Models of Assembly and Disassembly of Individual Microtubules: Stochastic and Averaged Equations

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Abstract. In this paper we present solutions of the master equations for the microtubule length and show that the local probability for rescues or catastrophes can lead to bell-shaped length histograms. Conversely, as already known, non-local probabilities for these events result in exponential length histograms. We also derive master equations for a stabilizing cap and obtain a new boundary condition which provides an explanation of the results obtained in dilution and cutting experiments.

Key words: Microtubules, Dynamic instability, Master equations.

1. Introduction

Microtubules are formed from the dimers of tubulin which is a protein existing in two main homologous varieties, namely: α -tubulin and β -tubulin. An individual MT is a hollow 25 nm radius cylinder built usually from 13 linear protofilaments each of which is composed of alternating α - and β -subunits, although different numbers of protofilaments (between 12 and 17) have been seen, especially in experiments in vitro (Chretien et al. 1995). The geometry of the MT lattice is a separate issue and the reader is referred to a recent review article for a more in-depth discussion (Amos 1995). Although tubulin subunits form a regular array, each microtubule is characterized by its polarity i.e. its distal (plus) end grows faster than the proximal (minus)-end.

Our interest in this paper is focused on MT polymerisation and depolymerisation processes which, due to their unique nature among biopolymers, have been termed the dynamic instability phenomenon (Mitchison and Kirschner 1984a, 1984b). In spite of over a decade of analyses of these effects, complete understanding of the microscopic nature of, and the physico-chemical mechanism behind, dynamic instability is still elusive.

A simple picture of the cycle of MT assembly and disassembly is illustrated in Figure 1. Table 1 gives an overview of the chemical reactions involved. The following discussion of experimental aspects will help to motivate our models. The

Figure 1. A reaction cycle diagram for MT assembly.

Table I. A list of key chemical reactions involved in microtubule assembly

Reaction type	Equation	Rate
1. Nucleation	$n_c T \rightarrow M_g(n_c)$	k_n
2. Growth	$M_g(n) + T \rightarrow M_g(n+1)$	k_g
3. Shrinkage	$M_g(n) \rightarrow M_g(n-1) + T$	k_s
4. Catastrophe	$M_g(n) \rightarrow M_c(n)$	k_c
5. Collapse (decay)	$M_c(n) \rightarrow M_c(n-1) + D^+$	k_d
6. Rescue	$M_c(n) \rightarrow M_g(n)$	k_r
7. Thermalization	$D^+ \rightarrow D$	k_t
8. Reactivation (pumping)	$D + \text{GTP} \rightarrow T + \text{GDP}$	k_p

T is GTP-tubulin, D^+ are depolymerization products, D is GDP-tubulin, $M_g(n)$ is a microtubule in the growing state which consists of n tubulin dimers, $M_c(n)$ is a microtubule in the collapsing state and n_c is the size of the critical nucleus in a model with homogeneous nucleation.

first reaction, the nucleation of a new MT, is in fact a very complicated process. We will not explicitly include the nucleation in the models since it can be effectively incorporated via an appropriate choice of the boundary and normalization condition. A constant number of MTs is consistent with experiments with stabilized seeds. Under these conditions the MTs have only one free end so that we can neglect their polarity.

The rate of growth increases almost linearly with GTP-tubulin concentration whereas the rate of shrinkage is constant (Horio and Hotani 1986; Walker et al. 1991). Only tubulin dimers to which GTP is bound are capable of binding to a MT.

Upon binding, GTP rapidly hydrolyses into GDP (Erickson and O'Brien 1992) except perhaps for the top layer or two which is commonly referred to as the GTP-cap (Pantaloni and Carlier 1986; Bayley 1990). It is this chemical energy supply, transfer and dissipation which is at the center of the dynamic instability puzzle. The rates at which the growing and shrinking processes occur are quite different and additionally, significant fluctuations in growth and shrinkage velocities (beyond thermal noise levels) have been measured (Cassimeris et al. 1988; Walker et al. 1988; Gildersleeve et al. 1992; Shelden and Wadsworth 1993). The fourth reaction describes the transition from the growing to the collapsing state, called catastrophe. The exact dependency of the frequency of catastrophes from both the tubulin concentration and the growth velocity is not known but it seems to exhibit a decreasing character as a function of the tubulin concentration (Walker et al. 1988; O'Brien et al. 1990, 1992; Chretien et al. 1995).

