

Classification of microtubule histories

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A microtubule of a given length undergoes all possible scenarios of transitions between growing and shrinking phases, so-called microtubule dynamic instability. In this paper we utilize a minimal two-state model proposed by Hill [Proc. Natl. Acad. Sci. USA **81**, 6728 (1984)] that is equivalent to a two-state random walk. Using a technique for classifying discrete random walk configurations by introducing a counting variable in evolution equations, we have derived expressions for probability densities (which contain information about all transition histories) of phase transitions before the complete disappearance of a microtubule. As a result, the mean lifetime of a microtubule turns out to be equal to the total lifetime of growing and shrinking phases times the average number of transitions. An attractive feature of this simple model is that elementary formulas relating statistical averages to rate parameters are obtained.

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I. INTRODUCTION

Microtubules (MTs) are rigid, hollow, 25-nm-diam cylindrical polymers of tubulin $\alpha\beta$ heterodimers. Each dimer, which is roughly 5 nm in diameter and 8 nm long, is a 100-kDa tightly bound elongated complex formed from closely related α - and β -tubulin subunits. The cylindrical wall of the MT is composed typically of 13 protofilaments of tubulin dimers arranged head to tail in each filament and with all filaments having the same polarity. Thus one end of a MT has a crown of α -tubulin subunits while the other end has a crown of β -tubulin subunits. A 13-protofilament MT contains about 1625 tubulin subunits per micrometer.

On the basis of an *in vitro* study of a population of MTs at steady state, Mitchison and Kirschner [1] proposed that MT ends have the ability to alternate between persistent phases of growth and shortening. They termed this unusual behavior “dynamic instability.” Subsequently, the phenomenon of dynamic instability was observed directly *in vitro*, in real time, using video-enhanced microscopy [2–4]. In the latter studies it was observed that under steady-state conditions two populations of MTs coexist: a majority population, which elongates at a well-defined rate, and a minority population, which shortens at a significantly faster rate. Transitions from the growing phase to the shrinking phase are termed “catastrophes” and transitions from the shrinking phase to the growing phase are termed “rescues.” The transitions between the two phases occur rarely, but in a random or stochastic manner. Thus there are four parameters that characterize the observed behavior: the elongation and shrinking rates v_+ and v_- , respectively, and the catastrophe and rescue rates f_+ and f_- , respectively. Since MTs have a polar character, the rates observed at opposite ends are in

general different. Typical elongation and shortening rates are observed to be 2 and 27 $\mu\text{m}/\text{min}$, respectively [4,5], at a guanosine triphosphate (GTP) tubulin concentration of 10 μM . These rates of length change correspond to the gain (loss) of 54 (730) dimers/sec, assuming 13-protofilament MTs and a tubulin dimer length of 8 nm. Typical catastrophe and rescue rates are observed to be 0.2 and 2.0 min^{-1} .

In all the above experiments, MTs exhibit dynamic instability in the presence of tubulin dimers to which GTP is bound. Mitchison and Kirschner [1] suggested that after the GTP tubulin is incorporated into an elongating MT, the GTP is subsequently hydrolyzed to guanosine diphosphate (GDP). Thus the main body of the MT consists of a GDP-tubulin lattice with a stabilizing cap (or tip) of GTP tubulin. If the hydrolysis reaction overtakes the addition of GTP tubulin, the stabilizing cap is lost and a catastrophe occurs, i.e., the MT enters a shrinking phase. The exact nature and extent of a GTP-tubulin cap still remains a subject of intense study. See the recent paper by Flyvbjerg *et al.* [6], who suggest a different model of the competition between the addition of GTP tubulin at the growing tip and the hydrolysis of GTP tubulin in the MT body. This paper [6] also contains references to numerous experimental investigations of the dynamic instability of MTs. Other more general studies of the cap model have involved Monte Carlo simulations of 13-protofilament MTs, by Chen and Hill [7] and later by Bayley *et al.* [8]. Taking a different tack (using probabilistic and spectral analysis), Odde *et al.* [9] have analyzed the experimentally observed distribution of elongation times and found small departures from the exponential distribution of the two-state model. Although the approach developed in this paper is applicable to a multistate model, here we investigate MT histories for the two-state model because simple exact formulas relating statistical averages to rate parameters of the two-state model can be obtained. These results are a useful first approximation for more elaborate multistate models and have intrinsic interest in their own right. In addition, *in vitro* studies, which bear on the cap model, are summarized in reviews [10–12]. In most of these studies the rates of elon-

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gation and shortening as well as the lifetimes of the elongating and shortening phases were measured as a function of GTP-tubulin concentration. The elongation rate is expected to be proportional to the GTP-tubulin concentration. There is a critical value C_{cr} of the GTP-tubulin concentration [4,5] such that for higher concentrations the average length of a population of MTs increases proportional to the time, while for concentrations less than C_{cr} the average length decreases with time. If nucleating sites for MT assembly are present in the latter case, a stable population of MTs will be maintained as long as the concentration of GTP tubulin is maintained.

Two related minimal two-state models of dynamic instability that depend upon the four parameters v_+ , v_- , f_+ , and f_- have been proposed and studied extensively. One, introduced by Hill [13], is an example of a continuous time two-state biased random walk in a discrete length space [14] and the other, introduced by Dogterom and Leibler [15], is the continuous length space analog of the Hill model. In these models, $C < C_{cr}$ ($C > C_{cr}$) is equivalent, in terms of the four rate parameters, to $v_+f_- < v_-f_+$ ($v_+f_- > v_-f_+$). For the case $v_+f_- < v_-f_+$, Hill [13] determined that the steady-state fractional population of MTs in either of the two phases is a decreasing exponential function of MT length. Verde *et al.* [16] reached the same conclusion for the Hill model and Dogterom and Leibler obtained a similar result [15]. Rubin [17] for the Hill model and Bicout [18] for the Dogterom-Leibler model calculated the mean first-passage time to zero length of either an elongating or a shrinking MT. The results for the two different models are, for all practical purposes, identical.

