In this study, a two-state mechanochemical model is presented to describe the dynamic instability of microtubules (MTs) in cells. The MT switches between two states, the assembly and disassembly states. In assembly state, the growth of MTs includes two processes: free GTP-tubulin binding to the tip of protofilament (PF) and conformation change of PF, during which the first tubulin unit that curls outwards is rearranged onto the MT surface, using the energy released from the hydrolysis of GTP in the penultimate tubulin unit. In the disassembly state, the shortening of MTs also includes two processes, the release of GDP-tubulin from the tip of PF and the curling of one new tubulin unit out of the MT surface. Switches between these two states, which are usually called rescue and catastrophe, happen stochastically with external force-dependent rates. Using this two-state model with parameters obtained by fitting the recent experimental data, detailed properties of MT growth are obtained. I find that MT is mainly in the assembly state, its mean growth velocity increases with both the external force and the GTP-tubulin concentration, and an MT will shorten on average without an external force. To know more about the external force and GTP-tubulin concentration-dependent properties of MT growth, and for future experimental verification of this two-state model, 11 critical forces are defined and discussed numerically.

In eukaryotic cells, microtubules (MTs) serve as tracks for motor proteins (1–6), give shape to cells, and form rigid cores of organelles (7–10). They also play essential roles in the chromosome segregation (11–18). During cell division, MTs in spindles constantly grow and shorten by addition and loss of the enzyme tubulin (GTPase) from their tips. The attached duplicated chromosomes are stretched apart (through two kinetochores) from one another by the opposing forces (produced by MTs based on different spindles). Recently, many theoretical models have been designed to understand the roles of MTs during chromosome segregation (19–25). One essential point, to understand how MTs help chromosome segregation during cell division, is to know the mechanism of MT growth and shortening. In this study, inspired by the mechnochemical model for molecular motors (26) and the GTP-cap model and catch bonds model for MT (8, 27), a two-state mechnochemical model will be presented.

Electron microscopy indicates that an MT is composed of $n$ parallel protofilaments (PFs, usually $12 \leq n \leq 15$ and $n = 13$ is used in this work), which form a hollow cylinder (7, 8, 28). Each PF is a filament that made of head-to-tail associated $\alpha\beta$ heterodimers. At the tip of an MT, PFs curl outward from the MT cylinder surface. The tip might be in a shrinking GDP-cap state or growing GTP-cap state. In contrast to the tip in the shrinking GDP state, the growing GTP tip is fairly straight. In other words, in a GTP-cap state, the angle between the curled out segment of PFs and MT surface is less than that in the GDP state. In this work, I will only consider the growth and shortening of one single PF. I assume that each step of growth and shortening of one PF contributes $L$ (nm) to the growth and shortening of the whole MT. Intuitively, $L = L_1/n$, where $L_1$ is the length of one $\alpha\beta$ heterodimer. In the numerical calculations, I use $L = 8$ nm/13 $\approx 0.615$ nm (19, 29, 45).

My two-state mechnochemical model for PF growth and shortening is schematically depicted in Fig. 1a and mathematically described by a two-line Markov chain in Fig. 1b. In this model, a PF stochastically switches between two states: the assembly and disassembly states. During the assembly state, a PF grows through two processes, $1 \rightarrow 2$ (i), the free GTP-tubulin binding process. The rate constant is $k_1$, and depends on the GTP-tubulin concentration ($[tubulin]$), and $2 \rightarrow 1$ (ii), a PF conformation change process. During which, using energy released from GTP hydrolysis, the curled PF segment is straightened with one PF unit (i.e. one $\alpha\beta$ heterodimer) rearranged onto the MT surface, i.e. to parallel to the MT axis approximately. During the disassembly state, each step of a PF shortening also includes two processes, (i') $2' \leftarrow 1'$, disassociation of GDP-tubulin from a PF tip to the environment and (ii') $1' \leftarrow 2'$, one new PF unit curls out from the MT surface (during which phosphate is released from the tip of the tubulin unit simultaneously). The communication among curved PF units in the processes $2 \rightarrow 1$ and $1' \leftarrow 2'$ might be explained by similar methods as in the Monod-Wyman-Changeux model (30) or the Koshland-Nemethy-Filmer model (31).

The two-state model presented here can be regarded as a generalization of the one employed by Akiyoshi et al. (27) to explain their experimental data, which is depicted in Fig. 2a. (My corresponding generalized two-state model, including bead detachment from MT, is depicted in Fig. 2b, see “Modified Model according to Experiments, including Bead Detachment from MT” for detailed discussion). The reasons that I prefer to use this generalized model are as follows. The measurements in
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FIGURE 1. Schematic depiction of the two-state mecanochemical model of PF growth and shortening (a) and its corresponding two-line Markov chain (b). In assembly state, the growth of a PF is accomplished by two processes, one GTP-tubulin binds to the tip of PF (with GTP-tubulin concentration dependent rate \( k_1 \)), and one PF unit rearranges onto the MT surface (with external force dependent rate \( k_2 \)). The energy used in the second process comes from the GTP hydrolysis in the penultimate tubulin unit. One tubulin unit binding to the tip of PF is assumed to be equivalent to \( L \) (nm) growth of the whole MT (\( L = 0.615 \) nm is used in this work (19, 29)). Similarly, in the disassembly state, the shortening of PF also includes two processes, one PF unit detaches from the tip of PF and one new PF unit curls out the MT surface. In this depiction, the same described previously (25), a segment five dimers in length is assumed to curls out from the MT surface.

