Stochastic Dynamics of Microtubules: A Model for Caps and Catastrophes

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We introduce and solve a phenomenological model for the so-called *catastrophes*, the abrupt transitions from the growing to the shrinking state of microtubules. The model may explain existing experimental results and resolve some long-standing apparent contradictions. In particular, the model reproduces observed catastrophe rates and waiting times for catastrophes upon sudden dilution. It may also explain why recent experiments fail to measure the GTP content in growing microtubules and provides a mechanism for so-called *coupled hydrolysis*.

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Microtubules (MTs) are long and extremely rigid polymers assembled from tubulin—a protein found in eukaryotic cells. They form an important part of the cellular scaffold and provide a network of "rails" for active intracellular transport. They also play a crucial role during cell division, forming a dynamic structure which spatially separates duplicated chromosomes. Ten years ago, Mitchison and Kirschner discovered that the polymerization of MTs from tubulin is a very unusual process: A MT can repeatedly, and apparently randomly, switch between persistent states of assembly and disassembly (see Fig. 1, inset) in a *constant* concentration of tubulin [1,2]. This behavior is observed *in vivo* as well as *in vitro* and is referred to as *dynamic instability*.

This switching between growing and shrinking states at one concentration is unusual for a polymer. It is achieved by an increase in the chemical potential of the monomers after assembly. The energy required to do this is provided by hydrolysis of a GTP nucleotide bound to assembling monomers. While thermodynamics thus can explain the coexistence of the growing and the shrinking states, it cannot explain the dynamics of the transitions between these states. An interesting possibility, suggested by Mitchison and Kirschner [1,2], is that transitions occur as a consequence of competition between assembly and GTP hydrolysis. A growing microtubule assembles by the addition of GTP tubulin, which is later converted to GDP tubulin. In Mitchison and Kirschner's scenario, a growing microtubule has a stabilizing *cap* of GTP tubulin [2,3]. If hydrolysis overtakes the addition of new GTP tubulin, the cap is gone, and the MT's end undergoes a change to the shrinking state—a so-called *catastrophe*. Though it is known that GTP hydrolysis precedes disassembly, it may not be the rate-limiting process in the change to a disassembly favoring state, however. Conformational changes of tubulin or structural changes of the MT are other candidates; see [4,5] for recent reviews.

Despite the large amount of experimental and theoretical work devoted to the cap model, it is still the subject of controversy [4,6–8]. At the center of the debate are seemingly contradictory results about catastrophes coming from two types of experiments:

(1) When MTs are grown in pure GTP-tubulin solutions at various constant concentrations, the frequency of catastrophes is one every *few minutes* and decreases with increasing concentration [9,10]. This suggests that the stabilizing cap is longer, hence less apt to be lost, at larger concentrations. But no cap model has been able to relate concentration with frequency of catastrophe in a manner quantitatively resembling the observed relationship.

(2) In *dilution experiments* the concentration of tubulin is abruptly reduced to zero, resulting in catastrophes within *seconds*, independent of the initial concentration [11,12]. This suggests that the cap is short and independent of the concentration at which it is formed.

In this Letter, we show how a simple stochastic cap model may resolve these apparent contradictions. Their wide temporal range of behavior—from seconds to minutes—is explained in terms of tubulin assembly and

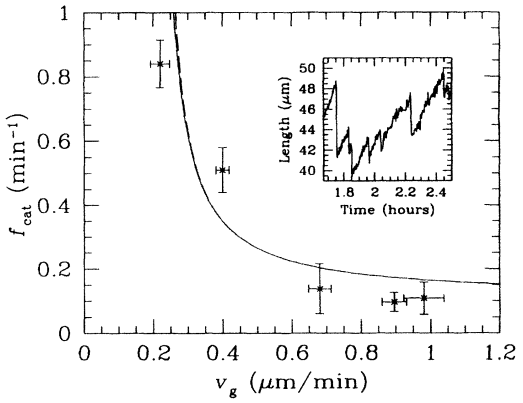

FIG. 1. Catastrophe rate, f_{cat}, versus growth velocity, v_g. Dots with error bars: experimental results [9]. The horizontal error bars represent the standard error in the mean (SEM) v_g for the sample, the vertical bars the SEM for the catastrophe rate. Full and dashed curves: first (α_0/t_0) and second (Dr/v) expression for the catastrophe rates in Eq. (5) fitted to experimental results. Inset: length as a function of time for a single microtubule. Data are taken from Ref. [20].