In either model, the governing equations correspond to a biased two-state random walk in which the drift decreases (increases) the length [14,19] for $v_+f_- < v_-f_+$ ($v_+f_- > v_-f_+$) [18]. Our principal interest in this paper is in calculating for the two-state model the average number of transitions for a MT of given length and phase before the MT reaches zero length for the first time. In addition, we calculate from the probability density of reaching zero length for the first time at time t the probability density of MTs that have made n transitions into the shrinking state at the time of their disappearance. We will use the Dogterom-Leibler model in our calculations and merely note that the results do not differ significantly from those obtained for the Hill model. In Sec. II we outline a method for counting transitions between states in the Dogterom-Leibler model. The method, which generalizes to the continuum a technique for classifying discrete lattice random walk configurations according to the number of visits of each configuration to a selected set of lattice sites [20,21], consists in introducing a counting variable in the evolution equations. Then we calculate the lifetime distribution of a MT of given length and phase as a function of the counting variable. These distributions are then generating functions in the counting variable that contain information about all transition histories of a MT. In Sec. III we exploit these generating functions to calculate the probability densities of MT phase changes before complete disassembly, the average number of phase changes, and fluctuations about the average. The relation between these quantities and the mean lifetime of a MT before disappearance is established in Sec. IV. Finally, a summary of results is given in Sec. V.

II. LIFETIME DISTRIBUTIONS OF A MICROTUBULE

Let $P_+(x,t)$ and $P_-(x,t)$ be the probability density distributions of finding at time t a MT with the length x in state $+$ and $-$, respectively. These distributions satisfy the differential equations

$$\frac{\partial P_+}{\partial t} = -v_+ \frac{\partial P_+}{\partial x} - f_+ P_+ + f_- P_-, \quad (2.1a)$$

$$\frac{\partial P_-}{\partial t} = v_- \frac{\partial P_-}{\partial x} + y f_+ P_+ - f_- P_-, \quad (2.1b)$$

with the initial condition

$$P_-(x,0) = (1 - \alpha) \delta(x - x_0), \quad P_+(x,0) = \alpha \delta(x - x_0). \quad (2.1c)$$

We treat the nucleating site as an absorbing point and model the complete disassembly of a MT by requiring that

$$P_+(x,t) = 0 \text{ if } x = 0. \quad (2.2)$$

To account for passages of a MT through the shrinking phase, a counting variable y ($0 < y \leq 1$) has been introduced in Eq. (2.1b). By inserting y , one can keep track of the number of different transition histories from the $+$ to $-$ state. Since MTs disappear only from the shrinking phase, the quantity of interest for a MT initially in the growing phase is the number of passes into the shrinking phase.

For a MT initially of length x_0 in either the growing or the shrinking phase, changes in length are governed by Eqs. (2.1a) and (2.1b). The size-time history of a MT, i.e., all possible scenarios of transitions between the growing and shrinking phases until complete disassembly, is encompassed in these equations. For the classification of MT histories according to the number of passages into the shrinking phase, we are interested in the lifetime distribution of a MT, i.e., the probability density per unit of time that the MT of length $x_0 > 0$ in state $+$ or $-$ completely disassembles to zero for the first time at t . This lifetime distribution is given by the flux $v_- P_-(0,t)$ of MTs reaching the nucleating site. As noted in Sec. I, two regimes of MT behavior can be distinguished depending upon whether the rate constants satisfy the condition $v_+f_- < v_-f_+$ (drift decreases length) or $v_+f_- > v_-f_+$ (drift increases length). An equivalent representation of these inequalities can be formed from the ratio ϵ of the average growth step length v_+/f_+ to the average shrinking step length v_-/f_- defined as

$$\epsilon = \frac{v_+f_-}{v_-f_+}. \quad (2.3)$$

When $\epsilon < 1$, all MTs eventually shrink to zero, while for $\epsilon > 1$, the fraction of MTs that shrink to zero is less than one. In this case the flux $v_- P_-(0,t)$ is proportional to the probability density distribution of MT lifetimes conditioned on reaching the nucleating site. Although the MT history of this fraction can be studied, the remainder of this paper will deal only with the regime $\epsilon < 1$ in which MTs have a steady-state length [13,15,18] given by

$$\frac{1}{l} = \frac{f_+}{v_+} - \frac{f_-}{v_-} \Rightarrow l = \frac{v_+ v_-}{v_- f_+ - v_+ f_-}. \quad (2.4)$$

In this latter case, all MTs will shrink to zero fast enough so that the lifetime probability distribution is normalized [22]

$$\int_0^\infty v_- P_-(0,t) dt = 1. \quad (2.5)$$

Denoting by $F_+(t|x_0;y)$ and $F_-(t|x_0;y)$ the respective probability density distributions of MT lifetimes starting at $x_0 > 0$ in states $+$ (i.e., $\alpha=1$) and $-$ (i.e., $\alpha=0$), we then have

$$F_+(t|x_0;y) = v_- P_-(0,t) \quad \text{for } \alpha=1, \quad (2.6a)$$

$$F_-(t|x_0;y) = v_- P_-(0,t) \quad \text{for } \alpha=0. \quad (2.6b)$$

In our calculations, we only need the Laplace transforms of $F_+(t|x_0;y)$ and $F_-(t|x_0;y)$ and in the remaining sections of the paper we will omit the subscript zero on x . The Laplace transform solution of Eqs. (2.1a) and (2.1b) with boundary condition (2.2) is given in Appendix A. It follows from the Laplace transform expression of $v_- P_-(0,t)$ given in Eq. (A8) that

