Steady states of a microtubule assembly in a confined geometry

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We study the steady state of an assembly of microtubules in a confined volume, analogous to the situation inside a cell where the cell boundary forms a natural barrier to growth. We show that the dynamical equations for growing and shrinking microtubules predict the existence of two steady states, with either exponentially decaying or exponentially increasing distribution of microtubule lengths. We identify the regimes in parameter space corresponding to these steady states. In the latter case, the apparent catastrophe frequency near the boundary is found to be significantly larger than that in the interior. Both the exponential distribution of lengths and the increase in the catastrophe frequency near the cell margin is in excellent agreement with recent experimental observations.

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Microtubules are long, rigid polymers which play an important role in several cellular processes. In usual circumstances, microtubules form part of the cytosekelton network and provide rigidity to the cellular stucture. Microtubules are, however, highly dynamic structures, and constantly switch stochastically between states of growth or shrinkage, and may disappear altogether and nucleate again. This interesting behavior is called dynamic instability, following its discovery by Mitchison and co-workers [1–3]. When a growing microtubule starts shrinking by losing its monomer units, it is said to have undergone "catastrophe" and the reverse transition is called "rescue". The basic monomer unit of a microtubule is a dimer of α and β tubulin, which is approximately 8 nm in length. The $\alpha-\beta$ dimers are arranged head-to-tail along a microtubule in protofilaments (usually 13 per microtubule).

The highly dynamic nature of microtubules originates from the hydrolysis of β-tubulin bound Guanine Tri-Phosphate (GTP). Following hydrolysis, the GTP is converted to Guanine Di-Phosphate (GDP) [4], and the GDP-bound tubulin does not polymerize as well as its GTP counterpart. The microtubule is thus a potentially unstable structure, and alternates between states of polymerization and depolymerization. The stability of microtubules has been attributed to the existence of a "GTP cap" on a growing microtubule (i.e., a patch of *T*-tubulin at the end, while most of the microtubule is made of *D*-tubulin) [4], although convincing experimental support for this model is still lacking [5]. In this model, the microtubule becomes unstable and depolymerizes when the cap is lost following stochastic fluctuations in its length. It has also been suggested that the stabilization of microtubules is primarily due to the strong coupling between the rates of hydrolysis and polymerization [5]. Conformational changes in tubulin subunits following hydrolysis is also believed to initiate catastrophe. The relative rates of catastrophe and rescue, combined with the rates of growth and shrinkage determine the character of a given population of microtubules.

Theoretical and numerical models of microtubule dynamics based on the concepts of dynamic instability have been studied for more than a decade now [6,7]. An elegant and simple mathematical model which incorporated most of the important features of microtubule dynamics is due to Dogterom and Leibler [7], which shall be the basis of our study here. In this model, microtubules are assumed to nucleate and grow from a flat substrate, and the dynamics is characterized by the velocity of growth (v_g) and shrinkage (v_s), and the frequencies of catastrophe (ν_c) and rescue (ν_R). In the absence of any boundary which restricts the growth, a steady state is achieved when $\nu_R p_g < \nu_C p_s$, characterized by an exponentially decaying distribution of lengths. When this condition is not satisfied, no steady state is reached, and the length distribution is Gaussian, with the mean length increasing linearly with time, and the width evolving diffusively.

Inside cells, the growth of microtubules is constrained by the presence of the cell boundary. Experimental observations have shown that the parameters of microtubule dynamics show a strong dependence on the proximity to cell boundary [8]. In particular, the catastrophe frequency is markedly higher near the periphery, compared to the cell interior. The obvious explanation for this difference is that the growing microtubule loses its "GTP cap" upon hitting the cell boundary and is transformed to a shrinking state. In addition, the length distribution of microtubules is found to be exponentially increasing, with a possible dip near the boundary.

In this Brief Report, we show that the exponentially increasing length distribution of microtubules can be understood from the Dogterom-Leibler equations, and is a new steady state which is a direct consequence of the presence of the cell boundary. We compute the steady state distribution exactly, and find excellent agreement with experimental observations. We also show that the observed increase in the apparent catastrophe frequency near the cell margin can be understood quantitatively within this model.

