The effect of minus ends on the microtubule steady state

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Abstract

Dynamic instability describes microtubule assembly in which individual microtubules exhibit alternating phases of elongation and rapid shortening. This dynamic is significantly different for the plus ends and minus ends of a microtubule. In this work, by considering the contribution of the plus and minus ends, we have investigated the behavior of T-tubulin concentration in the steady state in a regeneration system in the presence and absence of minus ends of microtubules.

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1 Introduction

A microtubule (MT) is a protein polymer that is involved in several biological functions in eukaryotic cells. Microtubules are vital cell structures that can be formed from molecules of globular protein tubulin(GTP-tubulin) in vitro [1]. Tubulin is an approximately 8×10^{-9} μm-long dimer composed of two monomers α and β [2]. This arrangement produces an inherent polarity in their structure, resulting in differences in their ends. The fast growing end is called the plus end and the slow growing end is minus end. The studies of Mitchison and Kirschner [3, 4], proved that dynamic instability is the mechanism of microtubule assembly and dis-assembly, a process in which the polymers alternate between polymerization and depolymerization [5]. Microtubules were observed to undergo dynamic instability at both ends. In this paper, we have created a model for the dynamics of microtubules which grow from both ends. In the following sections, the model and basic assumption for a cyclic polymerization is introduced, the dynamics of polymerization is described and the steady state solution and behavior of T-tubulin concentration in the steady state is presented.

2 Dynamics of growing and shrinking of microtubules

Under appropriate conditions and in the presence of GTP, a microtubule can be nucleated and switch between polymerization and depolymerization phases. This dynamic instability can be caused by the hydrolysis of GTP to tubulin protein (GDP) [6]. In some experiments and in the absence of excess free GTP a net microtubule assembly phase and a net microtubule disassembly phase occur sequentially [5] while in other experimental systems either excess GTP or GTP-regenerating systems can be considered. In these systems there is a cyclic polymerization-depolymerization dynamics of the protein tubulin. In GTP-regeneration systems depolymerized microtubules directly convert into GDP tubulin dimers, D-tubulin. If an excess of GTP is available, D-tubulin in solution will exchange its unit of GDP for GTP and the tubulin-T resulting from such an exchange step is identical to the initial tubulin-T dimer. These systems have been studied by several groups [7, 8, 9]. In this paper the regenerating system is considered as a basis for microtubules which grow from both ends. In Fig. 1, a schematic view of the model is shown. The regeneration factor α is global and unique for both ends while there is a separate cycle of polymerization for each end.

If we consider the two different phases of microtubules and neglect concentration variations, the following detailed balance equations can be written for the dynamics of microtubules [6, 10]:

$$\partial_t P_{g_{(i)}} = -f_{c_{(i)}} P_{g_{(i)}} + f_{r_{(i)}} P_{s_{(i)}} - v_{g_{(i)}} \partial_l P_{g_{(i)}}, \tag{1a}$$

$$\partial_t P_{s_{(i)}} = +f_{c_{(i)}} P_{g_{(i)}} - f_{r_{(i)}} P_{s_{(i)}} + v_{s_{(i)}} \partial_l P_{s_{(i)}}. \tag{1b}$$

where i stands for the (+) or (-) end of a microtubule and f_c is the frequency of catastrophe and f_r is frequency of rescue and v_g and v_s are velocity of growing and shrinking of a microtubule.

We assume that the plus and minus ends of microtubules experience independent stochastic processes, so that by choosing appropriate dynamical parameters $(v_{s_i}, v_{g_i}, f_{c_i}, f_{r_i})$ and with the help of Eq. (1), the dynamic instability behavior of each end can be described mathematically. The above equations have to be supplemented by boundary conditions. In this work, we consider a spontaneous nucleation with the rate ν instead of nucleation on stable a centrosome.

Immediately after nucleation, some of the total T-tubulin concentration in the solution interacts with the polymerization cycle of the plus end and some with the minus end. The time variation of T-tubulin concentration in each cycle can be written using the following equations [9, 11]:

$$\partial_t C_{T_{(i)}} = -\gamma v_{g_{(i)}} \int_0^{a(b)} P_{g_{(i)}}(l,t) \mathrm{d}l + \alpha C_{d_{(i)}}, \tag{2a}$$

$$\partial_t C_{d_{(i)}} = +\gamma v_{s_{(i)}} \int_0^{a(b)} P_{s_{(i)}}(l,t) \mathrm{d}l - \alpha C_{d_{(i)}}. \tag{2b}$$

where "i" indicates the polymerization cycle of the plus end or minus end and γ is a length factor describing the number of tubulin dimers that are incorporated in a unit length of microtubules. The length distribution or age of the plus ends has been considered between 0 and a and for the minus ends, between 0 and b.