$$\hat{F}_-(s|x;y) = e^{-\lambda(s,y)x}, \quad (2.7a)$$

$$\begin{aligned} \hat{F}_+(s|x;y) &= \left[\frac{s+f_- - v_- \lambda(s,y)}{y f_-} \right] e^{-\lambda(s,y)x} \\ &= g(s,y) \hat{F}_-(s|x;y), \end{aligned} \quad (2.7b)$$

where $\lambda(s,y)$ is given by

$$\begin{aligned} \lambda(s,y) &= \frac{[v_+ f_- - v_- f_+ + (v_+ - v_-)s]}{2v_+ v_-} \\ &+ \left\{ \left[\frac{[v_+ f_- - v_- f_+ + (v_+ - v_-)s]}{2v_+ v_-} \right]^2 \right. \\ &\left. + \frac{s(s+f_+ + f_-) + (1-y)f_+ f_-}{v_+ v_-} \right\}^{1/2}. \end{aligned} \quad (2.8)$$

Note that by setting $y=1$, Eqs. (2.7b) and (2.7a) reduce to the expressions previously obtained by Bicout [18].

III. STATISTICS OF TRANSITION HISTORIES

By inserting the counting variable y in the MT evolution equation (2.1b), $\hat{F}_+(s|x;y)$ and $\hat{F}_-(s|x;y)$ become generating functions in the variable y that contain information about the set of all possible transition histories of a MT.

A. Probability distribution of transits into the shrinking phase

Consider the expansion of $\hat{F}_+(s|x;y)$ and $\hat{F}_-(s|x;y)$ in powers of y at $s=0$,

$$\hat{F}_\pm(0|x;y) = \sum_{n=0}^{\infty} \Omega_n^\pm(x) y^n. \quad (3.1)$$

$\Omega_n^\pm(x)$ is the probability that a MT initially at x in the growing (+) or shrinking (-) phase disappears to the nucleating site after having passed exactly n times into the shrinking state. If the MT starts in the shrinking state and disappears without ever entering the growing phase, $n=0$. It follows from Eq. (3.1) that the sums over n of the probabilities $\Omega_n^-(x)$ and $\Omega_n^+(x)$ are normalized to one since $\hat{F}_\pm(0|x;1) = 1$ and the individual probabilities are given by the expression

$$\Omega_n^\pm(x) = \frac{1}{n!} \left. \frac{\partial^n \hat{F}_\pm(0|x;y)}{\partial y^n} \right|_{y=0}. \quad (3.2)$$

For a MT initially in the $-$ state, the probabilities $\Omega_0^-(x)$ and $\Omega_1^-(x)$ obtained from Eq. (2.7a) are

$$\Omega_0^-(x) = \exp\left\{-\frac{f_-}{v_-}x\right\}, \quad (3.3a)$$

$$\Omega_1^-(x) = \left(\frac{1}{1+\epsilon}\right) \frac{f_- x}{v_-} \exp\left\{-\frac{f_-}{v_-}x\right\}. \quad (3.3b)$$

Similarly, the probabilities $\Omega_0^+(x)$ and $\Omega_1^+(x)$ are

$$\Omega_0^+(x) = \left(\frac{1}{1+\epsilon}\right) \exp\left\{-\frac{f_-}{v_-}x\right\}, \quad (3.4a)$$

$$\Omega_1^+(x) = \frac{\epsilon}{(1+\epsilon)^3} \left[1 + \left(\frac{1+\epsilon}{\epsilon}\right) \frac{f_- x}{v_-} \right] \exp\left\{-\frac{f_-}{v_-}x\right\}, \quad (3.4b)$$

where ϵ is defined in Eq. (2.3). General expressions for $\Omega_n^\pm(x)$ are given in Appendix B. Since $\epsilon < 1$, these probabilities decrease as the number n of transitions into the shrinking phase gets larger and, in addition, they fall off exponentially on the scale of the average shrinking step length v_-/f_- .

On the other hand, $\Omega_n^+(0)$ is given by

$$\Omega_n^+(0) = \frac{\epsilon^n}{(1+\epsilon)^{2n+1}} c_n, \quad c_n = \frac{1}{n+1} \binom{2n}{n}, \quad (3.5)$$

where c_n is the Catalan number. $\Omega_n^+(0)$ is the probability that a newly nucleated MT completely disassembles after n passages into the shrinking state. As can be seen from Table I, depending on ϵ the new MT is most likely to disappear after having made one phase change $+\rightarrow-$. This general trend holds as well for any MT length. Indeed, when $x=l$ (the steady-state length) and $\epsilon=0.1$, for example, the probabilities of disappearance are $\Omega_n^+(l) = 0.813, 0.151, 0.029$ and $\Omega_n^-(l) = 0.895, 0.090, 0.005$ for $n=0, 1, 2$. This supports the experimental observation of catastrophe events in which the length of a MT suddenly shrinks to zero in a single run.

B. Average number of transits into the shrinking phase and fluctuations

Another quantity that is accessible and relevant to the life history of a MT is the average number of transitions into the

TABLE I. Probability of disappearance after n cycles of a newly nucleated MT for different values of ϵ .

n	$\Omega_n^+(0)$	
	$\epsilon=0.1$	$\epsilon=0.5$
0	0.90909	0.6667
1	0.07510	0.1482
2	0.01240	0.0658
3	0.00257	0.0366
4	0.00059	0.0228
5	0.00015	0.0152

shrinking state executed before disappearance. This average number $\langle n_{\pm}(x) \rangle$, which is a function of starting MT length x , can be easily obtained from

$$\langle n_{\pm}(x) \rangle = \sum_{n=0}^{\infty} n \Omega_n^{\pm}(x) = \left. \frac{\partial \hat{F}_{\pm}(0|x;y)}{\partial y} \right|_{y=1}. \quad (3.6)$$

Differentiating Eq. (2.7a) and (2.7b) with respect to y and setting $y=1$, we find

$$\langle n_{+}(x) \rangle - \langle n_{+}(0) \rangle = \langle n_{-}(x) \rangle = \left(\frac{1}{1-\epsilon} \right) \frac{f_{-}x}{v_{-}}, \quad (3.7)$$

where $\langle n_{+}(0) \rangle$, the average number of passes through the shrinking state experienced by a newly nucleated MT before disappearance, is

$$\langle n_{+}(0) \rangle = \frac{\epsilon}{1-\epsilon}. \quad (3.8)$$

This shows that $\langle n_{+}(0) \rangle$ is smaller than, equal to, or greater than one for ϵ smaller than, equal to, or greater than $\frac{1}{2}$, respectively, consistent with the data of Table I. For example, $\langle n_{+}(0) \rangle = 0.11, 1, 19$ for $\epsilon = 0.1, 0.5, 0.95$.

