Microtubule Dynamics may Embody a Stationary Bipolarity Forming Mechanism Related to the Prokaryotic Division Site Mechanism (Pole-to-Pole Oscillations)

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Abstract. Cell division mechanisms in eukaryotes and prokaryotes have until recently been seen as being widely different. However, pole-to-pole oscillations of proteins like MinE in prokaryotes are now known to determine the division plane. These protein waves arise through spontaneous pattern forming reaction – diffusion mechanisms, based on cooperative binding of the proteins to a quasistationary matrix (like the cell membrane or DNA). Rather than waves, stationary bipolar pattern formation may arise as well. Some of the involved proteins have eukaryotic homologs (e.g. FtsZ and tubulin), pointing to a possible ancient shared mechanism. Tubulin polymerizes to microtubules in the spindle. Mitotic microtubules are in a highly dynamical state, frequently undergoing rapid shortening (catastrophe), and fragments formed from the microtubule ends are inferred to enhance the destabilization. Here, we show that cooperative binding of such fragments to microtubules may set up a similar pattern forming mechanism as seen in prokaryotes. The result is a spontaneously formed, well controllable, bipolar state of microtubule dynamics in the cell, which may contribute to defining the bipolar spindle.

Key words: microtubule dynamics, cell division | bipolarity | pole-to-pole oscillations | Turing structures

1. Introduction

Cell division (cytokinesis) in eukaryotes is preceded during mitosis by the spontaneously established bipolar spindle, consisting of microtubules formed from polymerized heterodimers of α , β -tubulin. Individual microtubules are known to be in a highly dynamical state, with growth being interrupted by rapid shortening (catastrophe) phases (dynamic instability) [14, 49, 81]. A number of microtubule associated proteins (MAPs) and plus-end tracking proteins (+TIPs) are known to be involved in the precise control of growth rates and catastrophe frequency [9, 10, 22, 31, 84].

The spontaneous organization of microtubules into a bipolar spatial structure and the role of the spindle to determine the division plane of the cell is still a

much studied, somewhat enigmatic subject. Many models have been proposed, but experiments from different cell types seem to indicate that spindle assembly may be governed by a combination of modular principles and that their relative contribution may vary in different cell types [36, 50, 85].

Recently, insight into cell division has been extended from a hitherto unlikely source, prokaryotic cells. These cells were for a long time considered to have a very crude mechanism for genome separation, but the actual mechanism seems to be highly dynamic, relying on well-studied phenomena of spontaneous pattern formation arising in non-equilibrium systems. Indeed, it was found that recurrent waves of MinD,E proteins run back and forth (so-called pole-to-pole oscillations) in the rod-shaped cells. The mean concentration is lowest in the middle of the cell, thus allowing the tubulin-like GTPase FtsZ to assemble a ring of polymer underneath the membrane at midcell as a prelude to cell division [20, 25, 64].

Partitioning loci encoding Walker box ATPases related to MinD are present on many prokaryotic plasmids and chromosomes. Partitioning loci function to segregate plasmids and perhaps chromosomes to daughter cells at cell division. The ParA protein of *E. coli* also shows recurrent waves within the nucleoid region, and a similar situation is present for the ParA-homologous protein *Soj* in *Bacillus subtilis*. The wave periods are much longer than those for the MinD,E system, indicating that these waves constitute autonomous pattern generating systems [15, 44, 63].

The first proposed model for prokaryotic protein waves [47], M-deB in the following, assumes that these proteins diffuse in the cytoplasm, but under appropriate conditions, they may associate cooperatively with the membrane of the cell. This introduces an autocatalytic step in the formation of the bound state of the protein, a prerequisite for pattern forming reaction-diffusion mechanisms. Another crucial feature is the large spread in effective diffusion rates of the two forms of the proteins, the free and the bound form. Under appropriate conditions for the involved rates, such an autocatalytic system with differential diffusion spontaneously generates stable spatial concentration patterns (Turing structures), from homogeneous initial concentrations with small random fluctuations. This pattern forming mechanism is well studied both theoretically and experimentally [13, 54, 66, 79]. For an accessible account of these ideas in a biological context, see [46].