The catastrophe is followed by the collapse of a large fraction of the assembled protein. The collapse rate in vitro is typically 10–20 times higher than the rate of growth (Mitchison and Kirschner 1984a; Horio and Hotani 1986; Walker et al. 1988; Gildersleeve et al. 1992; Chretien et al. 1995). Since the disassembled tubulin contains GDP, it is assumed that the hydrolysis energy may have been stored at least partially in an MT in the form of mechanical stress that could contribute to the causes of catastrophic disassembly (Caplow et al. 1994; Semenov 1996). It is also noteworthy that the geometry of growing MTs (mainly straight protofilaments) differs from that of shrinking ones (curved protofilaments, sometimes called ram's horns) (Trinczek et al. 1993; Chretien et al. 1995). The above tends to imply that polymerisation involves the formation of axial bonds while depolymerisation consists of breaking the already weakened lateral bonds (Melki et al. 1989). It is, therefore, conceivable that mechanical stress may explain the stochastic nature of the dynamic instability phenomenon. It would also be very interesting to have various possible amounts of unhydrolyzed GTP in a microtubule as this could explain both the variability of growth velocities and elastic coefficients.

The rescue reaction is the recovery from the collapsing towards the growth state. The frequency of rescues has been found to be almost linearly proportional to tubulin concentration (Walker et al. 1988).

While the cycle of growth, catastrophe, collapse and rescue is repeated over and over again in the life history of a single MT, it exhibits little regularity or periodicity (Cassimeris et al. 1988; Drechsel et al. 1992; Gliksmann et al. 1993; Billger et al. 1996).

In order to close the reaction cycle the depolymerization products D^+ need to be recycled into assembly-competent GTP-tubulin T . Since there is evidence for intermediate products after disassembly such as rings and oligomers (Melki et al. 1989; Marx and Mandelkow 1994) we have divided this reactivation into two steps. First the breakage of rings or oligomers into dimers (thermalization) and second the exchange of GDP by GTP on the dimer (reactivation, pumping). A possible significance of this type of extension has been emphasized by Odde et al. (1996)

in connection with a γ -type probability distribution in the obtained cumulative histograms.

Many other dependences of kinetic parameters in the reaction cycle have been reported in literature such as the GTP/GDP concentrations (Engelborghs and Van Houtte 1981), specific drugs, e.g. colchicine (Vandecandelaere et al. 1994), microtubule-associated proteins, e.g. tau (Drechsel et al. 1992), cell cycle effects (Belmont et al. 1990; Verde et al. 1992) and ionic specifications of the solution (Carlier and Pantaloni 1978; O'Brien et al. 1990; O'Brien et al. 1992; Simon et al. 1992).

Various experimental and theoretical studies were aimed at extracting characteristic features of life histories of MTs in order to build mathematical models (Mitchison and Kirschner 1984a; Chen and T.L. 1985; Horio and Hotani 1986; Walker et al. 1988; Cassimeris et al. 1988; Bayley et al. 1991; Martin et al. 1993; Vandecandelaere et al. 1995). The most common statistical tools included the construction of histograms and correlation functions (Walker et al. 1988; Gliksman et al. 1993). For example in a dilution experiment, a histogram of delays before catastrophes for both ends has a characteristic Poissonian shape which is suggestive of statistically independent, small probability events (Walker et al. 1991). Histograms for length distributions have an exponential tail and sometimes a peak corresponding to relatively short microtubules (Verde et al. 1992; Mejillano et al. 1992). One of the main objectives of the present study is to determine precise criteria for the existence of bell-shaped (as opposed to exponential) probability distributions for MT lengths as seen in some experiments (Cassimeris et al. 1986). A very important conclusion was reached on the basis of an inverse proportionality of the frequency of catastrophes to the growth velocity of an MT (Drechsel et al. 1992). It has been demonstrated that the above is consistent with the presence of a GTP cap at the plus end (Flyvbjerg et al. 1994). A recent paper by Odde et al. (1996) investigated the validity of the standard assumption that the growth and shrinkage of MTs is governed by first-order chemical kinetics. The analysis was based on the distribution of growth times and resulted in the conclusion that for the minus ends the histograms are purely exponential but not so for the plus ends. This might imply that the catastrophe probability is an increasing function of length indicating a form of memory process.

In the present paper we wish to develop and evaluate the usefulness of simple stochastic models consistent with our knowledge about the mentioned chemical reactions. Of course, in the past, a number of authors (Verde et al. 1992; Martin et al. 1993; Gliksman et al. 1993; Vandecandelaere et al. 1994; Flyvbjerg et al. 1994, 1996; Odde et al. 1996) have presented models which contain a number of features in agreement with experimental results. We intend to build on these models and discuss specific assumptions which need to be implemented in order to obtain an adequate theoretical framework. Henceforth we will assume that the probability for a certain reaction is proportional to the probability of finding the appropriate reactants at the reaction point, which is often but not always proportional to the product of reactant concentrations and the reaction rate (Atkins 1990; Chen and

Figure 2. A life history diagram for an individual MT without rescues.

Figure 3. A histogram of length distribution for a model without rescues (a) drawn linearly; (b) drawn semilogarithmically.

Hill 1987). We will not use effective (concentration dependent) reaction rates, in contrast to some of the earlier approaches. Furthermore, we assume that the same reaction rates should hold for all concentrations of reactants.

