Role of GTP remnants in microtubule dynamics

Sumedha, Michael F Hagan, and Bulbul Chakraborty

Martin Fisher School of Physics, Brandeis University, Waltham, MA 02454, USA

We study a model of microtubule assembly/disassembly in which GTP bound to tubulins within the microtubule undergoes stochastic hydrolysis. In contrast to models that only consider a cap of GTP-bound tubulin, stochastic hydrolysis allows GTP-bound tubulin remnants to exist within the microtubule. We find that these buried GTP remnants enable an alternative rescue mechanism, and enhances fluctuations of filament lengths. Our results also show that in the presence of remnants microtubule dynamics can be regulated by changing the depolymerisation rate.

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Microtubules are semiflexible polymers that serve as structural components inside the eukaryotic cell and are involved in many cellular processes such as mitosis, cytokinesis and vesicular transport. In order to perform these functions, microtubules (MTs) continually rearrange through a process known as dynamic instability (DI), in which they switch from a phase of slow elongation to rapid shortening (catastrophe), and from rapid shortening to growth (rescue). Despite extensive experimental efforts over several decades, it has not been possible to discriminate between models that have been proposed to explain DI. In this letter, we study a minimal model of DI that involves stochastic hydrolysis (SH), a mechanism that has received relatively little attention compared to interfacial hydrolysis (IH) that forms the basis of cap models. With recent advances in experimental techniques, it has become possible to quantify MT dynamics at nano-scale and, thereby, provide more stringent tests of models. We make predictions that clearly distinguish between SH and IH mechanisms, and experimental tests of these predictions should clarify the nature of hydrolysis in MT dynamics.

MTs are formed by assembly of α − β tubulin dimers, which are polar and impart polarity to MTs. MTs grow mainly from the end that has exposed β tubulin, and are composed of (typically) 13 linear protofilaments.

While a free tubulin dimer has a GTP molecule bound to each monomer, incorporation into a MT activates the β-tubulin monomer for hydrolysis of its associated GTP. GDP-bound tubulin is less stable within the MT lattice and hence a GDP-bound tubulin at the tip of a MT has a higher rate of detachment (depolymerization) than a GTP-bound tubulin. While it is established that GTP hydrolysis is essential to DI, the mechanism of the process is not fully understood. Models in the IH class assume that all hydrolysis occurs at a sharp interface between GDP-bound and GTP-bound tubulins, whereas in SH-based models, hydrolysis occurs stochastically, anywhere in the MT. We study a minimal model of DI that involves stochastic hydrolysis. In contrast to models that only consider a cap of GTP-bound tubulin, stochastic hydrolysis allows GTP-bound tubulin remnants to exist within the microtubule. We find that these buried GTP remnants enable an alternative rescue mechanism, and enhances fluctuations of filament lengths. Our results also show that in the presence of remnants microtubule dynamics can be regulated by changing the depolymerisation rate.

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1. Attachment: If the growing tip is a + monomer, it grows with rate λ by addition of a + subunit.
2. Detachment: A − monomer at the growing end detaches with rate µ, causing its shrinkage.
3. Hydrolysis: With rate 1 any + monomer in the MT can undergo hydrolysis to yield a − monomer.
4. Non-remnant Rescue: + subunits could attach to a growing end with a − monomer at the tip with rate pλ (p ≤ 1).

A previous study of the model for p > 0, demonstrated a transition from a phase of bounded to unbounded growth of the MTs. The present study will focus on low p and fluctuations in the bounded growth region of the phase diagram.
Recent experiments monitored the distribution of lengths of growing and shortening excursions in \textit{in vitro} systems of MTs. These experiments were able to resolve fluctuations at the monomer level, and the distributions were found to be exponential. In simulations of our model we find that for small values of $p$ the growth excursions are independent of $\mu$ and can be fitted well by $\exp(-i/\lambda)/\lambda$ ($i$ is the length of the excursion). The form of the distribution of shortening excursions changes non-trivially with $\mu$ (Fig. 4).