Refs. 32–34 indicate that the rate of catastrophe, i.e. transition from elongation to shortening, depends on the GTP-tubulin concentration of the solution. However, for the simple model depicted in Fig. 2a, the catastrophe rate \( k_c \) is independent of GTP-tubulin concentration. (It biochemically reasonable to assume that the elongation rate \( k_1 \) depends on GTP-tubulin concentration \( k_1 = k_1^0[tubulin] \), but there is no reason to write \( k_c \) as a function of [tubulin].) The “Results” show that for my generalized model, the catastrophe rate does change with [tubulin] because GTP-tubulin concentration will change the probabilities of a PF being in states 1 and 2 and consequently change the transition rate from assembly state to disassembly state. At the same time, for the simple model depicted in Fig. 2a, the distribution of the catastrophe time is an exponential. However, experimental measurements under a particular situation indicate that this distribution is clearly not an exponential (34). Certainly, from the parameter values listed in Table 3, one may find that the rates \( k_1^0 \) and \( k_3 \) are much larger than \( k_2^0 \) and \( k_5^0 \) so under low external force and high free GTP-tubulin concentration, the model depicted in Fig. 2a is a good approximation of my generalized model depicted in Fig. 2b. It should be pointed out that, although the model presented here looks more complex, there are only two more parameters than the one depicted in Fig. 2a.\(^3\)

\(^3\) To keep as few parameters as possible in my two-state model, I assume that the bead only can detach from an MT from substates 1 and 1'. The reasons are as follows: in assembly state, the experimental data in Refs. 27 and 43 (or see Fig. 3b) imply the growth speed of MT increases with external force. (Note that the definition of force direction in Ref. 27 is different from that in Ref. 43.) In this study, the force direction definition is the same as in Ref. 27, i.e., the force is positive if it points to the MT growth direction), so the corresponding force distribution factor \( \delta \) (see Equation 18) should be positive because the growth speed \( V_c = k_c L \) (see Equation 5). Consequently, the probability \( p_1/k_1^0 \) (that an MT in state 1 (see Equation 12) increases, but the probability \( p_2/k_2^0 \) (that an MT in state 2 decreases with external force, i.e. as the increase of external force, the assembly MT would more like to stay in state 1. Furthermore, from the experimental data in Ref. 27, one sees the detachment rate from the assembly state increases with external force. Therefore, the more reasonable choice is to assume that the bead can only detach from state 1 but not state 2. Through similar discussion, one also can see that it is more reasonable to assume that in disassembly state the bead can only detach from state 1. At the same time, the experimental data in Ref. 32 imply the catastrophe rate decreases with GTP-tubulin concentration [tubulin] (or see Fig. 4b). Because \( k_c = k_3^0[tubulin] \), the probability \( p_1 = k_3^0/k_3^0 + k_2^0 \) decreases with [tubulin], but the probability \( p_2 = k_2^0/k_3^0 + k_2^0 \) increases with [tubulin]. This is why I assume the catastrophe takes place at state 1.

The two-state mecanochemical model will be presented and theoretically studied under “Model” and then under “Results” based on the model parameters obtained by fitting the experimental data (mainly obtained in Ref. 27), and properties of MT growth and shortening are studied numerically. This includes external force and GTP-tubulin-concentration-dependent growth/shortening speeds (i), mean dwell times in assembly and disassembly state (ii), mean growth or shortening length before the bead, which is used in experiments to apply external force, and detachment from MT. To know more properties of MT dynamics, 11 “critical forces” (detailed definitions will be given in “Critical Forces of MT Growth”) are also numerically discussed under “Results,” followed by concluding remarks.

MODEL

As the schematic depiction in Fig. 1, a PF might be in two states: the assembly (growth) state and the disassembly (shortening) state. Each of the two states includes two substates, denoted by 1, 2 and 1', 2', respectively. Let \( p_1, p_2 \) be the probabilities that a PF is in assembly substates 1 and 2, respectively, and \( p_1, p_2 \) be the probabilities that the PF is in disassembly substates 1' and 2', then \( p_1, p_2, p_1, p_2 \) are governed by the following master equation,
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\[ V = (k_2p_2 - k_4p_2^l) L = (k_1 - k_2k_c/k_a)p_L \]  

(Eq. 3)

where \( L \) is the effective step size of MT growth corresponding to one step of PF growth, and \( V < 0 \) means the MT is shortening with speed \(-V\) on average.