The prokaryotic waves come about by the formation of a Turing-structure with high concentration in one end of the cell, low at the other. This structure is destabilized by an antagonist, only to reform at the other end of the cell, etc., and recurrent waves are thus produced. A modification to the M-deB mechanism was formulated, where the antagonist was non-moving. However, its effect is to convert the bound protein into an alternative form (say, ParA-ATP and ParA-ADP), which may act as a seed to destabilize the Turing-pattern [29].

The above schemes may be particular examples of a general class of pattern forming mechanisms, based on protein oligomerization upon a template (membranes, DNA, etc, see below) with resulting enhanced ATPase (or NTPase) function in the

oligomer state, which may bring the oligomer into an unstable internal state. An effector may then destabilize the oligomer and in the process produce seeds, which may spread and act as further destabilization agents. This kind of destabilization may apply to a large superfamily of proteins [80, 56, 40].

Thus NTPases may be an ancient class of proteins capable of setting up spontaneous spatial patterns by mechanisms related to the M-deB model. The actual protein used may thus have varied, and many particular pattern forming systems may be possible, which may contribute to the emerging picture that homologous proteins in prokaryotes and eukaryotes seem to have interchanged function. For example FtsZ forms the ring at the division site in prokaryotes [69], whereas this role is assigned to actin in eukaryotes. Weak homologs of actin, being involved in cytoskeletal morphogenesis, have been found in bacteria (Mreb, Mbl) [35, 82, 16, 7].

The eukaryotic homolog of FtsZ is tubulin, which however forms microtubules. The polymerization process of tubulin involves GTPase activity as well, and the microtubule is in a metastable state, stabilized by a cap at the plus end. Catastrophe exposes the plus end and the internal unstable protofilaments curl out in bulls-horn like structures. Products formed during depolymerization may act as seeds for further destabilization, much as described above.

Under the assumption that prokaryotic and eukaryotic cell division mechanisms may have common roots, the spatial pattern forming capabilities of such a seed mechanism will be explored here. The main idea is that fragments from the depolymerization process may interact cooperatively with microtubule ends during catastrophe, much like the above described proteins in prokaryotes. The quasi-stationary matrix to which binding occurs is now not the cell membrane or DNA, but the unfolding microtubules, see Fig. 1.

Figure 1. Microtubule during catastrophe. The plus end shows protofilaments curling outwards. If destabilizing proteins (solid circles) bind cooperatively (bfr) to the unfolding protofilaments and the free destabilizing proteins (fr) diffuse much faster than the bound form, this may create a so-called activator – depletor reaction – diffusion system, known to form spatial patterns. This may induce a stable, dynamic spatial pattern in the cell. If parameters are right, a bipolar pattern as in Fig. 5 may form spontaneously.

In prokaryotes, recurrent waves are formed. The core mechanism which creates the initial pattern may just as well form a bipolar spatial pattern, if parameters are right. If no antagonist is present to destabilize this pattern in the proposed catastrophe-induced pattern forming mechanism, no waves emerge but microtubule dynamics may be bipolar in space. The intrinsic dynamics is thus qualitatively the same everywhere in the cell, but quantitatively it becomes different in the spontaneously formed polar regions versus the equatorial regions.

The present study thus points to an ancestral role for the presented pattern types, and a link in generating bipolarity in prokaryotes and eukaryotes.

2. Model Overview

In subsection 2.1 the core model for spatial pattern formation is presented, as it was introduced for prokaryotes, the M-deB model [47]. The basic feature is protein binding to a matrix, in the form of a quasistationary structure like the cell membrane, with kinetics where the binding is cooperative that is autocatalytic. The bound protein and its free form constitute a so-called activator – depletor [46] reaction – diffusion system, which is well known to be capable of spontaneous formation of well-defined spatial concentration patterns, like high concentrations at two opposite, spontaneously created poles within the cell, and corresponding small concentrations in the equator region of a spherical cell.