We also emphasize that:

$$C_{T_{(+)}} + C_{T_{(-)}} = C_T. \tag{3}$$

The conservation of tubulin dimers can be expressed by the following condition:

$$C_{T_{(+)}} + C_{d_{(+)}} + \gamma L_{(+)} = mC_0, \tag{4a}$$

$$C_{T_{(-)}} + C_{d_{(-)}} + \gamma L_{(-)} = nC_0, \tag{4b}$$

$$m + n = 1, \tag{4c}$$

where C_0 is overall concentration of tubulin dimers. $L_{(i)}(t)$ is the integrated length of all microtubules per unit volume for each end and is equal to:

$$L_{(i)}(t) = \int_0^{a(b)} l \left[P_{g_{(i)}}(l,t) + P_{s_{(i)}}(l,t) \right] \mathrm{d}l, \tag{5a}$$

$$L(t) = L_{(+)} + L_{(-)}. \tag{5b}$$

Where L(t) is the total length average. With the help of Eqs. (2-5), the time variation of the T-tubulin concentration can be expressed by the following equation:

$$\partial_t C_T = -\gamma \int_0^a \left[v_{g_{(+)}} P_{g_{(+)}} + \alpha l (P_{g_{(+)}} + P_{s_{(+)}}) \right] \mathrm{d}l$$

$$-\gamma \int_0^b \left[v_{g_{(-)}} P_{g_{(-)}} + \alpha l (P_{g_{(-)}} + P_{s_{(-)}}) \right] \mathrm{d}l + \alpha (C_0 - C_T). \tag{6}$$

3 Stationary stage

By definition, steady state for microtubule dynamics implies time invariant kinetic parameters. The steady state solution for Eq. (1) is then:

$$P^0_{g,s_{(i)}}(l) = \frac{\nu}{v_{(g,s)_{(i)}}} \exp(A.l_{(i)}). \tag{7}$$

Where $A = \frac{f^0_{r(i)}}{v_{s_{(i)}}} - \frac{f^0_{c(i)}}{v_{g_{(i)}}}$. We have assumed that in the treadmilling steady state the length distribution of the plus end is between (0 to ∞) and the distribution for the minus end is (0 to b). An addition constraint should be implied to the steady state solution to fulfill the requirement of treadmilling steady state in vitro. This condition is:

$$\gamma v_{s_{(-)}} \int_0^b P^0_{s_{(-)}}(l) \mathrm{d}l = \gamma v_{g_{(+)}} \int_0^\infty P^0_{g_{(+)}}(l) \mathrm{d}l. \tag{8}$$

Through this assumption we have a condition for the length distribution of the minus end, i.e.:

$$b = \frac{1}{A} \ln \left(1 + A \frac{v_{g_{(+)}}}{f_{c_{(+)}}} \right). \tag{9}$$

In our calculation we assumed that the frequency of rescue in the steady state can be neglected for the plus end, the growing velocity is a factor of two greater for the plus end than minus end, the shrinking velocity is almost the same for the plus and minus end of a microtubule and catastrophe frequency of minus end is half of the catastrophe frequency of plus end. The above assumptions are compatible with the experimental data reported Walker et al. [5]. In the following, the behavior of T-tubulin concentration is studied in the treadmilling steady state and in the presence of dynamical instability for each end.

3.1 Behavior of T-tubulin concentration in the treadmilling steady state

In the stationary phase, the left hand side of Eq. (6) vanishes. We assume that the frequency of catastrophe and rescue both exhibit exponential C_T dependence [8]. With the help of Eq. (9), the stationary T-tubulin concentration C_T^0 can be determined by a nonlinear self-consistent equation:

$$C_0 - C_T^0 = \frac{\gamma\nu v_{g_{(+)}}}{f_{c_{(+)}}}\left(\frac{1}{\alpha} + \frac{1}{f_{c_{(+)}}}(1 + \frac{v_{g_{(+)}}}{v_{s_{(+)}}})\right) + \frac{\gamma\nu}{A\alpha}\left[\exp(Ab) - 1\right] + \frac{\gamma\nu}{A^2}(\frac{1}{v_{g_{(-)}}} + \frac{1}{v_{s_{(-)}}})\left[1 + \exp(Ab)(-1 + Ab)\right]. \tag{10}$$

Where $A = \frac{f^0_{r(-)}}{v_{s_{(-)}}} - \frac{f^0_{c(-)}}{v_{g_{(-)}}}$.

$$f_c = f\exp(\frac{-C_T}{C_f}), \tag{11}$$

$$f_r = f'\exp(\frac{+C_T}{C_{f'}}). \tag{12}$$

While f and f', C_f and $C_{f'}$ are constant. The behavior of T-tubulin concentration has been investigated via numerical analysis as shown below.

