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A Lateral Cap model of microtubule dynamic instability

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The co-existence and interconversion of growing and shrinking microtubules have been termed 'dynamic instability', and have been directly observed to occur under a variety of conditions in vitro and in vivo. Previous modelling was based on the concept of an extensive, fluctuating cap of tubulin-GTP to stabilise growing microtubules. A quantitative kinetic model is now presented in which only the terminal layer of the multi-start helical microtubule lattice contains tubulin-GTP molecules, comprising a 'Lateral Cap'. In Monte Carlo numerical simulation, this model readily reproduces the decisive experimental evidence of microtubule dynamics, and predicts a co-operative mechanism for microtubule transitions. The model also suggests how differing kinetic properties at opposite ends are the result of the intrinsic polarity of the microtubule lattice, reflecting the polarity of the tubulin α/β heterodimer.

Tubulin; Microtubule dynamics; GTP hydrolysis; Monte Carlo method; Computer simulation

1. INTRODUCTION

Microtubules are an essential component of the cytoskeleton of most eukaryotic cells. They exist as a dynamically changing array that confers directionality on many important biological processes, such as mitosis, secretion and intracellular transport. The phenomenon of dynamic instability [1] is most graphically illustrated by the direct observations of individual microtubules undergoing transitions between growing and shrinking states [2–4]. The biological implications [5,6] are that microtubules can be rapidly replaced, disassembled or reassembled in a controlled manner [7]. These dynamic properties are dependent on microtubule polarity, and the frequencies of transitions in vitro are known to be strong functions of the concentration of tubulin-GTP [3,4]. We have derived a kinetic model, based on rate constants for intrinsic association and dissociation reactions, which can be readily simulated using modest computing power, and which accounts quantitatively for the phenomenon of dynamic instability.

Many proposals have been made on the nature of the events which confer the behaviour of dynamic instability. The stabilisation of the growing state of dynamic microtubules has been attributed to the presence of a 'cap' of tubulin-GTP; when the cap is lost, the microtubule would shrink rapidly [8,9], but could recover 'rescue' by further tubulin-GTP addition [2–4,10–12]. One detailed model has been evaluated numerically [13]; the transitions between growing (G) and shrinking (S) microtubules derived from fluctuations in a substantial cap of potentially several hundred tubulin-GTP molecules.

Subsequently, several lines of experimental evidence have appeared which question the basic assumptions of this formulation. The Fluctuating Cap model incorporated the postulate of uncoupling of GTP hydrolysis from tubulin-GTP addition [9,14], which dictates as a general principle that a substantial proportion of the growing microtubule would be composed of tubulin-GTP. Direct measurements of the GTP content of microtubules during growth and at steady state [15–17] could not detect such caps. This indicates that uncoupling cannot be a general mechanism, and strongly suggests that GTP hydrolysis is effectively coupled to tubulin-GTP addition. Since the existence of a substantial fluctuating cap, (fundamental to the Chen and Hill formulation [13]), is strongly questioned, it becomes essential to seek an alternative mechanism for the origin of the dynamic transitions of microtubules.

2. MATERIALS AND METHODS

We now present a quantitative model which accounts for the available data without invoking extensive caps. The assumptions involved are:

(1) Tubulin-GTP is confined to a 'Lateral Cap', namely the terminal layer of the microtubule lattice, [cf. 4,15,18,19]. This is achieved via tight temporal coupling and specific spatial coupling between tubulin-GTP addition and GTP hydrolysis, such that GTP hydrolysis occurs on a previously terminal tubulin-GTP effectively synchronously with its incorporation into the microtubule lattice. A similar concept is discussed by Walker et al. [4] for a linear model.

(2) We use the idealised formulation of the A-type microtubule lattice as in fig.1 [20]; the definition of an individual binding site (following [13]) determines that addition is on the 5-start helical lat-

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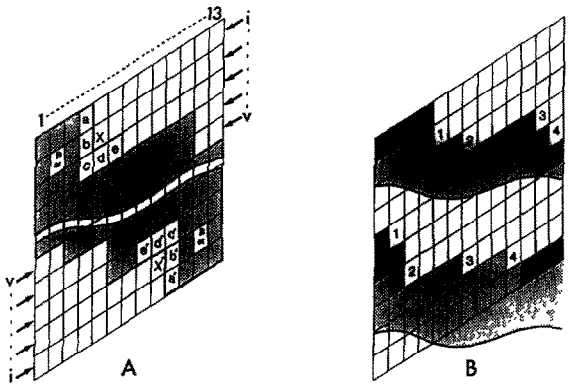

