Physical Aspects of the Growth and Regulation of Microtubule Structures

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The growth of microtubules through the so-called dynamical instability is analyzed within a simple theoretical model in which the polymers are nucleated by a flat surface. For an isolated microtubule the model predicts the existence of a transition between bounded and unbounded growth. It is also shown that this transition alters the assembly of dense structures, e.g., by drastically limiting the number of long microtubules grown from the surface. Coupled to the microscopic biochemical control of the growth, such physical effects seem to play an important role in the regulation of the formation of cellular structures (such as the mitotic spindle).

Microtubules (MTs) are long, rigid polymers made of tubulin—a globular protein found in eukaryotic cells [1]. They constitute an important part of the cellular scaffold (cytoskeleton) and provide a network of "rails" for an active intracellular transport [2,3]. MTs also play a crucial role in cell division; during mitosis they form the mitotic spindle, which first spatially organizes chromosomes and which is then used to divide the chromosomes between the two daughter cells [2,3].

Formation of a MT network or a mitotic spindle is a fascinating phenomenon whose mechanisms only begin to be elucidated [4]. On its very basis lies a process of out-of-equilibrium aggregation (polymerization) called "dynamical instability" [5]. A MT, nucleated in a homogeneous and isotropic solution, can under appropriate conditions constantly switch between assembly and disassembly states. This apparently random, "sawtooth" behavior (Fig. 1), observed both *in vivo* and *in vitro* [5,6], is driven by the hydrolysis of GTP nucleotides bound to the tubulin proteins (i.e., the transformation GTP→ GDP) [7]. The hydrolysis process provides the free energy necessary for conformational changes of tubulin: of the
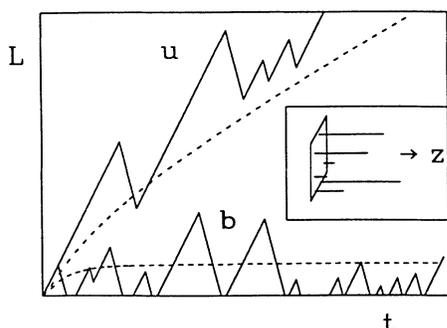

FIG. 1. Typical time sequence for a microtubule undergoing the dynamical instability: (u) unbounded growth, (b) bounded growth. The average over many microtubules with the same dynamical parameters is shown as dotted lines. Inset: A schematic view of the "semi-infinite" geometry chosen in this paper.

two forms of tubulin monomers (or more precisely dimers) it is the GTP tubulin which polymerizes, while the GDP tubulin prefers to stay unpolymerized or to form small oligomers. Although the process of hydrolysis takes place on free GTP tubulin, it is strongly accelerated for GTP tubulin forming a microtubule; it is believed that the bound nucleotides are quickly hydrolyzed in many extended regions of the microtubule. It seems that when such a transformed, extended region reaches the end of the microtubule a fast shortening (disassembly) takes place, until the moment when new GTP tubulin may be added from the solution and a new growth (assembly) period starts again. If the concentration of monomers in solution is high enough one observes a net growth of the polymer [7]. Compared with the usual reversible polymerization the growth through dynamical instability amplifies the length fluctuations, which may be very useful for an efficient regulation of the formation of cellular structures made out of MTs.

In this paper we consider some physical aspects of the phenomenon of dynamical instability which until now has been mainly studied from the cell biology and biochemistry points of view [8]. We use a theoretical model with a very simplified, semi-infinite geometry, in which infinitely rigid MTs grow perpendicularly to a nucleating planar surface (Fig. 1, inset). Each MT switches randomly between the assembly state $(+)$, in which it grows with the average speed v_+ proportional to the local monomer density c, and the disassembly state $(−)$, in which it shrinks with the average speed v_-. The frequencies of the transitions between the two states, f_{+-} (of the "catastrophies" from $+$ state to $−$ state) and f_{-+} (the "rescues" from $−$ state to $+$ state), determine, together with v_+,v_- and the nucleation rate of the surface, v, the behavior of the model [9–11]. We summarize now the main results obtained through mean-field theories and Monte Carlo simulations [8].

When the nucleated MTs are separately distributed, with typical spacings l much larger than the diffusion length, $l_D = D/v_+$ (where D is the diffusion constant for free tubulin), they grow independently from one another.