Another informative quantity for the MT dynamics is the variance of the number of transitions into the shrinking state $\langle \Delta n_{\pm}^2(x) \rangle = \langle n_{\pm}^2(x) \rangle - \langle n_{\pm}(x) \rangle^2$. This can be obtained by using the relation

$$\begin{aligned} \langle [n_{\pm}(x)]^2 \rangle - \langle n_{\pm}(x) \rangle^2 &= \sum_{n=0}^{\infty} n(n-1) \Omega_n^{\pm}(x) \\ &= \left. \frac{\partial^2 \hat{F}_{\pm}(0|x;y)}{\partial y^2} \right|_{y=1}. \end{aligned} \quad (3.9)$$

Combining this with Eq. (3.7), one can show that the fluctuation in the number of cycles is given by

$$\begin{aligned} &\frac{\langle \Delta n_{+}^2(x) \rangle - \langle \Delta n_{+}^2(0) \rangle}{[\langle n_{+}(x) \rangle - \langle n_{+}(0) \rangle]^2} \\ &= \frac{\langle \Delta n_{-}^2(x) \rangle}{[\langle n_{-}(x) \rangle]^2} = \frac{2\langle n_{+}(0) \rangle^2 + 2\langle n_{+}(0) \rangle + 1}{\langle n_{-}(x) \rangle} \end{aligned} \quad (3.10a)$$

$$= \left(\frac{1+\epsilon^2}{1-\epsilon} \right) \frac{v_{-}}{f_{-}x} \quad (3.10b)$$

and

$$\frac{\langle \Delta n_{+}^2(0) \rangle}{[\langle n_{+}(0) \rangle]^2} = \frac{2-\epsilon+\epsilon^2}{\epsilon(1-\epsilon)}. \quad (3.11)$$

For x fixed the fluctuation in the number of cycles diverges at $\epsilon=1$, as it would be expected for a symmetric one-dimensional random walk with an absorbing boundary. The standard deviation of the number of transitions is comparable to the average number of transitions for $x \sim v_{-}/f_{-}$ and $\epsilon \gg 1$ or for $x \sim l$ and ϵ close to one, both numbers diverging at the threshold.

IV. MEAN LIFETIMES

As an application of the above analysis, we calculate the mean lifetime of a MT that has cycled exactly n times into the shrinking phase. To proceed, we denote by $\tau_{+}(x)$ [$\tau_{-}(x)$] the mean lifetime of a MT given that it was initially in the growing [shrinking] phase with the length x . Using the formalism developed by Rubin [17], these times can be expressed as

$$\begin{aligned} \tau_{\pm}(x) &= - \left. \frac{\partial \hat{F}_{\pm}(s|x;y)}{\partial s} \right|_{s=0, y=1} \\ &= \lim_{y \rightarrow 1} \sum_{n=0}^{\infty} \Omega_n^{\pm}(x) \tau_n^{\pm}(x) y^n, \end{aligned} \quad (4.1)$$

where $\tau_n^{+}(x)$ [$\tau_n^{-}(x)$] is the mean lifetime of a MT that has transited n times from $+$ to $-$ when starting in the $+$ [$-$] state. It immediately follows from Eq. (4.1) that $\tau_n^{\pm}(x)$ can be derived from the relation

$$\Omega_n^{\pm}(x) \tau_n^{\pm}(x) = - \frac{1}{n!} \left. \frac{\partial^{n+1} \hat{F}_{\pm}(s|x;y)}{\partial s \partial y^n} \right|_{s=0, y=0}. \quad (4.2)$$

Substituting Eqs. (2.7a) and (2.7b) in Eq. (4.2) and then using Eqs. (3.3a), (3.3b), (3.4a), and (3.4b), we have (see Appendix C)

$$\tau_0^{-}(x) = \frac{x}{v_{-}}, \quad (4.3a)$$

$$\tau_1^{-}(x) = \tau_0^{-}(x) + \frac{1}{\kappa_{+} + \kappa_{-}}, \quad (4.3b)$$

$$\tau_0^{+}(x) = \tau_1^{-}(x), \quad (4.3c)$$

$$\tau_1^+(x) = \tau_0^+(x) + \frac{1}{\kappa_+ + \kappa_-} \left\{ 1 + \left[1 + \left(\frac{1+\epsilon}{\epsilon} \right) \frac{f_- x}{v_-} \right]^{-1} \right\}, \quad (4.3d)$$

in which the rates κ_{\pm} defined as

$$\kappa_+ = \frac{v_- f_+}{v_+ + v_-}, \quad \kappa_- = \frac{v_+ f_-}{v_+ + v_-} \quad (4.4)$$

can be regarded as the apparent catastrophe and rescue rates. It appears that $\tau_n^{\pm}(x)$ consists of two contributions: first a ballistic term x/v_- that accounts for shrinking propagation without any phase changes, and second a term that contains all information on cycle events and represents the diffusive aspect of MT evolution. Note in addition that at $x=0$ only the ‘‘diffusive’’ contribution is physically relevant:

$$\tau_n^+(0) = \frac{2n+1}{\kappa_+ + \kappa_-}. \quad (4.5)$$

Setting $y=1$ in Eqs. (2.7a) and (2.7b), differentiating the resulting expressions with respect to s , and taking the $s \rightarrow 0$ limit, we find that the mean lifetimes of a MT are given by [17,18]

$$\begin{aligned} \langle \tau_+(x) \rangle - \langle \tau_+(0) \rangle \\ = \langle \tau_-(x) \rangle = \left(\frac{1}{f_+} + \frac{1}{f_-} \right) \frac{f_- x}{(1-\epsilon)v_-} \end{aligned} \quad (4.6a)$$

$$= \left(\frac{1}{f_+} + \frac{1}{f_-} \right) \langle n_-(x) \rangle, \quad (4.6b)$$

where $f_+^{-1} + f_-^{-1}$ is the typical duration of a cycle of successive phase changes and $\langle n_-(x) \rangle$, given in Eq. (3.7), is the average number of transitions into the shrinking state executed by a MT before complete disassembly to the nucleating site. The mean lifetime for a newly nucleated MT is

$$\langle \tau_+(0) \rangle = \left(\frac{\epsilon}{1-\epsilon} \right) \frac{1}{\kappa_-} = \frac{\langle n_+(0) \rangle}{\kappa_-}. \quad (4.7)$$

Similarly, denoting the variance of the mean lifetimes by $\langle \Delta \tau_{\pm}^2(x) \rangle = \langle \tau_{\pm}^2(x) \rangle - \langle \tau_{\pm}(x) \rangle^2$, one can also relate the fluctuations of the mean lifetimes to the fluctuations in the number of phase changes given in Eq. (3.10b) as

$$\begin{aligned} \frac{\langle \Delta \tau_+^2(x) \rangle - \langle \Delta \tau_+^2(0) \rangle}{[\langle \tau_+(x) \rangle - \langle \tau_+(0) \rangle]^2} \\ = \frac{\langle \Delta \tau_-^2(x) \rangle}{[\langle \tau_-(x) \rangle]^2} = \frac{2(1+\epsilon)^2 v_-}{(1-\epsilon) f_- x} \end{aligned} \quad (4.8a)$$

$$= \left(\frac{2(1+\epsilon)^2}{1+\epsilon^2} \right) \frac{\langle \Delta n_-^2(x) \rangle}{[\langle n_-(x) \rangle]^2}. \quad (4.8b)$$

Equations (4.6b) and (4.8b) establish the one to one relationship between the statistics of phase changes and that of life-

times. Therefore, all remarks made about the number of phase changes apply as well to the MT lifetime.

V. SUMMARY

The evolution equations describing the dynamic behavior of MTs are an example of a biased two-state random walk with an absorbing boundary. Consequently, in the interesting case where the bias, or drift, is directed toward zero length (i.e., the location of the absorbing boundary), MTs have a finite lifetime before absorption. By generalizing a procedure used to classify discrete random walks [20,21] according to their histories, we have introduced a counting variable y in the MT evolution equation (2.1b). These equations encompass all possible transition histories of a MT before its disappearance. With the variable y , one can classify MT histories according to the number of transits into the shrinking phase prior to absorption. The solution of the generalized evolution equations can then be regarded as a generating function for quantities such as (i) the probability of transiting exactly n times into the shrinking phase before absorption $\Omega_n^{\pm}(x)$ in Eqs. (3.2), (B1), and (B6); (ii) the mean number of transits into the shrinking phase before absorption $\langle n_{\pm}(x) \rangle$ in Eqs. (3.6)–(3.8); and (iii) the mean lifetime of MTs that have undergone exactly n transits before disappearance $\tau_n^{\pm}(x)$ in Eqs. (4.1), (C3), and (C4). All of these can be computed directly from the time recording of a single MT length. Indeed, the time evolution of a MT length exhibits a sawtooth profile where peaks are formed by the alternation of growing and shrinking phases. The number of peaks is identically equal to the number n of times the MT enters the shrinking phase, i.e., the number of times the MT undergoes catastrophic events. Therefore, $\langle n_{\pm}(x) \rangle$ and $\Omega_n^{\pm}(x)$ can be obtained by simply counting and binning the number of peaks before complete disassembly of the MT and averaging over many realizations and $\tau_n^{\pm}(x)$ represents the average elapsed time taken for the MT to collapse to zero length after having entered the shrinking phase n times.

All the intimate details of MT histories $\Omega_n^{\pm}(x)$, $\langle n_{\pm}(x) \rangle$, and $\tau_n^{\pm}(x)$ discussed in previous sections depend on the initial length x of the MT. However, in applications one is interested in properties of the system prepared with an initial distribution of MT lengths that coincides with the steady-state distribution. Indeed, it has been shown [13,15,18] that for $\epsilon < 1$, there is a steady-state average length [Eq. (2.4)] for MTs. That is, in an ensemble of MTs at steady state, if MTs are renucleated when they reach zero length, the appropriate boundary condition for the evolution equations (2.1a) and (2.1b) is $v_+ P_+(0, t) = v_- P_-(0, t)$. Under the above conditions, it can be verified by direct substitution that the time-independent or steady-state solutions of Eqs. (2.1a) and (2.1b) have the exponential forms

$$\begin{aligned} p_{\text{eq}}^+(x) &= \left(\frac{v_-}{v_+ + v_-} \right) \frac{e^{-x/l}}{l}, \\ p_{\text{eq}}^-(x) &= \left(\frac{v_+}{v_+ + v_-} \right) \frac{e^{-x/l}}{l}. \end{aligned} \quad (5.1)$$