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transformation, processes which occur over time scales shorter than tenths of seconds. In formulating this model, we were inspired by the many previous attempts; see [7] for a review. Our model, however, neglects molecular details, contains only a few parameters to be fitted, and is sufficiently simple to allow its solution to be obtained analytically in most cases of interest. Its predictions agree well with the experimental results cited above.

A simple stochastic model.—Our model can be considered an effective theory for phenomena on length scales resolved with optical microscopy used in the studies of MT dynamics (typically $\sim 0.5\ \mu$m). It is designed to include as little as possible from the "microscopic" length scale set by the tubulin monomers ($\simeq 8$ nm). Therefore, we neglect the tubular shape of microtubules, the structure of the growing ends, the molecular details of the assembly process, and the nature of the transition that prepares the tubulin monomers for disassembly after they have been included in a MT. We assume only that there are monomers which polymerize in one state, A, with the potential to change to another state, B. Monomers assemble at the growing tip at a rate κ_g, and each adds a length δx to the MT. Thus the polymer will grow with average velocity $v_g = \kappa_g \delta x$. We also assume that the transformation from state A to state B is stochastic, occurring with rate κ_{AB}, when an A monomer neighbors a B monomer (an *induced transformation*), and rate κ'_{AB} when it does not (a *spontaneous transformation*); see Fig. 2. An existing interface between an A section and a B section of the polymer moves thus with average velocity $v_{AB} = \kappa_{AB}\delta x$, while new interfaces are created in the interior of an A section with rate (per unit length) $r = \kappa'_{AB}/\delta x$. Finally, fluctuations on the microscopic scale δx are modeled with an effective "diffusion constant" D as follows.

The A section located at the end of the polymer is the cap in our model: its disappearance results in a catastrophe

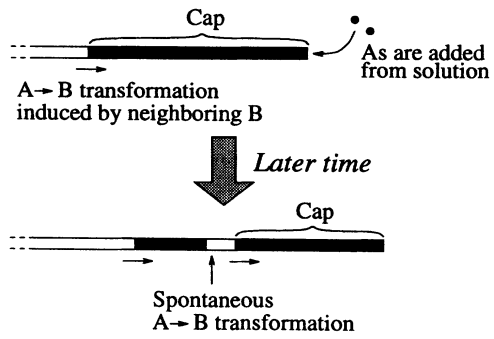

FIG. 2. A MT consisting of A (black) and B (white) monomers of length δx, growing by addition of A monomers with rate κ_g. Sections of A are transformed to B at rate κ_{AB}/monomer from their ends (induced) and rate κ'_{AB}/monomer from their interior (spontaneous). Spontaneous transformations produce B regions of zero length which spread by induced transformations; some spreading has already occurred by the time of the second view.

of the MT. We focus our attention on the evolution of this cap. Its length x increases at an average velocity $v = v_g - v_{AB}$, with fluctuations determined by $D = \frac{1}{2}(\kappa_g + \kappa_{AB})\delta x^2 = \frac{1}{2}(v_g + v_{AB})\delta x$. Occasionally, the cap is shortened to any fraction of its length by a spontaneous transformation in its interior.

Our model is summarized in the master equation for the distribution of cap lengths, $p(x,t)$,

$$\partial_t p = -v\partial_x p + D\partial_x^2 p - rxp + r\int_x^\infty dy\, p(y,t). \quad (1)$$

On the right-hand side of this equation, the first term describes the average growth of the cap's length between its abrupt shortening by spontaneous transformations in its interior. The second term describes the fluctuations, i.e., the random walk superposed on this average growth. The third term is the rate at which caps of length x are abruptly shortened. Finally, the last term is the rate at which caps longer than x are shortened to length x. We impose an absorbing boundary condition at $x = 0$, $p(0,t) = 0$, since a catastrophe occurs when the cap shrinks to zero length. The initial condition for Eq. (1) depends on the experiment under study.

This model resolves the apparent contradictions in the experimental results. At tubulin concentrations for which $v_g > v_{AB}$, the cap grows with velocity $v > 0$ between spontaneous transformations, abruptly reducing its size. Catastrophes are relatively infrequent, because they occur only when such a spontaneous transformation happens to occur close to the tip of the cap, *and* the short cap resulting from this happens to disappear by the fluctuations (parametrized by D).

By contrast, in a dilution experiment $v_g \approx 0$, hence $v \approx -v_{AB} < 0$. In this case the first term in Eq. (1) cooperates with the spontaneous transformations to quickly shrink the cap to zero size. This shrinking takes place roughly in two stages. Initially, spontaneous transformations dominate the shortening; eventually, the cap becomes short enough that it disappears because of the induced transformations. The fact that spontaneous transformations dominate the early stages of shortening results in the time required for the cap to disappear being nearly independent of the initial cap size.