Fig.1. The Lateral Cap model for dynamic microtubules: the idealised helical microtubule lattice is represented in two dimensions [20] and shows 13 parallel protofilaments in the five-start configuration (i to v at each end). The $\alpha\beta$ polarity of the lattice is shown arbitrarily with the β-subunit to the top. A: Top: the unhatched tubulin molecules **a** through **e** constitute the binding site for the incoming tubulin molecule at **X**. The Hydrolysis Rule specifies coupling of the addition of tubulin-GTP at position **X** and hydrolysis of tubulin-GTP to tubulin-GDP at position **c**. Bottom: the opposite end of the microtubule; the configurations **abcde** and **a'b'c'd'e'** are non-equivalent owing to the microtubule polarity. B: top: for the given Hydrolysis Rule, the tubulin-GTP (black) is present in maximally 18 positions, which we term the 'Lateral Cap'. Addition sites are numbered 1 to 4. The internal layers of the microtubule are composed of tubulin-GDP (grey). Bottom: the terminal layer is here composed of tubulin-GTP (black) and tubulin-GDP (grey). Loss of terminal tubulin molecules has exposed tubulin-GDP. Sites 1 to 4 differ in the composition of positions **b** and **d**; {**bd**} = **TT** (1), **TD** (2,3) and **DD** (4). Such configurations are involved in the transitions between growth and shrinking.

tice. We believe this representation is physically realistic, since it includes multiple sites for binding of tubulin dimers, and it distinguishes explicitly between lateral and longitudinal protofilament interactions.

(3) The Hydrolysis Rule (illustrated in fig.1A) specifies the coupling between addition at position **X** and hydrolysis at position **c**. This dictates that the maximum extent of tubulin-GTP present at a given end would be at the terminal 18 positions (from 13 protifilaments, plus the ends of the 5-start helix), fig.1B. We call this the 'Lateral Cap', to distinguish it from the extended longitudinal arrays of tubulin-GTP as in [13].

The nature of the microtubule lattice suggests that both lateral and longitudinal interactions are involved in the addition of an incoming tubulin-GTP molecule. We therefore allow rate constants to be modulated (by approximately one order of magnitude) by the nucleotide content at positions **b** and **d**, so that the relative affinity decreases from configuration {**bd**} = {**TT**}, through {**bd**} = {**TD**} or {**DT**}, to {**bd**} = {**DD**}. Tubulin-GTP associates and dissociates from site **X**, with rates $k(+T)_{bd}$ and $k(-T)_{bd}$, respectively. (Walker et al. [4] showed that the dissociation of tubulin-GTP is kinetically significant during the growth phase, and described this as the 'stochastic dissociation, coupled hydrolysis' model: in our formulation, this process is represented by $k(-T)_{bde}$). Tubulin-GDP only dissociates, and this is always from {**bd**} = {**DD**}. Further (minor) modulation of these affinities is introduced with occupancy of position **e** (and/or **a**): typically we allow occupancy of **e** to increase $k(+T)$ and decrease $k(-T)$ by 2-fold for {**bde**} = **NNN** relative to **NNm** where **N** = **T** or **D** and **m** = empty.

This assignment of rate constants is consistent with the specified Hydrolysis Rule, since both lateral interactions {**b,X**} and longitudinal interactions {**X,d**} are then involved in stabilising the terminal configuration and in coupling sites **X** and **c**. The Hydrolysis Rule '**X** to **c**' has been adopted to show the basic principle, although it is not unique; alternative couplings are possible, but extensively lateral '**X** to **b**', or exclusively longitudinal '**X** to **d**' appear to be notably less successful.

We have extensively simulated the growth and shrinking behaviour of a single microtubule as a function of the external (constant) tubulin-GTP concentration, using Monte Carlo methods. Using a random number generator, the choice is made, on a stochastic basis, of which reaction occurs first amongst several competing addition or dissociation reactions at the **n** sites at the end of a single microtubule. The new configuration is then the starting point for the next step in the simulation, and a plot of length versus time is derived for an extended time period which has the saw-tooth form of growing and shrinking phases as observed experimentally [2,4]. The net growth (or shrinking) rate is obtained from the overall length change divided by overall elapsed time. Over a sufficiently long period, this net growth rate gives the bulk population growth rate. The mean state lifetime is derived from the duration of growth (or shrinking) phases; up to 200 transitions are generally required to give a well-defined average value.