Let \( p_1, p_2 \) be the probabilities that a PF is in substates 1 and 2, respectively, provided the PF is in assembly state, then \( p_1, p_2 \) satisfy that shown in Equation 4.

\[ dp_1/dt = k_3p_2 - k_1p_1 = -dp_2/dt \]  

(Eq. 4)

One can easily show that at steady state, the mean growth speed of a MT with a PF in assembly state is as shown in Equation 5.

\[ V_g = k_3p_2L = k_1k_2L/(k_1 + k_2) \]  

(Eq. 5)

Similarly, the mean shortening speed of a MT with a PF in disassembly state is shown in Equation 6.

\[ V_s = k_3k_4L/(k_3 + k_4) \]  

(Eq. 6)

Modified Model according to Experiments (including Bead Detachment from MT)

To know the model parameters \( k_i, i = 1, \ldots, 4 \) and \( k_c, k_r \), I need to fit the model to experimental data. In recent experiments, Akiyoshi et al. (27) attached a bead prepared with kinetochore to MTs, and constant tension was applied to the bead using a servo-controlled laser trap. In their experiments, the force-dependent mean growth and shortening speeds of MTs were measured. Meanwhile, the rates of rescue and catastrophe, the force-dependent mean lifetime, during which the bead remains attached to MT, and mean detachment rates of the bead during assembly and disassembly states are also measured. Therefore, to fit these experimental data, the model depicted in Fig. 1 should be modified to include the bead detachment processes (see Fig. 2b).

For the model depicted in Fig. 2b, the formulations of mean growth velocity \( V \) and mean growth and shortening speeds \( V_g \) and \( V_s \) are the same as in Equations 3, 5, and 6. In the following, I will derive an expression of the mean lifetime of the bead on MTs. Let \( T_{1r}, T_{2r}, T_{1r}', T_{2r}' \) be the mean first passage times (MFPTs) of a bead to detachment, when the initial substates are 1, 2, 1', 2', respectively, and then \( T_{1r}, T_{2r}, T_{1r}', T_{2r}' \) satisfy (37–39) as follows.

\[ T_1 = 1/(k_1 + k_a + k_c) + k_1/(k_1 + k_a + k_c)T_2 + k_1/(k_1 + k_a + k_c)T_1' \]

\[ T_1' = 1/(k_1 + k_a + k_c) + k_1/(k_1 + k_a + k_c)T_2' + k_1/(k_1 + k_a + k_c)T_1' \]

\[ T_2 = 1/k_d + T_1 + T_2' = 1/k_d + T_1' \]  

(Eq. 7)

Then, the mean lifetime is as shown in Equation 8.

\[ T = p_1T_1 + p_2T_2 + p_1T_1' + p_2T_2' \]  

(Eq. 8)

where \( p_1, p_2, p_1', \) and \( p_2' \) can be obtained by the formulation in Equation 2.
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In assembly state, let \( T_{a1} \) and \( T_{a2} \) be the MFPTs to detachment of the bead initially at substates 1 and 2 respectively, and then \( T_{a1} \) and \( T_{a2} \) satisfy Equation 9.

\[
T_{a1} = \frac{1}{k_1 + k_a} + \frac{k_1}{k_1 + k_a} T_{a2}, \quad T_{a2} = \frac{1}{k_2} + T_{a1}
\]  
(Eq. 9)

One can easily show the following (Equation 10).

\[
T_a = \frac{k_1 + k_2}{k_2 k_a}, \quad T_{a2} = \frac{k_1 + k_a + k_2}{k_2 k_a}
\]  
(Eq. 10)

Therefore, the MFPT to detachment of the bead in assembly state is as shown in Equation 11,

\[
T_a = \bar{p}_1 T_{a1} + \bar{p}_2 T_{a2}
\]  
(Eq. 11)

where the steady state probabilities of Equation 12 are obtained from Equation 4.

\[
\bar{p}_1 = \frac{k_1}{k_1 + k_2}, \quad \bar{p}_2 = \frac{k_2}{k_1 + k_2}
\]  
(Eq. 12)

The mean detachment rate during assembly can then be obtained as \( K_a = 1/T_a = 1/(\bar{p}_1 T_{a1} + \bar{p}_2 T_{a2}) \), i.e. as shown in Equation 13.

\[
K_a = \frac{(k_1 + k_2) k_a}{(k_1 + k_2)^2 + k_1 k_a}
\]  
(Eq. 13)

Similarly, the mean detachment rate during disassembly are as follows,

\[
K_d = 1/T_d = 1/(\bar{p}_1 T_{a1} + \bar{p}_2 T_{a2})
\]

\[
= \frac{(k_1 + k_2) k_a}{(k_1 + k_2)^2 + k_1 k_a}
\]  
(Eq. 14)

with steady state probabilities \( \bar{p}_1 = k_d/(k_1 + k_d) \), \( \bar{p}_2 = k_d/(k_1 + k_d) \).

Let \( T_{c1} \) and \( T_{c2} \) be the MFPTs of an MT to catastrophe from substates 1 and 2, respectively, and then \( T_{c1} \) and \( T_{c2} \) satisfy (see Fig. 2b).

\[
T_{c1} = \frac{1}{k_1 + k_c} + \frac{k_1}{k_1 + k_c} T_{c2}, \quad T_{c2} = \frac{1}{k_2} + T_{c1}
\]  
(Eq. 15)

The mean rate of catastrophe is then \( K_c = 1/T_c = 1/(\bar{p}_1 T_{c1} + \bar{p}_2 T_{c2}) \). The explicit expression can be obtained by replacing \( k_a \) with \( k_c \) in Equation 13.

\[
K_c = \frac{(k_1 + k_2) k_c}{(k_1 + k_2)^2 + k_1 k_c}
\]  
(Eq. 16)

Similarly, the mean rate of rescue is as follows.

\[
K_r = \frac{(k_1 + k_2) k_r}{(k_1 + k_2)^2 + k_1 k_r}
\]  
(Eq. 17)