Let us consider a set of microtubules nucleating from a substrate, and growing by the addition of tubulin dimers in

the direction perpendicular to the plane of the substrate (the z axis). For simplicity, we ignore the three-dimensional structure of individual microtubules, and treat them as one-dimensional polymers. Nucleation is assumed to take place at empty nucleation sites at a rate ν. A microtubule in the growing state adds T-tubulin at a rate p_g per unit time, and a microtubule in the shrinking state loses tubulin at a rate p_s per unit time. Also, a microtubule in the growing state switches to the shrinking state at a rate ν_c (catastrophe frequency), and a microtubule in the shrinking state switches to the growing state at a rate ν_R (rescue frequency). Both rescue and catastrophe are assumed to be purely stochastic events. The length of a "monomer unit" in our effectively one-dimensional polymer is denoted by δ (which is approximately $8\ \mathrm{nm}/13 = 0.6\ \mathrm{nm}$, since a microtubule has 13 protofilaments of tubulin arranged in parallel). We use δ as our unit of length for the rest of the paper (and, consequently, all length variables will be dimensionless).

Our principal aim in this paper is to study explicitly the steady state of the system in the presence of a boundary. We assume that this boundary is located at $z = l^*$. We denote by $p_+(l,t)$ the fraction of sites in the substrate which have microtubules of length l at time t in growing state, and $p_-(l,t)$ denotes the same fraction in shrinking state. By convention, the fraction of vacant sites in the lattice at time t is denoted $p_-(0,t)$ and $p_+(0,t) = 0$ at all times t. The discrete equations for the dynamics of this assembly, including growth, shrinkage, catastrophe, and rescue events are given by

$$\frac{\partial p_-(0,t)}{\partial t} = -\nu p_-(0,t) + p_s p_-(1,t) \tag{1}$$

and

$$p_+(0,t) = 0,$$

$$\frac{\partial p_+(1,t)}{\partial t} = \nu p_-(0,t) - p_g p_+(1,t) - \nu_C p_+(1,t) + \nu_R p_-(1,t), \tag{2}$$

$$\frac{\partial p_+(l,t)}{\partial t} = p_g[p_+(l-1,t) - p_+(l,t)] + \nu_R p_-(l,t) - \nu_c p_+(l,t), \quad 1 < l < l^*, \tag{3}$$

$$\frac{\partial p_-(l,t)}{\partial t} = p_s[p_-(l+1,t) - p_-(l,t)] + \nu_c p_+(l,t) - \nu_R p_-(l,t), \quad 1 \leqslant l < l^*. \tag{4}$$

The presence of the boundary affects the dynamics of the system in the following way: When a growing microtubule reaches a length l^*, it is instantaneously transformed to the shrinking state with length l^*. The equations representing this process are given by

$$\frac{\partial p_-(l^*,t)}{\partial t} = p_g p_+(l^*-1,t) - p_s p_-(l^*,t),$$

$$p_+(l^*,t) = 0. \tag{5}$$

To find the steady state of the system, we set all time derivatives to zero. For simplicity, we also omit time from the expressions for all steady state quantities. From Eqs. (1) and (2) we get the following relations:

$$p_-(0) = \frac{p_s}{\nu} p_-(1), \tag{6}$$

$$p_+(1) = \frac{\nu p_-(0) + \nu_R p_-(1)}{p_g + \nu_c}. \tag{7}$$

After combining Eqs. (6) and (7), we find that

$$p_-(1) = \frac{p_g + \nu_c}{p_s + \nu_R} p_+(1), \tag{8}$$

and, after using Eq. (6) again,

$$p_-(0) = \frac{p_s}{\nu}\left[\frac{p_g + \nu_c}{p_s + \nu_R}\right] p_+(1). \tag{9}$$