We can fit the experimental data on the distribution of excursions with our model by taking $\lambda = 12; \mu \approx 0.1; 0 \leq p \leq 0.001$ (Fig. 1). The fitting provides tight bounds on the parameters, if we demand good fits to both positive and negative excursions simultaneously. For the low values of $p$ obtained from the fits, rescue due to remnants dominates DI. Rare non-remnant rescues do influence the steady state but have very little effect on the statistics of fluctuations. Hence, in order to highlight the effect of remnants on DI we analyze in detail the $p = 0$ model, which we call the GTP remnants model since the only mode of rescue is via remnants. The presence of remnants and the correlation of their location with rescue events is consistent with recent experiments. In particular, Perez et al. [9] observe GTP-bound tubulin within mature, MT growth.

The probability distribution of total length of the MT $L(t)$ at time $t$ follows the following equation:

$$
\frac{dP(L, t)}{dt} = \lambda(1 - n_0(t))[P(L - 1, t) - P(L, t)] + \mu n_0(t)[P(L + 1, t) - P(L, t)]
$$

where $n_0(t)$ is the probability of having a GDP at the tip of MT and is given by:

$$
\frac{dn_0(t)}{dt} = 1 - n_0(t) - \mu n_0(t)P(+- > , t)
$$

The average length at any time $< L(t) > = \lambda(1 - \exp(-t))$. Similarly, the average number of GTP-bound tubulins at time $t$ is $< T(t) > = \lambda t \exp(-t)$. Hence, the average time at which the amount of GTP goes to zero scales roughly as $\ln(\lambda)$. This is different from the exponential dependence of $t_N$ on $\lambda$ for large $\mu$ indicated by the scaling collapse in Fig. 2. As seen in Fig. 2, $< t_N >$ increase monotonically from a value of order $O(\ln \lambda)$ to a value of order $\exp(\lambda)$ as we increase $\mu$ from 0 to $\infty$.

Another limit where we can obtain exact results for $< L(t) >$ and $< T(t) >$ is $\lambda \to \infty, \mu \to \infty$. In this limit,
The average time at which the number of GTP-bound subunits goes to zero ($< t_N >$) as a function of $\ln \mu$. The inset shows a scaling collapse of the plots, with $y$-axis scaled by $e^\theta/\lambda$ and the $x$-axis displaced by $\lambda$, for $\lambda = 8, 10, 12$. Right: The average growth velocity ($v = < L(t_N) > / < t_N >$) for indicated values of $\lambda$ as a function of $\ln \mu$. In the inset, we have scaled $v$ by $\mu(\lambda - 1)/(\mu + 1)$ to show the scaling collapse for $\lambda = 5, 8, 10, 12$ and $\mu > 1$. The dataset which does not fall on the same curve corresponds to $\lambda = 3$.

$P(+-, t)$ can be assumed to be equal to unity for all $t$. We found numerically that most catastrophes were of $O(1)$ and hence this approximation provides a sensible description of the MT behaviour in the growing phase for any $\lambda$ and $\mu$. With $P(+-, t) = 1$, we get,

$$P(L, t) = (\lambda)^L/2 I_L \left( \frac{2\mu\lambda^{1/2}}{1 + \mu} \right) \exp \left( -\mu(\lambda + 1)t \right)$$

where $I_L(x)$ is the modified Bessel function of first kind and Eq. (4) leads to:

$$< L(t) > = \frac{\mu(\lambda - 1)}{(1 + \mu)}t$$

FIG. 3: (a)-(c) The distributions of times at which the amount of GTP in the MT goes to zero ($t_N$). The $x$-axis is scaled by $\ln \lambda$ (the average value of $t_N$ for $\mu = 0$). The distribution shifts to the right with increasing $\mu$ until it saturates. (d) The distribution of maximum lengths for $\lambda = 5$. The distributions of maximum lengths and $t_N$ have similar dependencies on $\mu$.