Typical experimental values have been published by Walker et al. (1988) and pertain to an in vitro assay at a tubulin concentration of $10 \mu\text{M}$. In the calculations for Figures 2 to 7 we have used the parameters for the plus end: growth velocity $v_g = 3.3 \frac{\mu\text{m}}{\text{min}}$, shortening velocity $v_s = 1.6 \frac{\mu\text{m}}{\text{min}}$, mean growth time $t_g = 33 \text{ s}$, mean

Figure 4. An illustration of the solutions $P(x, t)$ of Eq. (10) spatial form and time development.

Figure 5. A life history diagram for an individual MT with an evenly distributed rescue probability.

Figure 6. A histogram of length distribution for a model with an evenly distributed rescue probability.

Figure 7. A histogram of length distribution for a model with a localized rescue frequency given by Eq. (25).

shrinkage time $t_s = 14$ s, catastrophe frequency $f_{cat} = 0.03$ s⁻¹, rescue frequency $f_{res} = 0.07$ s⁻¹, collapse velocity $v_d = 44$ $\frac{\mu\text{m}}{\text{min}}$.

2. A Stochastic Model Without Rescues

The behavior of an individual MT is clearly stochastic, primarily due to the dynamic instability but also because growth and shrinkage events are given by probabilities and are not known to take place with absolute certainty. Initially we ignore the intermediate steps involving free tubulin (reactions 7 and 8 in Table 1) assuming a constant concentration of T . Furthermore in all models we assume that the collapse is instantaneous. This adiabatic approximation is in good agreement with the fact that the collapse velocity is much higher than the growth velocity. We have also examined models with finite collapse velocity but our results show no qualitative difference. Therefore, we have not distinguished between a growing and a shortening subpopulation of MTs. Thus, the key reactions to be included are:

The probabilities t_x are just the transition rates t_{rx} for a given process multiplied by the probability of finding the reactant molecules in a given reaction region and thus for a single MT are given by

$$\begin{aligned}
 t_1 &= c_T t_{r1} \\
 t_2 &= t_{r2}
 \end{aligned} \tag{2}$$

$$t_3 = t_{r3}.$$

Here c_T denotes the concentration of T . The connection between the transition rates t_{rx} with the reaction constants k_x is

$$t_{rx} = k_x \Delta t \quad (3)$$

where Δt is the time step in the simulation. For the stochastic equations in this paper we use r which is a random number in the interval $0 \leq r \leq 1$. For example, a stochastic equation which describes the length evolution $\ell(t)$ as a function of time t for an individual microtubule can be written as

$$\ell(t + \Delta t) = \begin{cases} \ell(t) & \text{for } 0 < r \leq t_1 \\ \ell(t) - a & \text{for } t_1 < r \leq t_1 + t_2 \\ 0 & \text{for } t_1 + t_2 < r \leq t_1 + t_2 + t_3 \\ \ell(t) & \text{for } t_1 + t_2 + t_3 < r \leq 1 \end{cases} \quad (4)$$

where a is the length of a tubulin dimer. Figure 2 provides an illustration of a resulting time series (life history of an individual MT) for a particular choice of parameter values. We now intend to generalize the results of this section to a more experimentally suitable case of a number of microtubules present but at concentration values low enough to preclude synchronization, competition for T or any significant interactions or correlation effects. Our goal is to derive equations which can deal with evaluating ensemble averages for sets of microtubules.

3. The Development of an Average Picture: The Master Equation Approach

We now consider the situation where a large number of MTs is present so that an experimentalist can measure various associated averaged quantities for an ensemble of MTs with different lengths. It is possible, of course, to calculate such quantities through a repeated application of a computational procedure described in the previous section, i.e. by averaging the results of stochastic recurrence relations. However, a much more elegant and direct approach to this problem is to derive and then to try to solve the underlying master equation. We do not aim at providing a deterministic description because it is not suitable for this problem due to a large scatter of data. Instead, we wish to develop evolution equations for probabilities describing available states of microtubules.

The master equation describes the evolution of the probability distribution $P(n, t)$ which characterizes the system in a given state n (i.e. the number of segments assembled to form a microtubule) at a time instant t . In order to calculate $P(n, t)$ we only need to know the transition probabilities for: (a) processes leading into the given state n (rate in) and, (b) processes leading out of state n and into any other possible state (rate out). Furthermore, these probabilities t_{rj} are simply proportional to the reaction rates k_j (see Equation (3)). A generic master equation always takes the form

$$\frac{d}{dt}P(n, t) = (\text{rate in}) - (\text{rate out}). \quad (5)$$

When specified for our reaction scheme in Eq. (1) the master equation becomes

$$\frac{d}{dt}P(n, t) = c_T t_{r1} P(n-1, t) + t_{r2} P(n+1, t) - (c_T t_{r1} + t_{r2} + t_{r3}) P(n, t) \quad (6)$$

for $n > 0$. The master equation for $n = 0$ follows again from Eq. (5), but it also secures the time independence of the norm $\sum_{n=0}^{\infty} P(n, t) = 1$ and has the form

$$\frac{d}{dt}P(0, t) = t_{r2} P(1, t) + t_{r3} \sum_{n=1}^{\infty} P(n, t) - c_T t_{r1} P(0, t). \quad (7)$$