To make contact between model and experimental results, we consider the constants v, r, and D appearing in Eq. (1). They are functions of v_g, v_{AB}, δx, and r. v_g can be found directly by measuring the growth velocity of the MT. δx is known, equal to 0.6 nm [13]. The remaining constants, r and v_{AB}, are specific to our model and not directly observable in current experiments. MTs are polar objects, and their two ends, conventionally called plus and minus, behave differently. We expect v_{AB} to be different at the two ends, while the rate r of spontaneous transformations should be the same at both ends. Thus we need only three parameters, $v_{AB}^{(+)}$, $v_{AB}^{(-)}$, and r, to describe the dynamics of the two ends of a MT.

Equation (1) can be solved analytically; see below. We compare its predictions with recent experiments. Figure 1 shows the catastrophe rate as a function of growth velocity for the plus end of MTs [9]. Figure 3 compares with the results of dilution experiments for both ends, taken from Ref. [12]. From the top half of this figure, we see that the waiting time before catastrophe is indeed nearly independent of the initial growth velocity. This allows one to group the data and show the distribution of waiting times as done in the bottom portion of this figure. The theoretical curves in Figs. 1 and 3 were fitted simultaneously to the experimental data using $v_{AB}^{(+)}$, $v_{AB}^{(-)}$, and r as fitting parameters, giving $v_{AB}^{(+)} = 0.21 \pm 0.01\ \mu\text{m min}^{-1}$, $v_{AB}^{(-)} = 0.22 \pm 0.01\ \mu\text{m min}^{-1}$, and $r = 360 \pm 20\ \mu\text{m min}^{-1}$. With these values, the cap of a growing microtubule contains roughly $40\sqrt{v/v_{AB}}$ monomers, as long as $v_g \geq 0.3\ \mu\text{m min}^{-1}$.

The level of agreement between theory and experiment shown in Figs. 1 and 3 is surprising, given the simplicity of the theory. It is encouraging that one can model the collective behavior of a complex system of proteins quantitatively, and that with a model so simple it can be solved analytically.

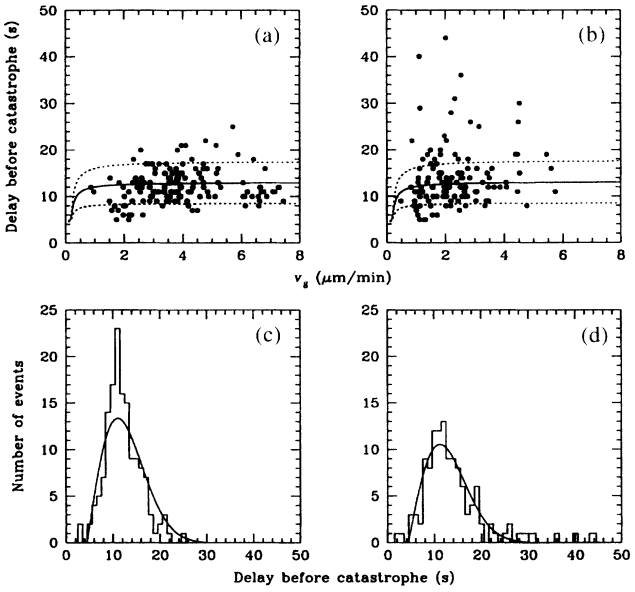

FIG. 3. Delays before catastrophe following dilution. Data taken from Ref. [12]. (a) and (c): plus end; (b) and (d): minus end. (a) and (b): delay as a function of initial growth velocity. Each point represents a single measurement on a MT. Curves are theoretical mean (solid) and standard deviation (dashed) of the delay, from Eqs. (6) and (7). (c) and (d): histograms showing the experimental distribution of delays before catastrophe. The curves are fits of the theoretical distribution given in Eq. (6). Dilution was initiated at $t = 0$ and required some time (6.1 s when used as a free parameter in our fit) for completion.