**Force and GTP-Tubulin Concentration Dependence of Transition Rates**

From the experimental data in Ref. 27 (or see Fig. 3), one sees that some transition rates in my model should depend on the external force. Because the processes \( 1 \rightarrow 2 \) and \( 2' \leftrightarrow 1' \) are accomplished by binding a tubulin unit to, and releasing tubulin unit from, the tip of a PF (see Figs. 1 and 2b), I assume that \( k_1 \) and \( k_2 \) are force-independent. Similar to the methods demonstrated in the models of molecular motors (26, 40) and models for adhesive of cells to cells (41), the external force \( F \) dependence of rates \( k_3, k_4, k_r, k_c, k_d \) are assumed to be as follows.

\[
k_j = k_0 e^{F \delta_j / k_B T}, \quad l = 2, 4, a, d, r, c
\]  
(Eq. 18)

Henceforth, the external force \( F \) is positive if it points in the direction of MT growth.

Meanwhile, the rate \( k_1 \) should depend on the concentration of free GTP-tubulin in solution. Similar to the method in Ref. 26, I simply assume \( k_1 = k_0^{[\text{tubulin}]} \).

**Critical Forces of MT Growth**

For a better understanding of the external force-dependent properties of MTs and the experimental verification of the two-state model, in the following, I will define altogether 11 critical forces. Corresponding numerical results will be presented in the next section.

1. **Critical Force \( F_{c1} \)**—This is defined by \( V_a(F_{c1}) = V_s(F_{c1}) \), i.e. at \( F_{c1} \), the average speeds of assembly and disassembly are the same. From Equations 5 and 6, one sees that \( F_{c1} \) satisfies the following.

\[
k_1 k_2(F_{c1}) (k_3 + k_4(F_{c1})) = k_3 k_4(F_{c1}) (k_1 + k_2(F_{c1}))
\]  
(Eq. 19)

2. **Critical Force \( F_{c2} \)**—At this critical value of force, the mean velocity of MT growth is zero. Formulation (3) gives \( k_1 k_2(F_{c2}) = k_3 k_4(F_{c2}) \), i.e. the following.

\[
F_{c2} = \frac{k_3 T}{(\delta_r - \delta_c)} ln \frac{k_3 k_0^2}{k_4 k_0^2}
\]

\[
= \frac{k_3 T}{(\delta_r - \delta_c)} ln \frac{k_3 k_0^2}{k_4 k_0^2} [\text{tubulin}]
\]  
(Eq. 20)

3. **Critical Force \( F_{c3} \)**—At this critical value of force, \( p_1 + p_2 = \rho_1 + \rho_2 \), i.e. the probabilities that MTs in assembly and disassembly states are the same. From expressions in Equation 2, one easily sees \( F_{c3} \) that satisfies the following.

\[
k_1(F_{c3}) k_2(F_{c3}) (k_3 + k_4(F_{c3})) = k_3(F_{c3}) k_4(F_{c3}) (k_1 + k_2(F_{c3}))
\]  
(Eq. 21)

4. **Critical Force \( F_{c4} \)**—At this critical value of force, the detachment rates during assembly and disassembly states are the same. In view of Equations 13 and 14, one can get \( F_{c4} \) by \( K_a(F_{c4}) = K_d(F_{c4}) \).

5. **Critical Force \( F_{c5} \)**—At this critical value of force, the mean dwell times of an MT in assembly and disassembly states are the same.

Let \( T_{g1} \) and \( T_{g2} \) be the MFPTs of the bead to detachment or catastrophe of the MT with initial substates 1 and 2, respectively. Then, \( T_{g1}, T_{g2} \) satisfy (see Fig. 2b and Refs. 37 and 38).
The critical force $F_{c,5}$ can then be obtained by $T_g(F_{c,5}) = T_g(F_{c,6})$.

6. Critical Force $F_{c,6}$—At this critical value of force, the mean lifetime of the bead on MT attains its maximum, i.e. $T(F_{c,6}) = \max_g T(F)$ with $T$ given by formulation 8.

7. Critical Force $F_{c,7}$—At this critical value of force, the mean growth length of MT attains its maximum. The mean growth length of an MT can be obtained by $l_+ = V_T T$, with $V, T$ satisfy formulations 3 and 8, respectively.

8. Critical Force $F_{c,8}$—At this critical value of force, the mean shortening length of MT attains its maximum. The mean shortening length of an MT can be obtained by $l_- = -V_T T$, with $V, T$ satisfy formulations 3 and 8, respectively.

9. Critical Force $F_{c,9}$—The rates of catastrophe and rescue are the same, i.e. $K_c(F_{c,9}) = K_c(F_{c,9})$ (see Equations 16 and 17). Under critical force $F_{c,9}$, the average switch time between growth and shortening, i.e. $1/K_c$ and $1/K_c$, are the same. This indicates that the mean duration for each growth and each shortening period is the same.

10. Critical Force $F_{c,10}$—At this critical value of force, $V/T \mid_{g} = V/T \mid_{g}$ Here, $V/T \mid_{g} = l_-$ the mean growth length before bead detachment or catastrophe, and $V/T \mid_{g} = l_-$ is the mean shortening length before bead detachment or rescue. The formulations of $V/T \mid_{g}$, $T \mid_{g}$, and $T \mid_{g}$ are in Equations 5 and 6 and 24 and 25.