3. RESULTS AND DISCUSSION

Fig.2 shows, as found experimentally [13,14], that there are two distinct regimes, bulk growth ([tubulin-GTP] $> C_c$) and rapid shrinking ([tubulin-GTP] $< C_c$), with zero growth defining the critical or steady-state concentration, C_c. A most important new feature of this formulation, which provides detailed quantitative results (fig.3) is that the Lateral Cap model predicts the dependence on [tubulin-GTP] of the lifetimes of the states G and S, based solely on rate constants for intrinsic association and dissociation reactions. We evaluate the state lifetimes and their inverse (i.e. transition frequencies, c.f. [4]), see fig.3. The lifetimes of states S and G (fig.3A and B, respectively) are non-linear functions of [tubulin-GTP], emphasizing the potential cooperativity of the transition behaviour. In the frequency plots (fig.3C and D), the frequency of transition S to G appears close to linear with [tubulin-GTP] over a limited range, (fig.3C), though the frequency of transition G to S is more curved.

While the absolute values presented here have not been refined by fitting procedures, the calculated values for the growing state lifetime, T_G = 20–1000 s, and for the shrinking state lifetime, T_S = 4–15 s may be compared with the reported observed values [4]. At the experimental C_c ($=7\,\mu$M), T_G was measured as 200 s for the (+) end and 300 s for the (−) end, and T_S = 50 s for the (+) end and 12 s for the (−) end [4]. (In vivo values of T_S = 23 s and T_G = 71 s have also been measured [6]). Thus the values calculated for the standard kinetic set are somewhat lower in T_S and show greater concentration dependence than reported in T_G. The simulation shows that stronger concentration dependence is predicted further away from the C_c. Other sets of kinetic constants show basically similar lifetime patterns as fig.3A,B but with characteristically different values of C_c. This is consistent with the known sensitivity to transition behaviour to solution conditions.

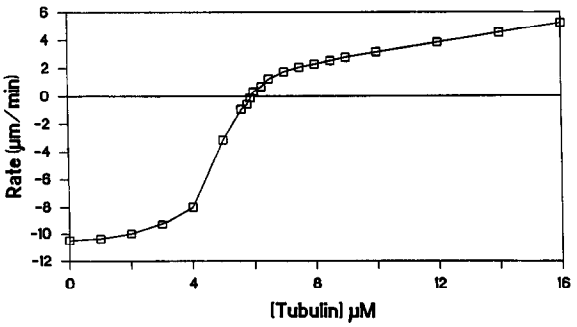

Fig.2. Quantitative simulation of microtubule dynamics as a function of tubulin-GTP concentration, using the Lateral Cap model: calculated bulk growth rate for a microtubule population as a function of [tubulin-GTP]; kinetic parameters for {**bde**} = **TTN, TDN** (or **DTN**) and **DDN** (where **N** = **T** or **D**) are $k(+T)_{bde}$ = 2.5, 2.0, 0.66 × 10^6/M per s and $k(-T)_{bde}$ = 0.625, 4.166 and 33.33/s, respectively; and $k(-D)_{DDD}$ = 58.3/s. The value of [tubulin-GTP] at which no growth occurs (the critical or steady-state concentration) is 5.8 μM for this set of rate constants.

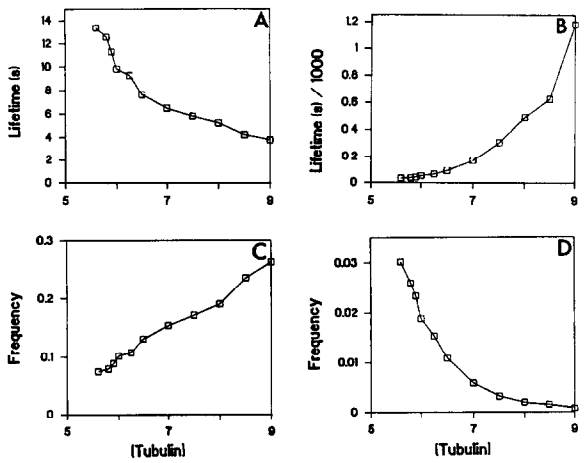

Fig.3. Quantitative predictions of microtubule transition properties as a function of tubulin-GTP concentration. Calculated mean lifetimes (in seconds) of the shrinking state (A) and the growing state (B) of individual microtubules as a function of [tubulin-GTP] for the standard kinetic set given in fig.2. The corresponding inverse plots of frequency of transition S to G, and transition G to S are given in C and D, respectively. The analysis of the growth and shrinking of an individual microtubule is described in the text.