In such an ensemble, all MT lengths x and states \pm are present with the statistical weights $p_{\text{eq}}^+(x)$ and $p_{\text{eq}}^-(x)$. These

weights can be used to calculate the ensemble average of $\Omega_n^\pm(x)$, $\langle n_\pm(x) \rangle$, and $\tau_n^\pm(x)$. For illustration, we just give the ensemble average of the number of transits into the shrinking phase before disappearance

$$\begin{aligned} \langle n \rangle_{\text{eq}} &= \int_0^\infty \langle n_+(x) \rangle p_{\text{eq}}^+(x) dx \\ &+ \int_0^\infty \langle n_-(x) \rangle p_{\text{eq}}^-(x) dx \\ &= \frac{\epsilon}{1-\epsilon} \left[\frac{1}{1-\epsilon} + \frac{v_-}{v_+ + v_-} \right] \end{aligned} \quad (5.2)$$

and the ensemble average of the lifetime of a MT before complete disassembly

$$\begin{aligned} \langle \tau \rangle_{\text{eq}} &= \int_0^\infty \langle \tau_+(x) \rangle p_{\text{eq}}^+(x) dx \\ &+ \int_0^\infty \langle \tau_-(x) \rangle p_{\text{eq}}^-(x) dx \\ &= \left(\frac{1}{f_+} + \frac{1}{f_-} \right) \langle n \rangle_{\text{eq}} + \left(\frac{v_-}{v_+ + v_-} \right) \frac{1}{f_+}. \end{aligned} \quad (5.3)$$

It follows that the average lifetime is equal to the total time of growing and shrinking phases times the average number of transitions, plus a noncycling contribution that is the ensemble averaged lifetime of newly nucleated MTs having transited only once into the shrinking phase to zero length.

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APPENDIX A: DERIVATION OF THE EXPRESSION OF $\hat{P}_-(X, S)$

Defining the Laplace Fourier transform as

$$\begin{aligned} \hat{P}_\pm(k, s) &= \int_0^\infty dx \int_0^\infty dt e^{ikx-st} P_\pm(x, t), \\ &= \int_0^\infty dt e^{-st} \tilde{P}_\pm(k, t), \\ &= \int_0^\infty dx e^{ikx} \hat{P}_\pm(x, s), \end{aligned} \quad (A1)$$

Eqs. (2.1a) and (2.1b) can be transformed into

$$\begin{aligned} (s + f_+ - ikv_+) \hat{P}_+ - f_- \hat{P}_- &= \alpha e^{ikx_0}, \\ yf_+ \hat{P}_+ - (s + f_- + ikv_-) \hat{P}_- &= - (1 - \alpha) e^{ikx_0} + v_- \hat{P}_-(0, s), \end{aligned} \quad (A2)$$

in which we have used the initial condition in Eq. (2.1c), the absorbing boundary condition $\hat{P}_+(0, s) = 0$, and the fact that $P_\pm(\infty, t) = 0$. For instance, $\hat{P}_-(k, s)$ can be obtained from Eq. (A2) as

$$\begin{aligned} \hat{P}_-(k, s) &= \frac{\begin{vmatrix} s + f_+ - ikv_+ & \alpha e^{ikx_0} \\ yf_+ & - (1 - \alpha) e^{ikx_0} + v_- \hat{P}_-(0, s) \end{vmatrix}}{\begin{vmatrix} s + f_+ - ikv_+ & -f_- \\ yf_+ & - (s + f_- + ikv_-) \end{vmatrix}} \\ &= \frac{(s + f_+ - ikv_+) v_- \hat{P}_-(0, s)}{v_+ v_- (ik - \lambda_1)(ik + \lambda_2)} - \frac{[(1 - \alpha)(s + f_+ - ikv_+) + \alpha y f_+]}{v_+ v_- (ik - \lambda_1)(ik + \lambda_2)} e^{ikx_0}, \end{aligned} \quad (A3)$$

where the roots λ_1 and λ_2 are given by

$$\begin{aligned} \left. \begin{array}{l} \lambda_1 \\ \lambda_2 \end{array} \right\} &= \mp \frac{[v_+ f_- - v_- f_+ + (v_+ - v_-)s]}{2v_+ v_-} \\ &+ \left\{ \left[\frac{[v_+ f_- - v_- f_+ + (v_+ - v_-)s]}{2v_+ v_-} \right]^2 \right. \\ &\left. + \frac{s(s + f_+ + f_-) + (1 - y)f_+ f_-}{v_+ v_-} \right\}^{1/2}. \end{aligned} \quad (A4)$$

In the argument of the square root of $\lambda_{1,2}$, the counting variable is assumed to be less than one, although ultimately the limit $y \rightarrow 1$ is taken. Therefore, that argument is positive

for real $s > 0$. Moreover, in our case of interest $v_+ f_- - v_- f_+ < 0$, we thus have $\lambda_1 > 0$ and $\lambda_2 \geq 0$. Let us invert the Fourier transform of $\hat{P}_-(k, s)$,

$$\hat{P}_-(x, s) = \frac{1}{2\pi} \int_{-\infty}^{\infty} e^{-ikx} \hat{P}_-(k, s) dk. \quad (A5)$$