11. Critical Force $F_{c,11}$—At this critical value of force, $V/K_c = V/K_c$. Here, $V/K_c = l_-$ the mean growth length before catastrophe, and $V/K_c = l_-$ is the mean shortening length before rescue. The formulations of $K_c, K_c$ are in Equations 16 and 17.

It needs to be clarified that the definitions for $F_{c,1}, F_{c,2}, F_{c,3}, F_{c,9}, F_{c,11}$ are unrelated to bead detachment, but the definitions for $F_{c,4}, F_{c,5}, F_{c,6}, F_{c,7}, F_{c,8}, F_{c,10}$ are related. Therefore, the values of $F_{c,1}, F_{c,2}, F_{c,3}, F_{c,9}, F_{c,11}$ obtained in this theoretical study can be verified by various experimental methods as described (13, 32–34, 42–44), but the values of $F_{c,4}, F_{c,5}, F_{c,6}, F_{c,7}, F_{c,8}, F_{c,10}$ can only be verified by a similar experimental method to that in Ref. 27. For the sake of convenience, and based on the above definitions and numerical calculations under “Results” (see Figs. 7 and 8),

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**TABLE 1**

Basic properties of the critical forces as defined under “Critical Forces of MT Growth”

See also Figs. 7 and 8.

| $F < F_{c,1}$ | $F = F_{c,1}$ | $F > F_{c,1}$ |
|--------------|--------------|--------------|
| $V^* < V^*$ | $V^* = V^*$ | $V^* > V^*$ |
| $V < V^*$ | $V = V^*$ | $V > V^*$ |
| $p_1 + p_2 < p_1 + p_2$ | $p_1 + p_2 = p_1 + p_2$ | $p_1 + p_2 > p_1 + p_2$ |
| $K_c < K_c$ | $K_d = K_d$ | $K_c < K_c$ |
| $T_g > T_g$ | $T_g = T_g$ | $T_g < T_g$ |
| $l_+ < l_+$ | $l_+ = l_+$ | $l_+ < l_+$ |
| $l_- < l_-$ | $l_- = l_-$ | $l_- < l_-$ |
| $K_c = K_c$ | $K_d = K_d$ | $K_c < K_c$ |
| $l_+ = l_+$ | $l_+ = l_+$ | $l_+ > l_+$ |
| $l_- = l_-$ | $l_- = l_-$ | $l_- > l_-$ |
| $i_{F_{c,4}}$ | $i_{F_{c,5}}$ | $i_{F_{c,6}}$ |

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**TABLE 2**

The main symbols and their expressions (or definitions) used in this work

| Symbol | Biophysical meaning | Definitions |
|--------|---------------------|-------------|
| $V$    | Mean velocity of MTs | Eq. 3       |
| $V^*$  | Growth speed of MTs  | Eq. 5       |
| $V^*$  | Shortening speed of MTs | Eq. 6    |
| $T$    | Mean lifetime of bead | Eq. 8      |
| $K_c$  | Bead detachment rate (assembly) | Eq. 13 |
| $K_d$  | Bead detachment rate (disassembly) | Eq. 14 |
| $T_g$  | Time to detachment (assembly) | 1/$K_c$ |
| $T_g$  | Time to detachment (disassembly) | 1/$K_d$ |
| $K_c$  | Catastrophe rate | Eq. 16 |
| $K_d$  | Rescue rate | Eq. 17 |
| $p_{1,2}$ | Probability | Eq. 2 |
| $T_g$  | Mean growth time | Eq. 24 |
| $l_+ $ | Mean shortening time | Eq. 25 |
| $l_- $ | Time to detachment (assembly) | 1/$K_c$ |
| $l_- $ | Time to detachment (disassembly) | 1/$K_d$ |
| $l_+ $ | Time to detachment (assembly) | 1/$K_c$ |
| $l_- $ | Time to detachment (disassembly) | 1/$K_d$ |
| $k_{1,2}$ | Rate constants (Fig. 2b) | Eq. 18 |

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**RESULTS**

To discuss the properties of MT growth and shortening, the model parameters, i.e. $K_{0,1,2,3}$ and $K_{1,2,3}$, should be obtained first. By fitting the expressions of $V_{g} V_{s} T_{s} K_{0,1,2,3} K_{0,1,2,3}$, which are given in Equations 5, 6, 8, 13, 14, 16, and 17, respectively, to the experimental data (mainly measured in Ref. 27), these parameter values are obtained (Eq. 3 and Table 3; the fitting methods are discussed. The data corresponding to zero external force in Fig. 3, $b$ and $c$ (two black dots on vertical axis) are obtained by fitting the corresponding measurements in Ref. 32 with a constant (see the two lines in Fig. 4, $a$ and $b$) because, as implied by our model, the rates of MT shortening and rescue are independent of GTP-tubulin concentration. All the following calculations will be based on the parameters listed in Table 3. The curve in Fig. 4b is the theoretical prediction of GTP-tubulin concentration dependent basic properties of the 11 critical forces, $F_{c,9}$, are listed in Table 1. The main symbols used in this study are listed in Table 2.