Solving the model.—We now sketch the solution of Eq. (1). Equation (1) is equivalent to

$$\partial_t P = (-v\partial_x + D\partial_x^2 - r x)P, \tag{2}$$

where $P(x,t) = \int_x^\infty dy\, p(y,t)$. This can be seen quite easily by inserting the definition of $P(x,t)$ into Eq. (2). We introduce dimensionless variables $\xi = x/x_0$, $\tau = t/t_0$, and $\gamma = v t_0/2x_0$, where $x_0 = (D/r)^{1/3}$ and $t_0 = (Dr^2)^{-1/3}$. Defining $\tilde{P}(\xi,\tau) = \exp(-vx/2D)P(x,t)$, Eq. (2) takes the form $\partial_\tau \tilde{P}(\xi,\tau) = (\partial_\xi^2 - \xi - \gamma^2) \times \tilde{P}(\xi,\tau)$. Since $\tilde{P}$ by its definition must vanish for $\xi \to \infty$, its general form is

$$\tilde{P}(\xi,\tau) = \int d\alpha c(\alpha) \exp(-\alpha\tau) \mathrm{Ai}(\xi + \gamma^2 - \alpha), \tag{3}$$

where $\mathrm{Ai}(\xi)$ is the first Airy function [14]. The absorbing boundary condition, $p(0,t) = 0$, is equivalent to

$$-\mathrm{Ai}'(\gamma^2 - \alpha) = \gamma \mathrm{Ai}(\gamma^2 - \alpha), \tag{4}$$

which is solved only by a discrete set of relaxation rates, $\alpha_k(\gamma)$, $k = 0, 1, 2, \ldots$.

When $\gamma > 0$, there is a gap of order γ^2 between the slowest relaxation rate, α_0, and higher rates. Consequently, $\tilde{P}(\xi,\tau)$ quickly approaches its asymptotic form, so fast that faster modes do not show in experimental data, we find. Thus, all *observed* catastrophes are Poisson distributed in time with rate

$$f_{\text{cat}} = \alpha_0(\gamma)/t_0 \sim \frac{Dr}{v} = \frac{1}{2} r \delta x \frac{v_g + v_{AB}}{v_g - v_{AB}}, \tag{5}$$

where the approximation follows from the asymptotic form of the Airy function in Eq. (4) [14]. Equation (5) shows that the catastrophe rate has a finite limit, $r\delta x/2$, for $v_g/v_{AB} \to \infty$, the same for both MT ends. This prediction is consistent with experimental results for the minus end given in Fig. 7 of Ref. [11], but a significant test requires the collection of more data.

In dilution experiments, MTs are first grown in a high concentration of tubulin, giving a high assembly rate, v_g, then submitted to rapid and massive dilution to induce catastrophes and disassembly [11,12]. Under both these conditions, it is a good approximation to treat D as equal to zero. In this approximation we find the distribution in time of catastrophes upon sudden dilution, $p_{\text{cat}}(t)$, by solving Eq. (2), using for initial condition the steady-state solution that it has before dilution. We find

$$p_{\text{cat}}(t) = -\partial_t P(0,t) = \frac{\pi t}{2\langle t\rangle_{\text{cat}}^2} \exp\left(-\frac{\pi t^2}{4\langle t\rangle_{\text{cat}}^2}\right), \tag{6}$$

where the average delay $\langle t\rangle_{\text{cat}}$ before catastrophe is

$$\langle t\rangle_{\text{cat}} = \left[\frac{2r|v_f|}{\pi}\left(1 + \frac{|v_f|}{v_i}\right)\right]^{-1/2} \simeq \left(\frac{\pi}{2r|v_f|}\right)^{1/2}. \tag{7}$$

Here $v_i < 0$ and $v_f > 0$ are the predilution and postdilution cap growth velocities, respectively. The approximations employed here may be justified by comparing

the results given here with results obtained by exact numerical solution of Eq. (1) [15]. Here we just note that Eqs. (6) and (7) agree with experimental results, as shown in Figs. 1 and 3.

We have presented here a phenomenological model for catastrophes, without determining the underlying microscopic picture. Progress in structural studies should permit connecting the phenomenological and microscopic descriptions. Most likely, catastrophes will be described as a superposition of biochemical (e.g., GTP hydrolysis, release of products, etc.) and structural changes (e.g., conformational changes in tubulin, "closing" of protofilaments into tubes [16], etc.).

If we identify A monomers with GTP tubulin, our model also predicts the outcome of a third type of experiment, in addition to the two types mentioned in the introduction. These experiments carefully measure the GTP content of rapidly grown MTs, but fail to find any [17,18]. This indicates that the GTP content of MTs always is small and/or able to hydrolyze before the measurements are made. According to our model, the cap is not the only patch of A monomer at a MT end; see Fig. 2. But the cap is typically the *largest* patch of A monomers. Hence, the time $\langle t \rangle_{\text{cat}} \sim 8.6$ s obtained from Eq. (7) for $v_f \approx -v_{AB} = -0.22\ \mu\text{m min}^{-1}$ overestimates the average lifetime of A monomers/GTP in a MT. Thus our model predicts that virtually all GTP is hydrolyzed in the 20 s dead time after growth is interrupted and before measurements are done, in full agreement with the experimental observations [18].