In evaluating the transform by contour integration, closure of the contour containing the term e^{-ikx} is clockwise around $-i\lambda_1$, while for the term containing $e^{ik(x_0 - x)}$ closure is clockwise around $-i\lambda_1$ for $x > x_0$ and counterclockwise around $i\lambda_2$ for $x < x_0$. The result is

$$\begin{aligned}\hat{P}_-(x,s) = & -\frac{(s+f_+-v+\lambda_1)v-\hat{P}_-(0,s)}{v_+v_-(\lambda_1+\lambda_2)}e^{-\lambda_1x} \\ & +\frac{[(1-\alpha)(s+f_+)+\alpha yf_+]}{v_+v_-(\lambda_1+\lambda_2)}e^{-\lambda_2(x_0-x)} \\ & +\frac{(1-\alpha)\lambda_2}{v_-(\lambda_1+\lambda_2)}H(x_0-x)e^{-\lambda_2(x_0-x)}, \\ & x \leq x_0,\end{aligned}\quad (\text{A6a})$$

$$\begin{aligned}\hat{P}_-(x,s) = & -\frac{(s+f_+-v+\lambda_1)v-\hat{P}_-(0,s)}{v_+v_-(\lambda_1+\lambda_2)}e^{-\lambda_1x} \\ & +\frac{[(1-\alpha)(s+f_+)+\alpha yf_+]}{v_+v_-(\lambda_1+\lambda_2)}e^{-\lambda_1(x-x_0)} \\ & -\frac{(1-\alpha)\lambda_1}{v_-(\lambda_1+\lambda_2)}H(x-x_0)e^{-\lambda_1(x-x_0)}, \\ & x \geq x_0,\end{aligned}\quad (\text{A6b})$$

where $H(x)$ is the Heaviside step function defined as $H(x) = 1$ for $x > 0$ and $H(x) = 0$ for $x < 0$. The expression for $\hat{P}_+(x,s)$ can as well be derived using the same method.

We now focus on the case $x < x_0$. Set $x = 0$ in Eq. (A6a) and solve the resulting equation for $\hat{P}_-(0,s)$. Using the relation

$$\frac{yf_+}{s+f_++v+\lambda_2} = \frac{s+f_--v-\lambda_2}{f_-}, \quad (\text{A7})$$

we finally obtain

$$v_-\hat{P}_-(0,s) = \left[\frac{\alpha(s+f_--v-\lambda_2)}{yf_-} + (1-\alpha) \right] e^{-\lambda_2x_0}. \quad (\text{A8})$$

APPENDIX B: GENERAL EXPRESSIONS FOR $\Omega_n^\pm(x)$

By differentiating Eq. (2.7a) n times with respect to y and using Eq. (2.3) in the resulting expressions, we find that the

probability of n transitions for a MT initially in the shrinking phase is, for $n \geq 1$,

$$\begin{aligned}\Omega_n^-(x) = & \frac{\epsilon^n}{(1+\epsilon)^{2n}} \exp\left\{-\frac{f_-}{v_-}x\right\} \\ & \times \sum_{k=1}^n \frac{(2n-k-1)!}{n!(k-1)!(n-k)!} \left[\left(\frac{1+\epsilon}{\epsilon}\right) \frac{f_-x}{v_-} \right]^k.\end{aligned}\quad (\text{B1})$$

The structure of Eq. (2.7b) suggests expressing $\Omega_n^+(x)$ in terms of $\Omega_n^-(x)$. We have

$$\begin{aligned}\Omega_n^+(x) = & \frac{1}{n!} \sum_{k=0}^n \binom{n}{k} \left[\frac{\partial^k g(0,y)}{\partial y^k} \frac{\partial^{n-k} \hat{F}_-(0|x;y)}{\partial y^{n-k}} \right] \Bigg|_{y=0} \\ = & \sum_{k=0}^n \frac{\Omega_{n-k}^-(x)}{k!} \left[\frac{\partial^k g(0,y)}{\partial y^k} \right] \Bigg|_{y=0}.\end{aligned}\quad (\text{B2})$$

The remaining derivative can be easily evaluated to give

$$\Omega_n^+(x) = \sum_{k=0}^n \Omega_k^+(0) \Omega_{n-k}^-(x), \quad (\text{B3})$$

where $\Omega_n^+(0)$ is given in Eq. (3.5). However, it is possible to work out a general formula for $\Omega_n^+(x)$. Substitute Eqs. (B1) and (3.5) into Eq. (B3) and obtain

$$\begin{aligned}\Omega_n^+(x) = & \frac{\epsilon^n}{(1+\epsilon)^{2n+1}} \exp\left\{-\frac{f_-}{v_-}x\right\} \left\{ \frac{(2n)!}{n!(n+1)!} \right. \\ & + \sum_{k=0}^{n-1} \frac{(2k)!}{k!(k+1)!(n-k)!} \\ & \left. \times \sum_{i=1}^{n-k} \frac{(2n-2k-i-1)!}{(i-1)!(n-k-i)!} \left[\left(\frac{1+\epsilon}{\epsilon}\right) \frac{f_-x}{v_-} \right]^i \right\}.\end{aligned}\quad (\text{B4})$$

Next, using in this expression the identity

$$\sum_{k=0}^{n-1} \frac{(2k)!}{k!(k+1)!(n-k)!} \sum_{i=1}^{n-k} \frac{(2n-2k-i-1)!}{(i-1)!(n-k-i)!} Z^i + \frac{(2n)!}{n!(n+1)!} = \sum_{k=0}^n \frac{(k+1)(2n-k)}{!(n+1)!k!(n-k)!} Z^k, \quad (\text{B5})$$

we finally find

$$\begin{aligned}\Omega_n^+(x) = & \frac{\epsilon^n}{(1+\epsilon)^{2n+1}} \exp\left\{-\frac{f_-}{v_-}x\right\} \\ & \times \sum_{k=0}^n \frac{(k+1)(2n-k)!}{(n+1)!k!(n-k)!} \left[\left(\frac{1+\epsilon}{\epsilon}\right) \frac{f_-x}{v_-} \right]^k.\end{aligned}\quad (\text{B6})$$

Note that similar expressions, i.e., Eqs. (B1) and (B6) for the number of phase changes, were also obtained by Hill [13] using a different method.