In Fig. 2, the T-tubulin concentration in the treadmilling steady state as a function of regeneration factor is shown. Order of magnitude of growing and shrinking velocity obtained from Ref. [11].

4 Conclusion

In this work, we have presented a mathematical model for the dynamics of a microtubule in the regeneration system when it grows from both ends. We assumed that with separate sets of dynamical parameters, each end can experience dynamical instability and the system can reach the treadmiling steady state for a short time. We found that, in the steady state, plus ends have the exponentially decaying length distribution and minus ends have exponentially increasing distribution of length while the the growth of minus ends is restricted because of the treadmilling condition. In this system, the behavior of T-tubulin concentration in the steady state as a function of regeneration rate is analyzed through numerical calculations.

The amount of T-tubulin concentration is a function of the regeneration factor for a small amount of α and independent of regeneration factor when α is large enough, the reason being that a large regeneration rate causes the D-tubulin population to be constantly converted to T-tubulin.

Also, as anticipated, We see that the amount of T-tubulin concentration in the steady state decreases and therefore steady state polymer mass increases in the presence of free minus ends.

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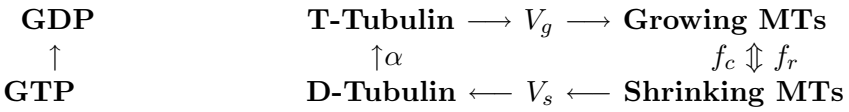

Figure 1: A microtubule can be polymerized from tubulin-T. Polymerization happened by the growing velocity V_g. Catastrophic and rescue frequencies are two dynamical parameters that can cause a transition between polymerization and depolymerization stage. In the depolymerization stage, shrinking microtubules convert to D-tubulin by the shrinking velocity V_s. The cycle becomes closed by regeneration of D-tubulin at the rate α back to T-tubulin dimers.

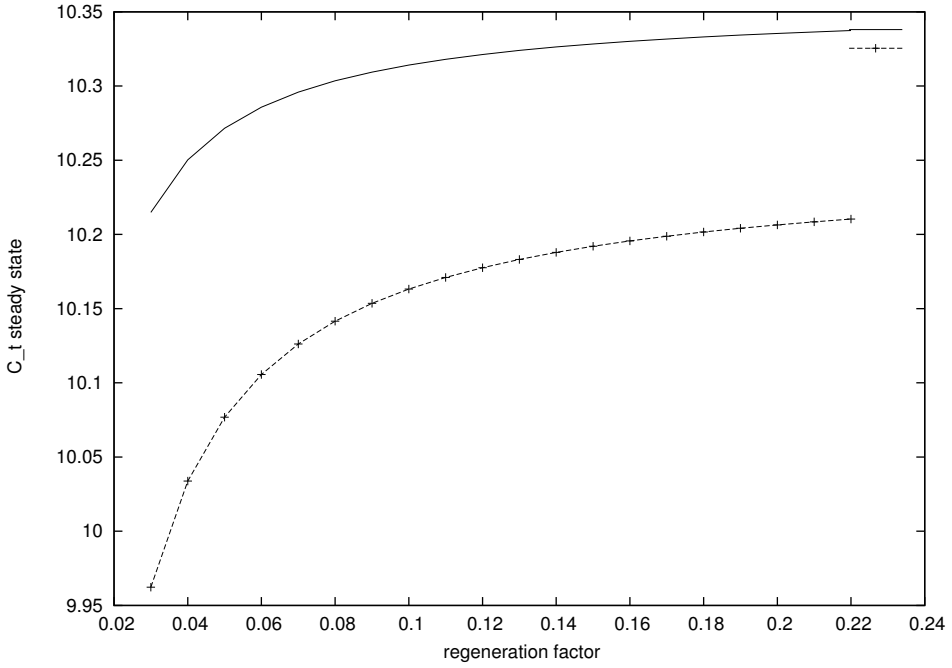

Figure 2: Solid line: the tubulin- T concentration for the stationary polymerization C_t^0 in the presence of the microtubule's plus ends. Point line: C_T^0 in the presence of both ends of microtubule. The parameters are $C_0 = 120$, $\gamma = 1$, $V_{g_{(+)}} = 2V_{g_{(-)}} = 0.1$, $V_{s_{(+)}} = V_{s_{(-)}} = 1$, $\beta_{(+)} = 0.1$, $\nu = 0.01$, $f = f' = 0.1$, $C_f = C_{f'} = 3$, $f_{c_{(-)}} = 0.5 f_{c_{(+)}}$.