Note that the $n = 0$ state is the seed state and it plays an important role in view of the boundary conditions provided through it. The stationary equations, this means $\frac{d}{dt}P(n, t) = 0$, can be solved with respect to the normalization condition $\sum_{n=0}^{\infty} P(n) = 1$. Figure 3 illustrates a numerical solution of this equation plotted linearly (a) and using a semi-logarithmic scale (b). It is obvious that the result describes a stationary-state histogram of microtubule lengths and it conforms to the well-known exponential law. It is straight-forward to take the continuum limit of the master equation. This is done by developing the probability $P(n \pm 1, t)$ as follows:

$$P(n \pm 1, t) \rightarrow P(x, t) \pm \frac{\partial}{\partial x} P(x, t) + \frac{1}{2} \frac{\partial^2}{\partial x^2} P(x, t) \pm \dots \quad (8)$$

When we develop Eq. (6) until first order terms we arrive at a first order linear partial differential equation (PDE) for $P(x, t)$:

$$\frac{\partial}{\partial t} P(x, t) = -(c_T t_{r1} - t_{r2}) \frac{\partial}{\partial x} P(x, t) - t_{r3} P(x, t) \quad (9)$$

where x is the continuum limit equivalent of n . Interestingly, the above PDE has a physically relevant exact solution of the form

$$P(x, t) = e^{-t_{r3}t} f(x - (c_T t_{r1} - t_{r2})t), \quad (10)$$

where f is an a priori arbitrary function which is subject to an arbitrary initial condition and of course must satisfy the normalization of probability $\int_0^{\infty} P(x, t) dx = 1$ and a boundary condition. This can easily be seen by inserting 10 into Eq. (9). Figure 4 shows how the solution $P(x, t)$ evolves with time from a single-peak shape to an exponential form. In order to bring the mathematical model developed here closer to reality we must include rescue events in the equations.

4. Models with Rescues

As mentioned earlier the collapse can be stopped by a rescue event. In order to account for rescues we modify our chemical reaction set as follows:

where the third reaction describes an incomplete collapse, i.e. allows for a rescue event to follow. Here m is a random integer number in the range:

$$0 < m < \frac{\ell(t)}{a} \tag{12}$$

which describes the size of the assembled tubulin material in a MT following a catastrophe event. A modified stochastic equation for the individual MTs length history is now given by (see Section 3 for comparison):

$$\ell(t+1) = \begin{cases} \ell(t) + a & \text{for } 0 < r \leq t_1 \\ \ell(t) - a & \text{for } t_1 < r \leq t_1 + t_2 \\ \ell(t) - m a & \text{for } t_1 + t_2 < r \leq t_1 + t_2 + t_3 \\ \ell(t) & \text{for } t_1 + t_2 + t_3 < r \leq 1 \end{cases} \tag{13}$$

Assuming for simplicity that m is evenly distributed within the above range of values we have illustrated this stochastic model in Figure 5 showing the life history of a single MT. For the probability that a collapsing MT of length n rescues at length $n - m$ we have

$$w(n, m) = w(n) = \frac{1}{n} \tag{14}$$

in this case. With the same assumption about the equal probability of rescues within the allowed range of values, the resulting continuous master equation can be derived by developing $P(x, t)$ up to first order as

$$\frac{\partial}{\partial t} P(x, t) = -(c_T t_{r1} - t_{r2}) \frac{\partial}{\partial x} P(x, t) - t_{r3} P(x, t) + t_{r3} \int_x^\infty \frac{1}{x'} P(x', t) dx'. \tag{15}$$

It can be demonstrated that the stationary solution of Eq. (15) satisfies the relationship:

$$P(x) \sim x e^{-\frac{t_{r3}}{(c_T t_{r1} - t_{r2})} x}. \tag{16}$$

This stationary solution is plotted in Figure 6 below. For this case we get in fact a bell-shaped solution, but clearly, the assumption about a uniform probability distribution for rescues is highly limiting and hence may result in solutions which are not appropriate for all experimentally observed situations. One might imagine several physical effects which would require a modification of this assumption. For

example, (a) an accumulation of stress-free tubulin dimers could occur at specific locations within the microtubule leading to a higher probability of rescues at these points. For instance, tubulin clusters close to the seed (older parts of a MT) could be more stable than those close to the growing tip. (b) The assembly of tubulin into a MT is closely related to the chemical energy pumping via GTP. GTP inside the MT is hydrolyzed into GDP and releases some energy, conceivably in the form of mechanical stress. Assuming a steady flux of energy flow through the MT may or may not result in an equal energy concentration throughout the structure. This could depend on a number of factors but in principle on: the rate of assembly and the rate of GTP hydrolysis.