For $l > 1$, we find the following relation between $p_+(l)$ and $p_-(l)$ from Eqs. (3) and (4) [using only $l > 1$ in Eq. (4)]:

$$p_-(l+1) - p_-(l) = \frac{p_g}{p_s}[p_+(l) - p_+(l-1)], \quad l \geqslant 2. \tag{10}$$

The general solution of this equation is

$$p_-(l) = \frac{p_g}{p_s} p_+(l-1) + C, \quad l \geqslant 2, \tag{11}$$

where C is an unknown constant. After substituting Eq. (11) in Eq. (3) [and after equating the left-hand side (LHS) of Eq. (3) to zero], we obtain the following equation for $p_+(l)$:

$$\left[1 + \frac{\nu_R}{p_s}\right] p_+(l-1) + \frac{\nu_R}{p_g} C = \left[1 + \frac{\nu_c}{p_g}\right] p_+(l), \quad l \geqslant 2. \tag{12}$$

The solution to this equation has the form

$$p_+(l) = A a^l + B, \quad l \geqslant 1 \tag{13}$$

as may be verified by direct substitution. After equating terms with the same power of l, we obtain the following expressions for the constants a and B:

$$a = \frac{1 + \dfrac{\nu_R}{p_s}}{1 + \dfrac{\nu_c}{p_g}}, \tag{14}$$

$$B = \left[\frac{\nu_R p_s}{\nu_c p_s - \nu_R p_g}\right] C. \tag{15}$$

The constant C may now be determined as follows. From Eq. (4), for $l = 1$, we have

$$p_s[p_-(2) - p_-(1)] = \nu_R p_-(1) - \nu_C p_+(1) \tag{16}$$

in the steady state, whereas from Eq. (11) we have another relation

$$p_-(2) = \frac{p_g}{p_s} p_+(1) + C. \tag{17}$$

We now substitute Eqs. (8) and (17) into Eq. (16) and solve for C, which gives $C=0$. From Eq. (15), this also implies $B=0$. It remains to determine the constant A, which is found using normalization

$$\Sigma_{l=0}^{l^*} p_-(l) + \Sigma_{l=1}^{l^*-1} p_+(l) = 1 \tag{18}$$

which may be written as

$$p_-(0) + p_-(1) + \left(1 + \frac{p_g}{p_s}\right) \Sigma_{l=1}^{l^*-1} p_+(l) = 1 \tag{19}$$

after using Eq. (11). We now use Eqs. (8), (9), and (13) in Eq. (19). The final result is

$$A = \left[\frac{p_g}{\nu} + \frac{p_g}{p_s + \left(\frac{p_g + p_s}{p_s}\right)\left[\frac{a^{l^*} - a}{a-1}\right]} \right]^{-1}. \tag{20}$$

The solution in Eq. (13) can also be written as

$$p_+(l) = A e^{\alpha l}, \quad \alpha = \ln\left[\frac{1 + \frac{\nu_R}{p_s}}{1 + \frac{\nu_C}{p_g}} \right]. \tag{21}$$

The complete length distribution is $p(l)=p_+(l)+p_-(l)$, and may now be written explicitly:

$$p(l=1) = A\left(\frac{p_g}{p_s} + a\right), \tag{22}$$

$$p(l) = A\left(1 + \frac{p_g}{a p_s}\right) e^{\alpha l}, \quad 1 < l < l^*,$$

$$p(l=l^*) = \frac{p_g}{p_s} \frac{A}{a} e^{\alpha l^*}. \tag{23}$$

If $\nu_R/p_s < \nu_C/p_g, \alpha < 0$, we have an exponentially decaying solution. On the other hand, if $\nu_R/p_s > \nu_C/p_g, a > 1$ and $\alpha > 0$, we have an exponentially increasing steady state distribution of lengths.