Neglecting concentration variations in the process of assembly and disassembly of MTs, we can write the following stochastic equations for the time evolution of the probability densities of growing, p_+, and shrinking, p_-, polymer tips:

$$\partial_t p_+ = -f_{+-} p_+ + f_{-+} p_- - v_+ \partial_z p_+ , \qquad (1)$$

$$\partial_t p_- = +f_{+-} p_+ - f_{-+} p_- + v_- \partial_z p_- . \qquad (2)$$

These equations can be solved analytically with appropriate boundary conditions (e.g., all microtubules having zero length at $t=0$). The main result is the prediction of a sharp transition or a threshold between an unlimited or "unbounded" growth, with the average speed $J > 0$ and a steady state or "bounded" growth, characterized by a well defined MT length distribution with $J = 0$ (Fig. 1) [11]. The transition takes place for $v_- f_{+-} = v_+ f_{-+}$ and thus can be reached by varying any of the four parameters v_+, v_-, f_{+-}, or f_{-+}. Since the dynamical parameters depend in general on the GTP-tubulin concentration c, one can reach the threshold also by crossing some critical value of the monomer density, $c = c_{cr}$. The precise c dependence of the dynamical parameters has not yet been firmly established [12]. If one assumes for instance the simplest scenario in which v_+ and f_{-+} depend linearly on c, while v_- and f_{+-} are c independent, $f_{-+} \equiv \omega c$, $v_+ \equiv u + c$, one obtains $c_{cr} = (f_{+-} v_- / \omega u_+)^{1/2}$. In the steady state the distribution of MT lengths L is exponential with the mean

$$\langle L \rangle \cong v_+ v_- / (v_- f_{+-} - v_+ f_{-+}),$$

and in the unbounded growth region the average length increases as $\langle L \rangle = Jt$, where

$$J = (v_+ f_{-+} - v_- f_{+-}) / f_{+-} + f_{-+} > 0 ,$$

while the distribution approaches asymptotically a Gaussian of width $s(D_{\text{eff}} t)^{1/2}$, where

$$D_{\text{eff}} = f_{-+} f_{+-} (v_+ + v_-)^2 (f_{-+} + f_{+-})^{-3}.$$

The presence of the sharp transition provides a very efficient mechanism of the regulation of MT structures: By varying only slightly the effective parameters of the dynamical instability, the cell (or more precisely the enzymes controlling the cell cycle) may change the distribution of polymer lengths [11,13]. The relevance of such a mechanism for mitotic MTs has indeed been demonstrated in recent experiments [11].

The existence of a transition between the bounded and unbounded growth also strongly influences the MT behavior for $l \le l_D$. To see this it is useful to consider first the case of the irreversible growth in the same "semi-infinite" geometry [8,14–16]. In this case, the growing rigid polymers leave a region depleted of monomers behind their advancing tips. The main consequence of the depletion is a progressive slowing down of the growth and formation of a wide distribution of the polymer den-
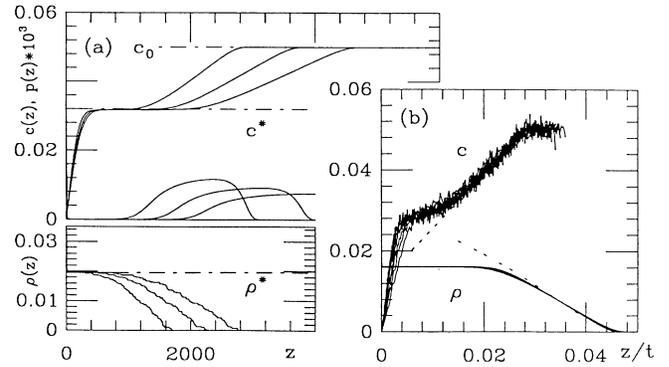

FIG. 2. (a) Average densities of monomers, $c(z)$ (upper curves), polymer tips, $p(z) = p_+(z) + p_-(z)$ (middle curves), and polymer fibers, $\rho(z,t)$ (lower curves), as functions of the distance z from the nucleating surface. The densities of monomers and of polymer tips shown here are obtained through numerical solutions of the mean-field equations, while the polymer densities are the results of Monte Carlo (MC) simulations, averaged over 10 runs [for "complementary" curves see (b)]. The values of the parameters are $c_0 = 0.05$; $D = 0.17$; $v_- = 0.05$; $f_{+-} = 0.0005$ (mean field) or 0.0002 (MC); $\omega = 0.15$, while in each set the three curves correspond to $t = (0.6, 0.8,$ and $1) \times 10^5$ MC steps. c^* and ρ^* indicate constant density solutions that separate the two MT subpopulations. (b) Scaling solutions for monomer and polymer densities. Monomer densities $c(z)$ are obtained through the MC simulations; polymer densities $\rho(z)$ are obtained through the numerical solutions of the mean-field equations [see (a)]. The values of the parameters are as in (a) with $t_{\max} = 1 \times 10^5$ MC steps. The dotted lines show the analytical solutions obtained for $t \to \infty$.