Assuming there is no major discrepancy and that the catastrophes start from the distal end, for every tubulin which disassembles we have a constant probability q (dependent on the rate of assembly) that the collapse is *not* stopped. The probability for rescue is then $(1 - q)$ and the probability that rescue occurs after $n - m$ collapse events is obviously

$$w(n - m) = \frac{1 - q}{1 - q^n} q^{n-m}. \quad (17)$$

The continuum limit of this master equation results in a integro-differential equation of the form

$$\frac{\partial P}{\partial t} = \frac{1}{2}(c_T t_{r1} + t_{r2}) \frac{\partial^2 P}{\partial x^2} - (c_T t_{r1} - t_{r2}) \frac{\partial P}{\partial x} - t_{r3} P + t_{r3} \int_x^\infty w(x' - x) P(x') dx'. \quad (18)$$

A formally similar equation has been recently derived by Flyvbjerg et al. (1994) in the context of the dynamics of the lateral cap. Fourier transforming the continuum – limit master equation directly yields the exact solution

$$P(x, t) = \int_{-\infty}^{+\infty} e^{-ik(x-At)} e^{-B(1-w(k))t+Ct} f(k) dk \quad (19)$$

where $w(k)$ is the Fourier transform of $w(x' - x)$ and $f(k)$ defines the initial condition (is its Fourier transform). A , B , and C are defined by the transition probabilities. In order for $P(x, t)$ to describe a stationary solution, $f(k)$ must select a specific value of the wavenumber k , namely k_0 such that

$$k_0 = i \frac{B}{A} \left[1 - w(k_0) + \frac{k_0^2}{B} \right] \quad (20)$$

under this condition, $k = k_0$, the stationary solution in real space takes the form:

$$P(x) \sim \exp \left[-\frac{B}{A} \left(1 - w(k_0) + \frac{k_0^2}{B} \right) x \right]. \quad (21)$$

The function $f(k)$ contains the important information regarding the initial condition and normalization; its form poses a significant mathematical problem. Once

again, as a consequence of the assumptions made, we have arrived at a stationary probability distribution which is exponential in character. Any other solution will evolve in time from the initial condition given to the asymptotic form which is exponential. It must be emphasized here that the conclusion reached about the exponential form of the histogram applies to all cases when w depends on the difference $(n - m)$. Conversely, for a histogram not to be exponential (e.g. bell-shaped), the function w must depend on n and m separately as we have seen in the first case (see Eq. (14)).

5. Finding Stationary Bell-Shaped Distributions

In many experiments the distribution of lengths of MTs in a stationary state has demonstrated a characteristic bell-shape form. Glicksman et al. (1993) pointed out that this type of behavior can be attributed to the correlations between the size of the free tubulin pool T_T and the assembled MTs. The main difference between this assumption and those made by us earlier is that increasing the size of a MT results in a corresponding decrease in the number of free tubulin dimers. Consequently, the master equation that results is almost identical to those obtained before

$$\begin{aligned} \frac{d}{dt}P(n, t) = & [T_T - (n - 1)]t_{r1}P(n - 1, t) \\ & + t_{r2}P(n + 1, t) + t_{r3} \sum_{n'=n+1}^{\infty} P(n')w(n' - n) \\ & - [(T_T - n)t_{r1} + t_{r2} + t_{r3}]P(n, t) \text{ for } n > 0. \end{aligned} \quad (22)$$

As before, the rescue probability is assumed to depend on the relative length of the catastrophe-to-rescue distance (see Eq. (17)). Furthermore we have replaced c_T through the quantity $(T_T - n)$ denoting the number of free tubulin dimers in solution which accounts for its depletion on MT assembly and provides a strong correlation effect between MTs and free tubulin. The numerical results for the stationary solution of (22) indicate bell-shaped behavior. But let us see what happens when we consider N MTs of different lengths and coupling them to a finite pool of free tubulin. The state of the system would then be given by specifying the lengths of all MTs $(n_1, n_2, \dots, n_N)$ and the number of free tubulin dimers would be: $T_T - n_1 - n_2 - \dots - n_N$. Somewhat surprisingly, in spite of the new complications arising in the master equations, a new symmetry arises which makes the problem still solvable:

$$P(\vec{n}) = P(n_1, n_2, \dots, n_N) = P(n_1 + n_2 + \dots + n_N) \quad (23)$$

i.e. the probability distribution only depends on the total amount of tubulin assembled in all MTs. Furthermore, $P(\vec{n})$ is the solution of the previously discussed

master equation. Consequently, one could hope that if $P(\vec{n})$ had a bell-shape form, so would $P_1(n_1)^*$ defined for a single MT. Unfortunately, this is not the case because the sum appearing in the defining relation for $P_1(n_1)$:

$$P_1(n_1) = \sum_{n_2} \sum_{n_3} \dots \sum_{n_N} P(n_1 + n_2 + n_3 + \dots + n_N) \quad (24)$$

rapidly destroys the presence of any peak in $P(\vec{n})$ and it can be demonstrated that as $n \rightarrow \infty$, $P_1(n_1)$ reverts back to an exponential form: $P_1(n_1) \sim e^{-cn}$. Since the above property is identically satisfied for a discrete case with an arbitrary number of tubulin dimers, it also holds in the continuum limit.