When we consider the behavior of the solution in the limit $l^* \to \infty$, from Eq. (20), we see that, in this limit, a steady state is possible only if $a<1$. For, if $a>1$, then $A \sim a^{-l^*}$ for large l^* and vanishes as $l^* \to \infty$. For $a<1$ and $l^* \to \infty$, $(a^{l^*} - a)/(a-1) \to a/(1-a)$, and so

$$A_{l^* \to \infty} = \left[\frac{p_g}{\nu} + \frac{p_g}{p_s + \left(\frac{p_g + p_s}{p_s}\right)\frac{a}{1-a}} \right]^{-1} \quad a < 1. \tag{24}$$

The exponentially decaying steady state length distribution when $l^*=\infty$, with $\nu_R/p_s < \nu_C/p_g$, has been predicted by Dogterom and Leibler in an earlier work [7]. The novel feature in the finite l^* case is the steady state with exponentially increasing distribution of lengths when $\nu_R/p_s > \nu_C/p_g$.

The exponentially increasing distribution of microtubule lengths has indeed been observed in experiments with real cells. Direct observation of microtubules inside cells has been made possible recently [8]. These experiments, done on centrosome-containing cytoplasts, observed almost persistent growth of microtubules almost up to the cell boundary. However, the catastrophe rate showed a dramatic increase within a zone about 3 μm near the cell margin (0.08 s^{-1}, compared to 0.005 s^{-1} in the cell interior). The other parameters describing the microtubule dynamics were $\nu_R \approx 0.12\ s^{-1}, v_g \approx 17.8 \pm 13.8\ \mu m/min, v_s \approx 28.8 \pm 14.1\ \mu m/min$. The parameters p_g and p_s are related to v_g and v_s as $p_g = v_g/\delta$ and $p_s = v_s/\delta$.

As a first test of our model, we compute the increase in catastrophe frequency near the cell margin. Let us consider all microtubules with length between l_1 and l^*. The total number of such microtubules is given by $N = \Sigma_{l_1}^{l^*} p(l)$. Using the expression for $p(l)$ from Eq. (23), we find that

$$N = \frac{A}{\alpha}\left[1 + \frac{p_g}{a p_s}\right][e^{\alpha l^*} - e^{\alpha l_1}].$$

The number of microtubules in this set undergoing catastrophe per unit time is given by

$$N^* = \nu_c N + p_g p_+(l^* - 1),$$

where the first term is the standard catastrophe term, and the second term represents the additional catastrophe events arising from the microtubules hitting the boundary. The apparent catastrophe frequency is given by $\nu_c^* = N^*/N$. After substituting for $p(l)$ and $p_+(l^*-1)$, we find that

$$\nu_c^* = \nu_c + \frac{p_g \alpha}{1 + \frac{p_g}{a p_s}} \frac{e^{-\alpha}}{1 - e^{-\alpha \Delta}}, \tag{25}$$

where $\Delta = (l^* - l_1)/\delta$. After substituting for all the numerical values and for $l^* - l_1 \simeq 3\ \mu$m as in experiments, we find that $\nu_c^* \simeq 0.0964\ s^{-1}$. This is in excellent agreement with the experimentally measured value of 0.08 s^{-1}.

It is also interesting to compare the experimentally measured value of α with the theoretical value. The observed steady state length distribution is found to fit well with an exponential function $P(l) \sim e^{\gamma l}$ with $\gamma^{-1} \simeq 5.8\ \mu$m.[8] We can convert this value to dimensionless units by multiplying with our unit of length, which gives $\alpha_{exp} = \delta \gamma^{-1} \simeq 1.03 \times 10^{-4}$. The theoretical value is found from Eq. (21), using the measured values of all the parameters, and turns out to be $\alpha \approx 1.5 \times 10^{-4}$. This is also in very good agreement with the experimental value. The discrepancy between the computed and observed values may be attributed to the significant experimental error in the measurements of v_g and v_s.

To conclude, we have studied the steady state of a microtubule assembly in a confined geometry, where the growth of individual microtubules is restricted in length. We found that, in addition to the exponentially decaying length distribution

in an infinite system, there is a novel steady state with exponentially increasing distribution of lengths. This prediction is in excellent agreement with experimental observations in real cells, and is thus a direct verification of the dynamical instability model of microtubule dynamics.

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