More importantly from a mechanistic point of view, the model illustrates a possible mechanism for switching, as a result of events and interactions in the multi-start terminal layer of the microtubule; the concentration dependence of the state lifetimes appears as the result of bimolecular addition reacions (rate = k_+ [*T*-GTP], with appropriate values of $k_+ = k(+T)_{bde}$) at individual sites at the microtubule end. Thus the model predicts that the transitions S to G ('rescue') and G to S ('catastrophe') are the stochastic result of events at a number of sites in the multi-start terminal layer or Lateral Cap. Thus, a distinction is made between rate constants, $k(+T)_{bde}$, for the intrinsic addition reactions which contribute to 'rescue' (tubulin-GTP adding to a tubulin-GDP rich end, i.e. **bde** = **DDD** or **DDm**) and those responsible for growth (tubulin-GTP adding to a tubulin-GTP rich end, i.e. **bde** = **TTT** or **TTm**). In the same way, the loss of tubulin-GTP via $k(-T)_{bde}$ is different from the loss of tubulin-GDP via $k(-D)_{bde}$.

This treatment uses a limited number of kinetic parameters, whose values are physically realistic compared to known processes of protein self-assembly [21]. It illustrates the requirement for extensive observations in order to define transition parameters to adequate statistical precision. The model appears robust with respect to the choice of parameters, consistent with observations of dynamic instability under a wide range of experimental conditions known to affect individual kinetic processes. The numerical simulation allows a rapid assessment of the way that changes in individual parameters due to different physical conditions and solution composition can affect overall dynamics. It can also treat the behaviour of the dynamic system under conditions removed from steady state, which are difficult to achieve experimentally. The results predict that the mean growth rate of an individual microtubule increases monotonically, but not linearly, with [tubulin-GTP] in the region of the critical concentration, owing to the presence of terminal tubulin-GDP, (cf. fig.1B). This effect significantly influences values of rate constants ($k(-T)$) obtained by extrapolation from experimental data for individual growth rates obtained over a necessarily limited experimental concentration range.

The question of the origins of the differential behavior of the two ends of the microtubule has been controversial. With an extended cap model, the caps at either end would require different properties throughout their (fluctuating) structures. The Lateral Cap model offers a simpler explanation: the structural difference in the two ends of a microtubule lattice (of parallel protofilaments [20]) is here limited to the difference in the polarity of molecules in the terminal layer (only) at either end, i.e. α and β tubulin subunits. Because of this α/β polarity, configurations {**(a')b'd'(e')**} and {**(a)bd(e)**} (fig.1A) are not equivalent. It is therefore reasonable that the two ends would have different kinetic parameters, and could, in principle, also have hydrolysis rules with different spatial coupling.

Restricting the tubulin-GTP to the terminal layer of a multi-start helix results in a successful quantitative treatment for microtubule dynamics in conformity with current observations [15–17]. It also provides a readily applied working model for further prediction and experimentation, as, e.g. the mode of action of anti-mitotic drugs [22], and the recovery of steady-state microtubules from mechanical shearing [23] or UV microbeam severing [24].

While this formulation of the Lateral Cap model is not necessarily unique, it illustrates an important prin-

ciple, namely that the spontaneous switching behaviour of microtubules can be explained in terms of events which occur in the terminal layer of a multi-start helix at a given microtubule end, without invoking an extended stabilising cap structure. This kinetic model allows for further refinement in terms of the molecular details of the hydrolysis reacion, which are not presently known. We note the suggestion of a role for tubulin-GDP-phosphate [25] as a component of the terminal structure, plus other (conflicting) data on the kinetic effects of added phosphate ion [26,27]. At this stage, it does not appear warranted to include in the model any additional species intermediate between tubulin-GTP and tubulin-GDP in order to model the dynamic properties of microtubules. A detailed treatment of the model is in preparation (Bayley, P.M., Schilstra, M.J., and Martin, S.R., in preparation).

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