However, after many unsuccessful attempts at finding a possible reason for the observed bell-shaped distribution of lengths, we eventually discovered a simple assumption that yielded the correct answer. Bell-shaped length distributions emerge naturally if we assume that the probability of rescues is not uniform but increases in some specific locations along a given MT – for example is significantly higher close to the seed. This is illustrated in Figure 7 below where we assumed that

$$t_r(x) = k_B e^{-\alpha x} \quad (25)$$

with $k_B = 100t_g$ and $\alpha = 0.12 \frac{1}{\mu\text{m}}$.

To summarize this part of our investigations, we have demonstrated that a transition from exponential to bell-shaped distribution functions must be intimately linked with a change of the internal state of a dynamic microtubule. More specifically, we believe that the assumption about every process being independent of the length of the MT must inevitably lead to an exponential histogram for an ensemble of non-interacting MTs. Conversely, when we assume that, e.g., the probability of rescues depends not only on the distance from the proximal end of a MT, we are able to obtain bell-shaped histograms. As we discussed earlier, the latter possibility may emerge naturally from relaxation processes following GTP hydrolysis and a redistribution of stresses in the protofilaments. In order to confirm this theoretical prediction, one ought to carefully analyze the available experimental data. In particular, the statistics of growth lengths and catastrophe sizes as well as rescue points should shed more light on these issues. The experimental data at our disposal did not provide an unequivocal corroboration of these predictions but indicated a degree of consistency within the experimental error.

6. Master Equation for the Cap

One of the key assumptions in the literature on the dynamic instability of MTs is that there exists a lateral cap which ensures the stability of a given MT. Its loss is a stochastic process which results in a collapse catastrophe. A recent paper by Flyvbjerg et al. (1994) provided an elegant physical discussion of the link between

* $P_1(n_1)$ denotes the probability of finding MTs with length n_1

the cap's spatial and temporal evolution on the one hand, and a collapse catastrophe on the other. Here, we show how the formalism developed in this paper leads to the master equation. The end result is virtually similar, except for the boundary condition which is chosen differently in our paper in order to have a complete consistency of the description for tubulin in the body of a MT and the seed. The chemical reactions that underly the cap's dynamics must account for the GTP hydrolysis and for the size of the cap. Hence, a MT is described by two coordinates: $M(m, n)$ where m is the number of internal segments of tubulin with GDP (less stable) while n stands for the number of segments of tubulin with GTP (the more stable lateral cap). If the cap disappears ($n = 0$) a catastrophe event takes place. The length of a microtubule is then governed by the reactions for:

The dynamics of the cap length is coupled to the total length of the microtubule

$$\ell(t) = a[m(t) + n(t)] \tag{27}$$

where a is the thickness of one tubulin dimer. For simplicity we drop this coupling, ending with a master equation, which only describes the dynamics for the cap*. This master equation has three parts:

$$\begin{aligned}
 \partial_t P(n, t) = & c_T k_g P(n-1, t) + (k_s + k_{ni}) P(n+1, t) + k_{ns} \sum_{n'=n+1}^{\infty} P(n', t) \\
 & -(T k_g + k_s + k_{ni} + n k_{ns}) P(n, t) \quad \text{for } n \geq 2,
 \end{aligned}
 \tag{28}$$

$$\begin{aligned}
 \partial_t P(1, t) = & (k_s + k_{ni}) P(2, t) + k_{ns} \sum_{n'=2}^{\infty} P(n', t) \\
 & -(c_T k_g + k_s + k_{ni} + k_{ns}) P(1, t) \quad \text{for } n = 1,
 \end{aligned}
 \tag{29}$$

* This can be done in good approximation by summing the master equations for $\tilde{P}(m, n, t)$ over m , and introducing $P(n, t) = \sum_{m=0}^{\infty} \tilde{P}(m, n, t)$.

$$\partial_t P(0, t) = (k_s + k_{ni})P(1, t) + k_{ns} \sum_{n'=1}^{\infty} P(n', t) - k_c P(0, t) \text{ for } n = 0. \quad (30)$$