Each patch of A monomers found on the trail of the cap in Fig. 2 shrinks with velocity $v_{AB}^{(+)} + v_{AB}^{(-)}$, independent of the MT's velocity of growth v_g. But the *number* of patches adjusts dynamically to v_g, ensuring that the total rate of conversion of A to B monomers equals the rate of addition of A monomers at steady growth. If this conversion is hydrolysis, this adjustment is the much sought mechanism for so-called "coupled hydrolysis."

When loss of the cap causes disassembly from the end of the MT, and disassembly reaches a left-behind patch of A monomers, it continues right through it, we propose. This implies the existence of additional elements of the dynamics, such as the $A \rightarrow B$ transformation is propagated in front of the disassembling tip. The reversal of disassembly to assembly, called *rescue,* thus requires something more than a leftover patch of A monomers, according to our model [19].

This issue of rescues and many other questions have to be resolved before we fully understand dynamic instability, even on a phenomenological level. We hope that the model presented here is a useful step toward a more physical, quantitative approach in the study of these fascinating phenomena.

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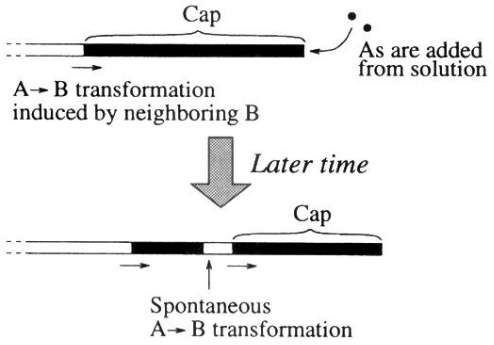
[1] T. Mitchison and M. Kirschner, Nature (London) **312**, 232 (1984).
[2] T. Mitchison and M. Kirschner, Nature (London) **312**, 237 (1984).
[3] M.-F. Carlier and D. Pantaloni, Biochemistry **20**, 1918 (1981).
[4] M. Caplow, Curr. Opinion in Cell Biol. **4**, 58 (1992).
[5] M.-F. Carlier, Int. Rev. Cytol. **115**, 139 (1989).
[6] Y. der Chen and T. L. Hill, Proc. Natl. Acad. Sci. U.S.A. **82**, 1131 (1985).
[7] H. P. Erickson and E. T. O'Brien, Annu. Rev. Biophys. Biomol. Struct. **21**, 145 (1992).
[8] S. R. Martin, M. J. Schilstra, and P. M. Bayley, Biophys. J. **65**, 578 (1993).
[9] D. N. Drechsel, A. A. Hyman, M. H. Cobb, and M. W. Kirschner, Mol. Biol. Cell **3**, 1141 (1992).
[10] R. A. Walker *et al.,* J. Cell Biol. **107**, 1437 (1988).
[11] W. A. Voter, E. T. O'Brien, and H. P. Erickson, Cell Motil. Cytoskeleton **18**, 55 (1991).
[12] R. A. Walker, N. K. Pryer, and E. D. Salmon, J. Cell Biol. **114**, 73 (1991).
[13] Each tubulin monomer has a size of 8 nm, but for MTs with 13 parallel protofilaments, each tubulin adds only 0.6 nm to the total length.
[14] *Handbook of Mathematical Functions,* edited by M. Abramowitz and I. A. Stegun (Dover Publications, New York, 1972), Chap. 10.4.
[15] H. Flyvbjerg, T. E. Holy, and S. Leibler (to be published).
[16] E. Karsenti (private communication).
[17] E. T. O'Brien, W. A. Voter, and H. P. Erickson, Biochemistry **26**, 4148 (1987).
[18] R. J. Stewart, K. W. Farrell, and L. Wilson, Biochemistry **29**, 6489 (1990).
[19] We find some experimental support for this hypothesis in the fact that catastrophes and rescues are affected very differently by chemical treatment. See, e.g., N. R. Gliksman, S. F. Parsons, and E. D. Salmon, J. Cell Biol. **119**, 1271 (1992); M. Caplow *et al.*, Biochemistry **28**, 8136 (1989).
[20] D. K. Fygenson, E. Braun, and A. Libchaber (unpublished).



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