance with the GTP or P<sub>i</sub> cap model, MTs containing T143G  $\beta$ -tubulin would have a larger amount of GTP<sub>E</sub> and/or P<sub>IE</sub> than wild-type derived MTs, while MTs containing T107W  $\beta$ -tubulin would have less GTP<sub>E</sub> and/or P<sub>IE</sub> than wild-type derived MTs.

The results showed that 7% of the T143G  $\beta$ -tubulin-containing MT is composed of tubulin-GTP<sub>E</sub>, a value that is about 3-fold higher than that found in the wild-type derived MT. A more pronounced difference between the two types of MTs was seen in the P<sub>IE</sub> content. MTs assembled from the mutated tubulin contained 8-fold more tubulin-(GDP-P<sub>i</sub>)<sub>E</sub> than wild-type derived MTs, suggesting that P<sub>i</sub> release, as well as GTP hydrolysis, is affected by the mutation. The T143G  $\beta$ -tubulin MT contained about 800 tubulin-GTP<sub>E</sub> and 4000 tubulin-(GDP-P<sub>i</sub>)<sub>E</sub> dimers, or 35% of the total dimers in the MT. The high levels of E-site GTP and P<sub>i</sub> in the T143G MT supports the proposed stabilizing role of tubulin-GTP<sub>E</sub> or tubulin-(GDP-P<sub>i</sub>)<sub>E</sub>. Moreover, the very high ratio of P<sub>IE</sub> to GTP<sub>E</sub> in the MTs containing T143G  $\beta$ -tubulin indicates that the threonine residue plays a role in P<sub>i</sub> release.

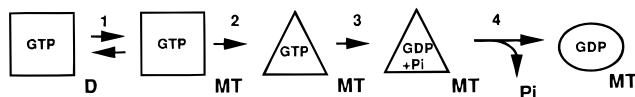


FIGURE 1: Model explaining the interrelationships among GTP hydrolysis, GTP<sub>E</sub> and P<sub>IE</sub> content, and MT stability. The model shows a tubulin-GTP<sub>E</sub> dimer adding to the end of a MT (reaction 1). Once incorporated into the end of the MT, the dimer undergoes a conformational change to form a stabilizing conformation (reaction 2). Hydrolysis (reaction 3), followed by release of P<sub>i</sub> (reaction 4), incorporates the dimer into the MT lattice in the GDP<sub>E</sub> form. In the case of the T107W dimer, the tubulin-(GDP-P<sub>i</sub>)<sub>E</sub> conformer is defective in that it cannot form the proper tubulin-GDP<sub>E</sub> conformation and dissociates rapidly from the end of the MT. In the case of the T143G dimer, P<sub>i</sub> release is very slow. Free dimers are designated with the subscript D. Dimers in the MT are designated with the subscript MT.

However, when we examined the E-site occupancy of the MTs containing the T107W  $\beta$ -tubulin, the more dynamic population of MTs, we did not find the expected lower GTP<sub>E</sub> and P<sub>i</sub> content when we compared them to wild-type MTs. In fact, the contents of the wild-type and T107W  $\beta$ -tubulin-containing MTs are similar (Table 2).

*Relationship among GTP and P<sub>i</sub> Content of MTs and MT Dynamics.* The results presented in this paper support the uncoupled mechanism of GTP hydrolysis upon dimer addition to a MT, and the existence of a tubulin-(GDP-P<sub>i</sub>)<sub>E</sub> intermediate in MT assembly. Although we cannot rule out the possibility that P<sub>i</sub> formation occurred during the sedimentation and workup of the MTs, it is not the likely explanation for the presence of P<sub>i</sub> in the MTs, as the greatest percentage of P<sub>i</sub> was found in the MTs containing the T143G  $\beta$ -tubulin and the lowest GTPase activity of the MTs studied. The data also suggest that the GTP or P<sub>i</sub> cap model alone may not be sufficient to explain the dynamic characteristics of yeast MTs. As predicted by the cap model, the GTP and P<sub>i</sub> content of stable MTs containing T143G  $\beta$ -tubulin was much higher than the content of MTs assembled from wild-type tubulin; however, we did not observe the expected lower content in the more dynamic MTs which contained T107W  $\beta$ -tubulin. Therefore, some other parameter in addition to the GTP<sub>E</sub> and P<sub>IE</sub> content of a MT must influence MT stability.

We propose that a structural element is also involved in controlling MT dynamics as described in the model shown in Figure 1. In this model, we envisage that a tubulin-GTP<sub>E</sub> dimer (square), newly added to the end of a MT (reaction 1), undergoes a conformational change to form an end-stabilizing tubulin-(GTP)<sub>E</sub> conformer (triangle, reaction 2). This conformational change may be influenced by surrounding tubulin dimers and lateral interactions with adjacent protofilaments (35) or may involve the conversion of a protofilament sheet to a closed MT (36). In wild-type MTs, GTP hydrolysis leads to the GDP-P<sub>i</sub> form (reaction 3) which undergoes another conformational change upon the release of P<sub>i</sub> to produce the GDP conformer incorporated into the lattice of the MT. We propose that in MTs containing T107W  $\beta$ -tubulin, the mutated dimers hydrolyze GTP effectively but after hydrolysis are defective in their ability to assume the GDP conformation and be incorporated into the lattice. The subunits are lost from the MTs at a rapid rate and release P<sub>i</sub>. Thus, the observed rate of GTP hydrolysis is much faster than that with wild-type MTs. One consequence of this is that elongation of MTs containing

T107W-mutated  $\beta$ -tubulin would be an inefficient process. From the steady-state GTP hydrolysis and elongation rates (Table 2 and C. A. Dougherty, C. R. Sage, A. Davis, and K. W. Farrell, unpublished data), we calculated that approximately 90 GTPs are hydrolyzed per elongation event, compared with only three to nine GTPs hydrolyzed per dimer added to wild-type derived MTs. By reducing the efficiency of subunit incorporation, MT dynamic instability could be increased. In the case of MTs containing T143G  $\beta$ -tubulin, reaction 4 ( $P_i$  release) is very slow. This results in MTs with a large content of GTP<sub>E</sub> and P<sub>IE</sub> that show little dynamic instability.

In conclusion, it appears that MT stability is modulated not only by the rate of GTP hydrolysis and  $P_i$  release but also by the rate at which tubulin subunits undergo conformational changes after adding to the MT end. The above results indicate there may be at least two tubulin conformational changes that are important for MT stability and that these changes are potential points for regulating MT dynamics in cells.

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