APPENDIX C: GENERAL EXPRESSIONS FOR $\tau_n^\pm(x)$

Differentiating Eq. (2.7a) once with respect to s and n times with respect to y and setting $s = y = 0$, we have

$$\begin{aligned} \Omega_n^-(x) \tau_n^-(x) &= \frac{x}{v_-} \Omega_n^-(x) + \left(\frac{1}{\kappa_+ + \kappa_-} \right) \\ &\times \left[\frac{-f_- x}{(1 + \epsilon) v_-} \right]^n \exp \left\{ - \frac{f_- x}{v_-} \right\} \\ &\times \sum_{k=0}^{n-1} \frac{(n+k)(n+k-1)!}{n! k! (n-k-1)!} \left[\left(\frac{1 + \epsilon}{\epsilon} \right) \frac{f_- x}{v_-} \right]^{-k}. \end{aligned} \quad (C1)$$

On the other hand, one can show by recurrence, for example, that for $Z \neq 0$

$$\begin{aligned} &\sum_{k=0}^{n-1} \frac{(n+k)(n+k-1)!}{k!(n-k-1)!} Z^{-k} \\ &= \sum_{k=1}^n (-1)^{n+k} (2k-1) \sum_{i=0}^{k-1} \frac{(k+i-1)!}{i!(k-i-1)!} Z^{-i}, \\ &= \sum_{k=1}^n (-1)^{n+k} (2k-1) \sum_{i=1}^k \frac{(2k-i-1)!}{(i-1)!(k-i)!} Z^{i-k}. \end{aligned} \quad (C2)$$

Using this relation and Eq. (B1) in Eq. (C1), we find

$$\begin{aligned} \tau_n^-(x) &= \frac{x}{v_-} + \left(\frac{1}{\kappa_+ + \kappa_-} \right) \sum_{k=1}^n (2k-1) \\ &\times \left[\frac{k! \Omega_k^-(x)}{n! \Omega_n^-(x)} \right] \left[\frac{-f_- x}{(1 + \epsilon) v_-} \right]^{n-k}. \end{aligned} \quad (C3)$$

Similarly, the differentiation of Eq. (2.7b) once with respect to s and n times with respect to y and identifying factors related to the shrinking phase, one can express $\tau_n^+(x)$ in terms of $\tau_n^-(x)$ and $\Omega_n^\pm(x)$ as

$$\tau_n^+(x) = \sum_{k=0}^n \frac{\Omega_k^+(0) \Omega_{n-k}^-(x)}{\Omega_n^+(x)} [\tau_{k+1}^-(0) + \tau_{n-k}^-(x)]. \quad (C4)$$

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- [1] T. Mitchison and M. Kirschner, *Nature (London)* **312**, 232 (1984); **312**, 237 (1984).
[2] T. Horio and H. Hotani, *Nature (London)* **321**, 605 (1986).
[3] H. Hotani and T. Horio, *Cell Motil. Cytoskeleton* **10**, 229 (1988).
[4] R. A. Walker, E. T. O'Brien, N. K. Pryer, M. F. Soboeiro, W. A. Voter, and H. P. Erickson, *J. Cell Biol.* **107**, 1437 (1988).
[5] R. A. Walker, N. K. Pryer, and E. D. Salmon, *J. Cell Biol.* **114**, 73 (1991).
[6] H. Flyvbjerg, T. E. Holy, and S. Leibler, *Phys. Rev. E* **54**, 5538 (1996).
[7] Y. Chen and T. L. Hill, *Proc. Natl. Acad. Sci. USA* **82**, 1131 (1985).
[8] P. M. Bayley, M. J. Schilstra, and S. B. Martin, *J. Cell. Sci.* **93**, 241 (1989); **95**, 33 (1990).
[9] D. J. Odde, L. Cassimeris, and H. M. Buettner, *Biophys. J.* **69**, 796 (1995); D. J. Odde, H. M. Buettner, and L. Cassimeris, *AIChE. J.* **42**, 1434 (1996).
[10] H. P. Erickson and E. T. O'Brien, *Annu. Rev. Biophys. Biomol. Struct.* **21**, 145 (1992).
[11] M. Caplow, *Curr. Opin. Cell Biol.* **4**, 58 (1992).
[12] L. Cassimeris, *Cell Motil. Cytoskeleton* **26**, 275 (1993).
[13] T. L. Hill, *Proc. Natl. Acad. Sci. USA* **81**, 6728 (1984).
[14] G. H. Weiss and R. J. Rubin, *Adv. Chem. Phys.* **52**, 363 (1983).
[15] M. Dogterom and S. Leibler, *Phys. Rev. Lett.* **70**, 1347 (1993).
[16] F. Verde, M. Dogterom, E. Stelzer, E. Karsenti, and S. Leibler, *J. Cell Biol.* **118**, 1097 (1992).
[17] R. J. Rubin, *Proc. Natl. Acad. Sci. USA* **85**, 446 (1988).
[18] D. J. Bicout, *Phys. Rev. E* **56**, 6656 (1997).
[19] G. H. Weiss, *J. Stat. Phys.* **15**, 157 (1976).
[20] R. J. Rubin, *J. Chem. Phys.* **43**, 2392 (1965).
[21] R. J. Rubin and G. H. Weiss, *J. Math. Phys.* **23**, 250 (1988).
[22] By adding the two differential equations (2.1a) and (2.1b), integrating the resulting equation over x from 0 to ∞ , and using the fact that $P_\pm(\infty, t) = 0$ and the absorbing condition $P_+(0, t) = 0$, we have $\int_0^\infty (\partial/\partial t) [P_+(x, t) + P_-(x, t)] dx = -v_- P_-(0, t)$. Now integrating this over time from 0 to ∞ and then using the initial conditions for $P_\pm(x, 0)$ leads to $1 - \int_0^\infty dx [P_+(x, \infty) + P_-(x, \infty)] = \int_0^\infty v_- P_-(0, t) dt$. When $\epsilon \leq 1$ (i.e., $C \leq C_{cr}$), all MTs will ultimately shrink to zero so that $P_\pm(x, \infty) = 0$. In this case we obtain the normalization in Eq. (2.5).