Eq. (30) shows, that we have to know $P(1) \dots P(\infty)$ to calculate $P(0)$. But in any case for $k_c \rightarrow \infty$, $P(0)$ will always vanish, or

$$P(0) = 0. \quad (31)$$

The first and second equation do not contain $P(0)$, so that any given value of $P(0)$ does not influence the rest of the cap. Since the disappearance of the cap corresponds to a collapse catastrophe, it is easy to demonstrate that within this formalism the frequency of catastrophes is given by:

$$f_{cat} = (Tk_g + k_s + k_{ni})P(1) + k_{ns} \sum_{n'=1}^{\infty} P(n'). \quad (32)$$

Taking the continuum limit of the first two equations, expanding them up to terms of second order, gives Eq.s (B1)–(B2) of Flyvbjerg et al. (1994). However, our ‘boundary condition’ is:

$$\partial_t P(1, t) = (k_s + k_{ni}) \left. \frac{\partial P(x, t)}{\partial x} \right|_{x=1} - (c_T k_g + k_{ns})P(1, t) + k_{ns} \sum_{n'=2}^{\infty} P(n', t), \quad (33)$$

unlike $P(1) = 0$ as proposed by the other authors. Eq. (33) is really not a usual boundary condition, because to solve for $P(1, t)$ we must know already $P(x, t)$ for $x > 1$. We find these coupled continuous equations hard to solve. In the following we want to describe two experimental situations:

1. The stationary state. Here the distribution for the total state of the MT is time independent. After every catastrophic decay with rescue, the cap will grow again starting with $n = 1$. On the other hand every MT disappearing because of catastrophic decay without rescue is replaced by a seed which means again a growing cap, starting with $n = 1$. As a result $P(n)$ is stationary and normalized ($\sum P(n) = 1$). In this case, we can use Eq. (28) but Eq. (29) has to be replaced by a new boundary condition $P(1) = C$, where C is a constant calculated later by the normalization condition. This is done by pumping as much MTs with a cap size of 1 into $P(1)$ as are lost due to catastrophes.
2. The system is for $t < 0$ in a stationary state. At $t = 0$ we then remove suddenly (or in a short time scale) all tubulin ($c_T \rightarrow 0$). The catastrophic decay events per time unit, will first have the stationary value, then it will grow, because after a characteristic time the caps are lost. Finally it will go down, because the number of MTs goes to zero. For this case the use of the correct boundary condition Eq. (29) or (33) is crucial in our opinion.

Figure 8. Catastrophe frequency f_{cat} in dependence of v_g . Crosses: Experimental data from Drechsel et al. (1992); Curve: Cap model with parameters $k_{ni} = 4.875 \frac{1}{s}$ and $k_{ns} = 1.2 \cdot 10^{-3} \frac{1}{s}$, as obtained by Flyvbjerg et al (1994).

Figure 9. Catastrophic decay events after dilution. Dots: Experimental data from Walker et al. (1991); Bars: Standard error of the mean; Curve: Cap model with parameters $k_{ni} = 4.875 \frac{1}{s}$, $k_{ns} = 1.2 \cdot 10^{-3} \frac{1}{s}$ and $k_g = 6.23 f(t) \frac{1}{s}$ where $f(t)$ is modeled after the experimental dilution curve in Walker et al. (1991) Figure 4; All values are normalized to $N(0) = 1$; Mean delays for both experimental and theoretical data is $\sim 12s$.

In Figures 8 and 9 we present results of calculations for both experimental situations (Walker et al. 1991; Drechsel et al. 1992). To avoid difficulties, with (33) we solved for both cases the discrete Eq.s (28) and (29) numerically. For the stationary case, however, we also checked our result by comparing with a continuous calculation. According to the different, but for the experimental conditions adequately chosen, boundary condition our results are different from those obtained by Flyvbjerg et al (1994).

7. Chemical Kinetics Formalism

In this paper we have focused on methods of constructing stochastic evolution equations for microtubules under conditions of sufficiently low tubulin concentrations such that nonlinear effects (in particular synchronization) are not present. The results obtained are much more general than those consistent with chemical kinetics methodology where only average concentrations are studied over the course of time. It should be stated here that starting from master equations and ignoring correlation effects one ends up with mean field equations which agree completely with chemical kinetics equations. We now wish to apply the methodology of the kinetics of chemical reactions to the assembly and disassembly phenomena of microtubules. For example the following four reactions (nucleation, growth, collapse and nucleotide exchange)

lead to the following differential equations for the concentrations:

$$\begin{aligned}
 \dot{c}_{M(n)} &= k_g c_T [\theta(n - n_c - 1) c_{M(n-1)} - c_{M(n)}] \text{ for } n > n_c \\
 &\quad - k_c c_{M(n)} + \delta(n - n_c) k_n n_c c_T^{n_c}, \\
 \dot{c}_T &= -k_g c_T \sum_{n=n_c}^{\infty} c_{M(n)} + k_p c_D - k_n n_c c_T^{n_c}, \\
 \dot{c}_D &= k_c \sum_{n=n_c}^{\infty} n c_{M(n)} - k_p c_D,
 \end{aligned} \tag{35}$$

where θ is the Heaviside theta function. Here, we assume a constant concentration of GTP. This is allowed as long c_{GTP} is much greater than the tubulin concentration. Furthermore, we neglect the loss of single tubulin-GTP-dimers. This is again the previously mentioned adiabatic approximation. It allows us to neglect the actual length distribution of the microtubules as we will see.