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4 In my fitting, I first obtained the parameters $K_{1,2,3}$, $K_{1,2,3}$, $K_{1,2,3}$, and $K_{1,2,3}$ by fitting formulations 5 and 6 to the experimental data of growth and shortening speeds respectively (see Fig. 3b), and then obtained $K_{1,2,3}$, $K_{1,2,3}$, and $K_{1,2,3}$ by fitting formulations 16 and 17 to the catastrophe and rescue rates (see Fig. 3c), $K_{1,2,3}$ and $K_{1,2,3}$ were determined by fitting formulations 13 and 14 to the corresponding data plotted in Fig. 3d. Finally, all of the parameters were slightly adjusted according to the experimental data about the mean lifetime of bead attachment to MT (see formulation 8 and Fig. 3d). All of the fitting was done by the nonlinear least square program *lsqnonlin* in Matlab. In each fitting, I randomly chose 1000 initial values of the parameters and adopted the parameter values that fit the experimental data best.
catastrophe rate $k_c$ by formulation (16). Compared with the experimental data measured in Refs. 32 and 34, these predictions look satisfactory.\textsuperscript{5}

\textsuperscript{5} The parameter values listed in Table 3 did not fit well to the GTP-tubulin concentration-dependent growth speed of MTs obtained in Refs. 32 and 34, because the corresponding data in Refs. 32 and 34 are much different from that in Ref. 27. Without external forces, but under similar GTP and tubulin concentrations, the growth speed of an MT measured in Ref. 32 is $\sim 43$ nm/s and $\sim 20$ nm/s in Ref. 34, but it is only $\sim 5$ nm/s in Ref. 27. In this study, I obtained the parameter values mainly based on the data measured in Ref. 27. One reason is that, from my model, if the GTP-tubulin concentration is non-zero, the growth speed of an MT will always be positive (see formulation 5), $V_g > 0$ if $k_2 > 0$. However, this might not be true for the data in Refs. 32 and 34. So, it might be impossible to get believable fitting parameters for formulation 5 from data in Refs. 32 and 34. Moreover, because the data in Refs. 32 and 34 cannot be described by a formulation like 5. In Ref. 43, the velocity-force data are measured under a tubulin concentration of $25 \mu M$. However, the zero force growth speed obtained there is $\sim 20$ nm/s, which is also much larger than that obtained in Ref. 27. Consequently, the theoretical results based on the parameter values listed in Table 3 also do not fit well to their data. One can verify that the velocity-force data in Ref. 43 can be well described by formulation 5 but with parameters $k_0 = 2.99 s^{-1} \mu M^{-1}$, $k_1 = 53.17 s^{-1}$ and $\delta_c = 4.13$. The difference among these experimental data might be due to differences of experimental techniques, methods, materials, or even temperature.

From Fig. 5a, one can see that the MT is mainly in assembly state. Further calculations indicate that the ratio of probabilities in assembly state to disassembly state, i.e. $(p_1 + p_2)/(p_1 + p_3)$, increases exponentially with external force $F$ (Fig. 6a). In experiments of Akiyoshi et al. (27), the external force $F$ is applied to the MT through a bead attached to its growing tip. Fig. 5b indicates that for $F \geq 16$ pN, the mean dwell time of the MT in assembly state before bead detachment is larger than that in disassembly state. Although the MT is mainly in assembly state, its mean growth velocity is negative under a small external force (Fig. 5c) because for such cases, the shortening speed is much larger than the growth speed (see Fig. 3b). But Fig. 5c indicates the mean velocity of MT growth always increases with external force. Similar to the mean growth velocity, the mean growth length of an MT, before bead detachment, might be negative (i.e. MT shortens its length in long time average, see Fig. 5d), although the MT spends most of its time in assembly state (Fig. 5b). Similar to the mean lifetime (Fig. 3d), the mean growth length of an MT also has a global maximum for external force (Fig. 5d). As mentioned under the “Introduction,” the chromosome segregation is accomplished by the tensile force generated during the MT disassembly. Fig. 5 tells us the critical force of one MT disassembly is $\sim 1.2$ pN under the present experimental environment (27). In Fig. 6b, the mean growth length of an MT before bead detachment is $\sim 15$ nm. This means that the MT spends most of its time in assembly state (Fig. 5b), although the MT spends most of its time in assembly state (Fig. 5b).

### TABLE 3

| Parameter | Value |
|-----------|-------|
| $k_1$ | $5.8 \times 10^2 s^{-1} \mu M^{-1}$ |
| $k_2$ | $1.0 \times 10^2 s^{-1}$ |
| $k_3$ | $7.5 \times 10^2 s^{-1}$ |
| $k_4$ | $41.9 s^{-1}$ |
| $k_5$ | $9.3 s^{-1}$ |
| $k_6$ | $57.1 s^{-1}$ |
| $k_7$ | $1.8 \times 10^4 s^{-1}$ |
| $k_8$ | $1.4 \times 10^4 s^{-1}$ |
| $\delta_a$ | 0.68 |
| $\delta_b$ | 3.71 |
| $\delta_c$ | 1.77 |
| $\delta_d$ | -2.88 |
| $\delta_e$ | -2.96 |
| $\delta_f$ | 0.33 |