sity ρ, with all lengths present [up to the maximal length, $\xi(t) = v_+ t$] [8,16]. This relatively simple behavior can be contrasted with the case of polymers growing through dynamical instability, where the effect of the depletion can be much more drastic (Fig. 2). In this case, provided the initial concentration $c(z) = c_0$ lies above c_{cr}, some polymers enter unbounded growth, creating again a region depleted of monomers. However, since near the surface the local concentration decreases below c_{cr}, any further unbounded growth from this region becomes in practice impossible. The result is a formation of two subpopulations of MTs [Fig. 2(a)]: an effectively growing "packet" [with lengths between $\xi_1(t) = J(c^*)t$ and $\xi_2(t) = J(c_0)t$] and the rest of MTs assembling and disassembling close to the surface [17]. The relative number n^* of MTs in the packet which escapes from the surface depends weakly on the exact initial conditions; however, it seems that for $c_0 \to c_{cr}$, n^* approaches zero with a more universal, power-law dependence (Fig. 3). It is interesting to notice that this unusual growth phenomenon can also be viewed as an "autoregulation" mechanism: The growing ensemble of MTs limits its own growth through the depletion of monomers which it creates.
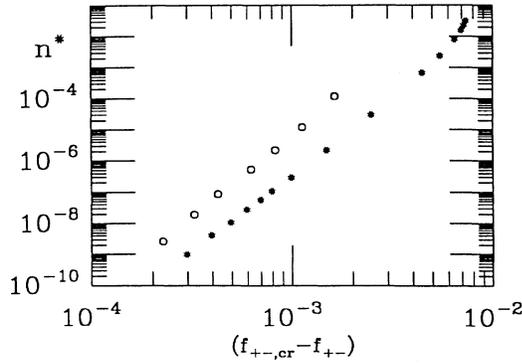
FIG. 3. The number of polymers nucleated from the unit area of the surface and growing through dynamical instability, $n^* = b\rho^*$, as a function of the dynamical parameter f_{+-}, at the approach to a critical value $f_{+-} \to f_{+-,\text{cr}}$. (Stars: parameters as in Fig. 2; circles: $c_0 = 0.05$, $D = 0.5$, $v_- = 0.08$, $\omega = 0.1$.)

We now address some quantitative issues related to the last results. Neglecting fluctuations in the process of assembly and disassembly of the MTs and assuming that the only density variations are along the z axis (perpendicular to the nucleating surface) we can write the one-dimensional mean-field equations [generalizing Eqs. (1), (2)] for the time evolution of the probability densities of growing [$p_+(z)$] and shrinking [$p_-(z)$] polymer tips as well as that of the monomer density $c(z)$:

$$\partial_t p_+ = -f_{+-}p_+ + f_{-+}p_- - u + \partial_z(cp_+) , \qquad (3)$$

$$\partial_t p_- = +f_{+-}p_+ - f_{-+}p_- + v - \partial_z p_- , \qquad (4)$$

$$\partial_t c = -v + p_+ + D\partial_{zz}c , \qquad (5)$$

where we have put the microscopic length scale b (the size of a protein) equal to 1. The polymer density $\rho(z)$ is related to $p_+(z)$ and $p_-(z)$ through $p_+ + p_- = -\partial_z\rho$. In writing the above equations we have neglected the effect of (slow) regeneration of the disassembled GDP tubulin to active GTP tubulin [8]. This last process involves short curved oligomers made out of GDP tubulin [7], which, however, seem not to participate in the growth phenomenon itself. We have therefore neglected their presence. At the nucleating surface we assume simple boundary conditions:

$$\partial_z c|_{z=0} = 0, \quad \partial_t s = -v + p_+|_{z=0} + v - p_-|_{z=0} ,$$
$$u + p_+|_{z=0} = vs , \qquad (6)$$

where s is the density of free nucleation sites and v is the nucleation rate. These equations can be solved through standard numerical methods [8]. In addition, in order to verify that the presence of thermal fluctuations does not alter the main conclusions of the mean-field theory we perform on-lattice Monte Carlo (MC) simulations with the Metropolis algorithm [8]. For the total number of monomers $N \leq 4 \times 10^4$ and the (simple-cubic) lattice

$B \times B \times 170B$, with $B = 20b$, we perform simulations of up to 10^5 MC steps, producing the maximal polymer lengths of the order of $140B$ without alternating significantly the monomer density c_0 in the region far away from the surface.