If we solve these equations, however, we do not see any oscillations. In chemistry we know of chemical oscillations which are explained by auto-catalytic reactions which make the equations nonlinear. Our idea for such a process is that decay can again induce decay. We already showed this possibility in our first reaction cycle. The MT decays into excited fragments which induce the decay of other MTs. We therefore have to add the following reaction.

$$M(n) + D^+ \xrightarrow{k_c^1} (n+1)D^+. \quad (36)$$

We can express the change in the equations by making k_c dependent on D^+ .

$$k_c(D^+) = k_c^0 + k_c^1 D^+. \quad (37)$$

This is a highly auto-catalytic reaction which we already proposed to be responsible for oscillations in microtubule polymerisation in an earlier publication (Bolterauer et al. 1996). The system of differential equations according to these chemical reactions can be further simplified by using new variables:

$$c_M = \sum_n n c_{M(n)} \quad \text{and} \quad c_N = \sum_n c_{M(n)}, \quad (38)$$

which correspond to the total polymerized tubulin mass concentration and the microtubule number concentration, respectively. As a consequence of the adiabatic approximation, the reduced system of differential equations becomes

$$\begin{aligned} \dot{c}_M &= k_g c_T c_N + k_n n_c c_T^{n_c} - (k_c^0 + k_c^1 c_{D^+}) c_M, \\ \dot{c}_N &= k_n c_T^{n_c} - (k_c^0 + k_c^1 c_{D^+}) c_N, \\ \dot{c}_T &= k_p c_D - k_g c_T c_N - k_n n_c c_T^{n_c} c_N, \\ \dot{c}_D &= k_t c_{D^+} - k_p c_D, \\ \dot{c}_{D^+} &= (k_c^0 + k_c^1 c_{D^+}) c_M - k_t c_{D^+}. \end{aligned} \quad (39)$$

A detailed analysis of the above system of equations indicated a possible transition to synchronized oscillations in total microtubule mass (Sept et al. 1998; Limbach et al. 1998). Thus for generating oscillations one requires that the reactions must be far away from equilibrium (pumping) and at least one auto-catalytic step must be present.

Oscillations in the microtubule ensemble for high-tubulin concentrations have been observed (Carlier et al. 1987; Pirollet et al. 1987; Lange et al. 1988; Melki et al. 1988; Wade et al. 1989; Marx et al. 1990; Obermann et al. 1990; Mandelkow et al. 1991) and a number of models have been proposed (Chen and Hill 1987; Mandelkow et al. 1988; Marx and Mandelkow 1994; Houchmandzadeh and Valade 1996; Jobs et al. 1997). Our intention in this section was to sketch a possible route to oscillations based on the already introduced formalism. A transition to oscillating behavior can be seen in Figure 10 which depicts our computer simulations based on Eq.s (39).

8. Summary and Conclusions

The main questions arising in the analysis of elongation data for microtubules can be listed as follows:

Figure 10. Transition from monotonic polymerisation to oscillatory behavior in the induced decay model. Tubulin dimer concentrations: upper curve 100 μM , middle curve 50 μM , lower curve 20 μM . Dynamic parameters: $k_n = 2 \cdot 10^{-8} \frac{1}{\text{s} \mu\text{M}}$, $k_g = 10 \frac{1}{\text{s} \mu\text{M}}$, $k_c = 0.001 + 0.014c_{\text{GDP}}^* \frac{1}{\text{s}}$, $k_t = 0.5 \frac{1}{\text{s}}$, $k_p = 0.05 \frac{1}{\text{s}}$, $n_c = 2$.

1. What causes a transition from exponential to bell-shaped histograms?
2. Is there length dependence in the rates for catastrophes and rescues?
3. Is there a velocity change in the growth process over time? If so, could this be related to the internal degrees of freedom of the MT lattice (e.g. lattice type or degree of local concentration of GTP bound to tubulin in a microtubule?)
4. Are there time-dependent correlations between catastrophe events in a given microtubule?
5. Does the lateral cap model sufficiently explain the key features of the dynamic instability phenomenon?

We have attempted to shed light on some of the above questions, especially (1), (2) and to a lesser degree (5). Some of these issues are inter-related and some require more detailed experimental studies, for example (3). We believe, however, that the theoretical framework within which rapid progress can be made should involve the master equation formalism. Our intention behind this paper was to provide the reader with a lucid amount of the main features and the key results.

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