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Dynamics of Microtubules

**FIGURE 3.** Theoretical results of the two-state model (see Fig. 2b) and experimental data obtained by Akiyoshi et al. (27). The detachment rates are obtained by formulations 13 and 14, the speeds are obtained by formulations 5 and 6, the switch rates are obtained by formulations 16 and 17, and the lifetime is obtained by formulation 8. The model parameters used in the theoretical calculations are listed in Table 3. The two black dots on the vertical axis of b and c are obtained by averaging the data in Ref. 32 (see the lines in Fig. 4, a and b).
growth length $l_g$, $l_s$ and mean shortening length $l_c$, $l'_s$ which are given in the definitions of critical force $F_{c10}$, $F_{c31}$ are also plotted as functions of external force. One can easily see that $l_g \leq l'_g$ and $l_s \leq l'_s$ because the mean dwell time of an MT in assembly state $T_g \leq 1/K_c$ and mean dwell time in disassembly state $T_s \leq 1/K_c$. But for a large external force, $l_c \approx l'_c$ because, for such cases, MTs leave disassembly state mainly by rescue.

As the assembly of an MT depends on the free GTP-tubulin concentration (in my model, the simple relation $k_1 = K_{11}^c$ [tubulin] is used, and the disassembly process is assumed to be independent of the GTP-tubulin concentration, which can be verified by the data in Ref. 32; see Fig. 4), the 11 critical forces defined in the previous section also depend on GTP-tubulin concentration. For convenience, in my calculations (results are plotted in Figs. 7 and 8), [tubulin] = 1 indicates that the free GTP-tubulin concentration is the same as the one used by Akiyoshi et al. (27). From Figs. 7 and 8, one can see that the critical forces $F_{ci}$ for $i = 4, 5, 6$ increase, but others decrease with [tubulin]. For high GTP-tubulin concentration, $[I]F_{c2} \approx F_{c11}$ and $F_{c3} \approx F_{c9}$ because for $K_c \ll k_k$, equations $V_c/K_c = V_c/K_c$ and $K_c = K_c$ can be well approximated by $k_3K_c = k_3k_c$ and $k_kK_c = k_k(k_1 + k_2) = k_k(k_1 + k_2)$. Because the force distribution factors $\delta_2 > 0, \delta_3 < 0$ (see Table 3), from Equations 5 and 6, one can easily show that the growth speed $V_g$ increases, but the shortening

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**FIGURE 4.** GTP-tubulin concentration dependent data measured by Walker et al. (32), a, shortening speed of an MT and their average value, $b$, switch rates of an MT between assembly and disassembly where the curve is obtained by my theoretical model using the parameter listed in Table 3 (see formulation 16 with $k_1 = K^c_{11}$), the solid squares are experimental data from Ref. 34.

**FIGURE 5.** Properties of MT growth and shortening obtained by the two-state model (see Fig. 2b) with model parameters listed in Table 3. a, under external force, the MT is mainly in assembly state, both the probabilities $p_1$ and $p_2$ of an MT in assembly substates 1 and 2 increase with force but with $p_2 \gg p_1$. During disassembly state, the MT is mainly in substate 2, $p_2 > p_1$. Here, $p_1, p_2, \rho_1, \rho_2$ are calculated by formulations in 2, $b$, the mean time $T_g$ of MT in assembly state is always larger than that in disassembly state (denoted by $T_s$) for external forces < 16 pN (see formulations 24 and 25 for $T_g$ and $T_s$). Similar to the mean lifetime of bead attachment to the MT (see Fig. 3d), both $T_g$ and $T_s$ increase first and then decrease with the external force. c, the mean velocity of MT growth (see formulation 3 for $V$ and formulation 8 for $T$).
speed $V_g$ decreases with external force $F$. Therefore, $V_g < V_s$, if $F < F_{c1}$ (see Table 1). Equations 5 and 6 also indicate that the growth speed $V_g$ increases with [tubulin], but the shortening speed $V_s$ is independent of [tubulin]. Therefore, the critical force $F_{c1}$ decreases with GTP-tubulin concentration [tubulin] (Fig. 8a). But for high [tubulin], critical force $F_{c1}$ is almost a constant (see Fig. 7a) because, for saturated concentration, the growth speed $V_g$ tends to a constant (see Equation 5 and Fig. 9a). The decrease of critical force $F_{c1}$ with [tubulin] can be seen easily from expression (see Fig. 7b) (20). The decrease of critical forces $F_{c1}$, $F_{c2}$ implies that low GTP-tubulin concentration might be helpful to chromosome segregation. From expressions in Ref. 2, one can verify $(p_1 + p_2)/(p_1 + p_2) = k_1k_2/k_1k_2 + k_2$. So $(p_1 + p_2)/(p_1 + p_2)$ increases linearly with [tubulin] (Fig. 10a). At the same time, $\delta_1 + \delta_2 > 0$, $\delta_1 + \delta_2 < 0$, $\delta_1 < 0$ (see Table 3) imply $(p_1 + p_2)/(p_1 + p_2)$ also increases with external force $F$ (see Fig. 6a). Therefore, the critical force $F_{c1}$ decreases with [tubulin] (see Fig. 7c).