The influence of the bounded-unbounded growth transition, described above for an isolated MT, is clearly observed in the solutions of the full set of Eqs. (3)–(6) for MTs interacting through the diffusion field. Figure 2(a) depicts the formation of flat regions $c(z) = c^*$ and $\rho(z) = \rho^*$ separating two subpopulations of the MTs: those who "escaped" from the surface before the depletion had developed and those who were trapped in its vicinity. The time of separation of these two subpopulations diverges at the approach of c_{cr}. The escaping MTs form a packet [Fig. 2(a), middle curves] whose position as well as width grows linearly with t. In this linear region, for large enough t, we can obtain solutions of the mean-field equations in a simple scaling form. Indeed, with $y \equiv zt^{-1}u_+^{-1}$, the density profiles can be written as

$$c(y,t) = \tfrac{1}{2}\{y + [y^2 + 4(c_{\text{cr}}^2 + f_{+-}\omega^{-1}y)]^{1/2}\}$$

and

$$\rho(y,t) = (c_0^2 + c_{\text{cr}}^2)/c_0 - [c(y,t)^2 + c_{\text{cr}}^2]/c(y,t) .$$

Figure 2(b) shows that the mean-field solutions approach these asymptotic forms for large t. The scaling solutions in the linear regimes match the constant boundary values on the large-z side, and the plateau regions on the other. Figure 3 shows that near the transition one observes a power-law behavior: e.g., $n^* \propto (f_{+-} - f_{+-,\text{cr}})^q$, where $q = 4.8 \pm 0.3$. The value of this exponent does not seem to depend on the initial conditions or the values of physical parameters, such as D (Fig. 3).

What is the relevance of the physical phenomena described here to the cellular process? Recent experiments made in mitotic extracts have shown that the MT structures are indeed regulated through the local, biochemical modification of the dynamical instability parameters [11,13]. For instance, the enzymes called cyclin-B associated kinases, which become active during mitosis, may increase f_{+-} severalfold and in this way shorten drastically the average length of MTs (nucleated from the surface of centrosomes). Even more surprisingly [11], it seems that the cell may use the transition (threshold) between the unbounded and bounded growth to go from nonmitotic to mitotic structures. Whether the additional autoregulation mechanism through diffusive effects described above, which limits the density of growing microtubules, is important in cells (compared for example to the biochemical regulation of the nucleation properties of the centrosome) remains for the moment an open question. From the theoretical point of view one needs to extend our analysis to a spherical geometry, more appropriate for the mitosis problem [18]; more experiments dealing quantitatively with nucleation on centrosomes are also necessary. However, the results of the simple model presented here show

that the collective, stochastic phenomena play an important role in cellular assembly processes such as mitosis.

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[9] Typical values of these parameters for a "plus" end of a MT growing in a pure tubulin solution with $c \approx 10$ μM are $v_+ \approx 2$ μm/min, $v_- \approx 20$ μm/min, $f_{+-} \approx 0.004$ sec^{-1}, $f_{-+} \approx 0.05$ sec^{-1} (Ref. [6]). These values are modified *in vivo* by the presence of MT binding proteins and the enzymes controlling the cell cycle.

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[14] For the D.L.A. ($c_0 = 0$) problem in a similar geometry, see M. E. Cates, Phys. Rev. A **34**, 5007–5009 (1986), and G. Rossi, Phys. Rev. A **35**, 2246–2253 (1987). For a general overview of D.L.A. problems see, for instance, T. Vicsek, *Fractal Growth Phenomena*, (World Scientific, Singapore, 1992), 2nd ed. Note, however, that these problems deal with systems which, unlike microtubules, do not switch between growing and shrinking states.

[15] For a previous interesting attempt to solve the mean field theory of such irreversible growth, see K. Kassner, Phys. Rev. A **42**, 3637–3640 (1990).

[16] Equations (3)–(6) can be easily adapted for irreversible growth if we put $p_- \equiv 0$ and $f_{+-} \equiv 0$. In this case, both the mean-field theory as well as Monte Carlo simulations exhibit two distinct regions outside of the proximal (microscopic) zone next to the nucleating surface: (i) a diffusive region, with the width $\zeta(t) = \sqrt{2Dt}$, and (ii) a linear growth region, between $\zeta(t)$ and $\xi(t) = v_+ t$. The mean-field equations here are simple enough so that one can find scaling solutions in these two regions [15]: In (i), defining $x \equiv zt^{-1/2}$, one obtains $\rho(x,t) = c_0 + u_+^{-1} t^{-1/2} \times (2Dx^{-1} - 2\sqrt{2D})$ and $c(x,t) = (1/\sqrt{2D}) u_+^{-1} t^{-1/2} x^2$, whereas in (ii), with $y \equiv zt^{-1} u_+^{-1}$, one gets $\rho(y,t) = c_0 - y$ and $c(y,t) = y$. These scaling solutions show that irreversible growth in a semi-infinite geometry produces a strong, largely linear depletion of monomers together with a wide, mainly linearly decreasing distribution of polymers, including all lengths between 0 and $\xi(t)$. Numerical solutions of Eqs. (3)–(6) and the results of Monte Carlo simulations can be presented in the above scaling from [8,18]. The agreement with the scaling form derived analytically gives us some confidence in the validity of both numerical methods applied to the case of MT growth.

[17] Similar division is also present for a simple reversible growth; however, the dynamical instability strongly reinforces this effect (Ref. [8]).

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