The result that the MT is always in the growing phase for $\lambda > 1$ is a consequence of the assumption $P(+-, t) = 1$, which breaks down at some finite time $t$, for arbitrary values of $\lambda$ and $\mu$. From Fig. 2 (Right), however, it is seen that the growing stage is very well approximated by Eq. 5 as the velocity obtained from simulations matches well with the expression of average velocity in Eq. 5. In fact, for a given $t$, the distribution of lengths (Eq. 4) broadens with $\mu$ in a manner similar to that seen in the simulations (Fig. 3). The variance of distribution of $P(L, t)$ from Eq. 4 comes out to be $\frac{2\mu}{1 + \mu}$, which depends only on $\mu$ and $t$.

Similarly, one can solve for the distribution and mean of $T(t)$:

$$T(t) = \frac{\lambda\mu}{1 + \mu} - \frac{\lambda \exp(-(1 + \mu)t)}{\mu(1 + \mu)} - \frac{\lambda(\mu - 1) \exp(-t)}{\mu}$$

This equation also matches the simulation results, as we found that the average amount of GTP in the MT during the growing phase fluctuates around $\frac{\lambda\mu}{1 + \mu}$.

A number of other predictions can be made in this limit of large $\lambda$ and $\mu$, which applies to the growing phase, $t << t_N$.

FIG. 4 shows the time trace for MT length for a representative
run for \( p = 0 \) and \( p = 0.01 \). Analysis of these trajectories shows that a small, non-zero value of \( p \) introduces rare rescue events (indicated by arrows in the figure). These events change the overall length of MT, but the statistics of growth, rescue and catastrophes remain similar to \( p = 0 \). Measurements of these statistics should, therefore, provide tests of the remnants model of DI and provide clear distinction between IH models that do not have remnants and SH models which do.

Our model can be easily extended to accommodate more detailed features of MTs while keeping the basic mechanism of remnant-dominated rescues. For example, spatially varying hydrolysis rates due to the structure of MTs [7] can be modeled by quenching some GTP-bound sites in our 1-d model. Similarly, the effect of motors that have remnants and SH models which do.

Preliminary studies of the model with quenched GTP-states [14] can be modeled by assuming a depolymerizing rate \( \mu \) for both GTP and GDP-bound tubulins. Preliminary studies of the model with quenched disorder indicate that, although the time for which MT grows changes and there is a transition to unbounded growth as a function of percent of quenched sites, the distribution of excursions and velocity of growth remains unchanged, and therefore is a robust feature of the remnant model. Studies of the model mimicking motors also indicate that the basic features of the remnant model remain unchanged as long as \( \lambda > \mu + 1 \).

To summarize, we have studied the role of rescue due to remnants in MT dynamics, and shown that remnants give rise to features of DI that are very different from IH models. These features are robust, and with recent progress in experimental techniques [8, 10], should provide tools for resolving the mechanism of hydrolysis inside a MT. Some particularly notable features of SH models, where remnants play an important role, that distinguish them from IH models are: 1) The distribution of MT lengths is not exponential but instead depends on \( \lambda \) and \( \mu \), broadening as \( \lambda \) and \( \mu \) are increased. Measurements of rates of growth and shortening of individual MTs, both in-vivo and in-vitro, indeed exhibit variability that cannot be explained by exponential distributions [13]. 2) The distribution of times for which a MT grows before a complete collapse also has a broad, non-exponential distribution in SH models. 3) The velocity of growth, besides depending on the free tubulin concentration (through \( \lambda \)), also depends on depolymerisation and hydrolysis rates. Interestingly, at the same tubulin concentration, MTs exhibit much higher growth rates in-vivo in comparison to in-vitro [17]. In conclusion, our study of a minimal model of MT DI, leads to predictions that provide sensitive tests of the mechanism of hydrolysis, and of the presence of GTP remnants in MTs. Experimental tests of these predictions should lead to a better understanding of how DI is controlled through various agents both in-vivo and in-vitro.

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