Because the detachment rate $K_a$ increases and detachment rate $K_d$ decreases, with the external force $F$ (see Fig. 3a), and $K_a$ increases with but $K_d$ is independent of [tubulin] (see Equations 13 and 14), the critical force $F_{c5}$ increases with [tubulin] (see Fig. 7a). The increase of critical force $F_{c5}$ indicates that MTs will spend more time in assembly state at high GTP-tubulin concentration (see Table 1 and Figs. 7a and 5b). The increase of critical force $F_{c6}$ (see Fig. 8d) implies that the peak of the lifetime-force curve as plotted in Fig. 3d will move toward the right as the increase of [tubulin], but with an upper bound at around 4 pN (see Figs. 7d and 9d). Similarly, the decrease of critical force $F_{c7}$ (see Fig. 7d) means the peak of the mean growth length-force curve will move leftwards with the increase of [tubulin], and with lower bound at ~4.44 pN. Finally, critical forces $F_{c8}$, $F_{c9}$, $F_{c10}$, $F_{c11}$ all decrease with [tubulin]. I should mention that in Ref. 27 only experimental data for positive force cases are measured, and similar experimental methods as used in

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**FIGURE 6.** a, the ratio of the probability $p_1 + p_2$, that an MT is in assembly state to the probability $p_1 + p_2$, that an MT is in disassembly state increases exponentially with the external force, and under a positive external force, the MT mainly stays in the assembly state, although the assembly speed might be much lower compared with the disassembly speed (see Fig. 3b). b, the mean growth length $l_g$, $l_g^*$, and shortening length $l_s$, $l_s^*$ of an MT in one assembly/disassembly period. The difference between $l_g$, $l_s$ and $l_g^*$, $l_s^*$ is that in the calculation of $l_g$, $l_s$, the bead attached to the tip of MT, through which the external force is applied to the MT, is assumed to keep attached to the MT, or the external force is just applied by other methods (32–34, 43), so the MT can only leave the assembly state by catastrophe and leave the disassembly state by rescue.

**FIGURE 7.** Critical forces as defined under “Critical Forces of MT Growth,” in which [tubulin] means the concentration of GTP-tubulin is the same as the one used in the experiments of Akiyoshi et al. (27). To better understand the curves for $F_{c1}$, see Table 1.
Refs. 34 and 43 might be employed to apply negative force to MTs. At the same time, the mechanism of MT growth and shortening under negative external force cases might be completely different from that under positive external force cases, so for the results of critical forces plotted in Fig. 7, which have negative values, experimental verification should be first done before further analysis.
To better understand the GTP-tubulin concentration-dependent properties of MT assembly and disassembly, more figures are plotted in Figs. 9 and 10. One can see that the mean lifetime $T_1$, ratio $p_1/p_2$, and mean growth length $l_g$ all increase linearly with [tubulin]. From the corresponding formulations, one can easily see that the mean shortening speed $V_s$ and mean shortening length $l_s$ are all independent of [tubulin].

The mean velocity $V$ and mean growth speed $V_g$ also increase with [tubulin] but tend to an external force $F$-dependent limit. For such cases, the MT stays mainly in substate 2, i.e. $p_2 = 1$, see Fig. 5a. The mean growth length $V_T$ does not change monotonically with external force (see Figs. 5a and 9c) but increases with [tubulin] for high GTP-tubulin concentration cases.

CONCLUDING REMARKS

In this work, a two-state mechanochemical model of MT growth and shortening is presented. In assembly (growth) state, one GTP-tubulin will attach to the growing tip of the PF first and then, after the hydrolysis of GTP in the penultimate PF unit, the curved PF segment is slightly straightened with one new PF unit lying in the MT cylinder surface. In the disassembly (shortening) state, one tubulin unit will detach from the tip of PF, and then the GDP (or GDP-Pi) capped tip segment of PF will be further curved with one new tubulin unit out of the MT surface. The phosphate is assumed to be released simultaneously. The PF can switch between the assembly and disassembly states with external force dependent rates stochastically. Each assembly or disassembly process contributes to one step of growth or shortening of MT with step size $L = 0.615$ nm. This model can fit the recent experimental data measured by Akiyoshi et al. (27) as well.

From this model, interesting properties of MT growth and shortening are found. Under large external force or high GTP-tubulin concentration, the MT is mainly in assembly state. The mean lifetime of bead attachment to the MT and mean growth length during this period (in experiments, the external force is applied to MT through a bead attached to the growing tip of MT) increase first and then decrease with the external force. Roughly speaking, they all increase with the GTP-tubulin concentration; the growth speed of an MT increases with GTP-tubulin concentration but has an external force-dependent limit. For the sake of experimental verification, altogether 11 critical forces are defined, including the force under which the mean lifetime or mean growth length reach its maximum, the mean assembly speed is equal to the mean disassembly speed, the probabilities of MT in assembly and disassembly states are equal to each other, the detachment rates of bead during assembly and disassembly states are the same, the mean dwell times in assembly and disassembly states are the same, the mean growth velocity of MT vanishes, and etc. Almost all of the above critical forces decrease with the GTP-tubulin concentration, as high GTP-tubulin concentration is favorable for MT growth, and under low GTP-tubulin concentration, an MT will shortens its length in average. Roughly speaking, GTP-tubulin and external forces are helpful to MT assembly, but there exists optimal values of the external forces for the mean lifetime of the bead on MT and the mean growth length of MT.

Acknowledgments—I thank Michael E. Fisher (Institute for Physical Science and Technology, University of Maryland) for initial introduction and inspiration of the present study. I also appreciate the critical comments and valuable suggestions of the reviewers, due to which many changes have been done.
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