In subsection 2.2 the basic features of microtubule dynamics is presented. Nucleation from tubulin heterodimers and subsequent growth with the uptake of tubulin bound to GTP is incorporated in a kinetic model. The polymer is a GTPase, and inside the microtubule tubulin is bound to GDP. The microtubule consists of bundles of protofilaments with a tendency to bend to form curled rather than straight polymers. The plus end of the microtubule is prevented from curling out by a stabilizing cap [57]. By severing of the cap, catastrophe is induced, the protofilaments at the plus end bend outwards and the entire microtubule undergoes rapid shortening, while tubulin dimers and oligomers in the tubulin-GDP form are released [78]. Here a model [2] for microtubule dynamics is used, which incorporate induction of catastrophe by the oligomers created. This constitutes an autocatalytic step for catastrophe and the released oligomers may induce catastrophe on neighboring microtubules.

In Subsection 2.3 the present model is introduced, the main feature being that the oligomers bind cooperatively to the protruding curled protofilaments. Hereby a pattern forming system much as the one discussed in Subsection 2.1 is created, with the matrix in the form of the cell membrane replaced by the microtubules.

2.1. PATTERN FORMATION BY COOPERATIVE BINDING TO A MATRIX

The core model for spontaneous spatial pattern formation used here is also the basic model in the M-deB model [47]. The main feature is a component, say a protein or oligomer, x , which exists in two versions, a form x which may diffuse in the cytoplasm, and a form bx which binds cooperatively to a matrix such as the cell membrane. The kinetics adopted for this process in the M-deB model is

$$\frac{d[bx]}{dt} = k_1[x][bx]^\gamma - k_t[bx] \quad (1)$$

$$\frac{d[x]}{dt} = k - k_1[x][bx]^\gamma \quad (2)$$

Here, the rate for formation of the bound protein is proportional to $k_1[x][bx]^\gamma$ that is to the concentration of the free form multiplied with $[bx]^\gamma$ which with the Hill constant γ larger than one describes the cooperativity. Thus bx enhances its own formation. Following M-deB $\gamma = 2$ is used below. The free form is removed by the same rate.

If diffusion is added to these equations, and the diffusion constants satisfy $D(x) \gg D(bx)$, which is quite reasonable since x is free to diffuse while xb is bound to a quasi-stationary matrix, the above system constitutes a well-researched reaction – diffusion system. Indeed, the above equations are known as Sel'kov's model [70], originally proposed as a feasible biochemical model for concentration oscillations in time, but subsequently shown to be capable of spontaneous spatial pattern formation as well.

Many extensions to the Sel'kov model with additional terms have been proposed. In the following sections we will discuss yet another variant of this model, and the corresponding linear stability theory is briefly set out in the Appendix. In the M-deB model, Equations (1)–(2) are also extended with terms from an antagonist, but this will not be discussed here. For a further extension of the M-deB equations, and corresponding stability analysis, see [29].

2.2. MICROTUBULE DYNAMICS

The polymerization of tubulin heterodimers to form microtubules has been studied extensively, and the simplified treatment used here has been adapted from [2, 72]. An extension of these models to account for spatial waves have already been made by Sept [71].

To initiate polymerization, tubulin heterodimers bound to GTP (T_t in the following) at first need to react in a nucleation process, which for simplicity will be taken as

with rate constant k_n .

Polymerization then proceeds with the uptake of one tubulin hetero dimer per step

with rate constant k_g .

The corresponding rate equations are

$$\begin{aligned}
 &\dots \\
 \frac{d[P_i]}{dt} &= k_g[T_t][P_{i-1}] - k_g[T_t][P_i]
 \end{aligned} \tag{5}$$

$$\begin{aligned}
 \frac{d[P_{i+1}]}{dt} &= k_g[T_t][P_i] - k_g[T_t][P_{i+1}] \\
 &\dots
 \end{aligned} \tag{6}$$

from which it is seen that in the rate for the sum $C_n = \sum P_i$ from $i = nc$ terms cancel out and we are left with

$$\frac{d[C_n]}{dt} = k_n T_t^{nc} - k_g[T_t][P_N] \tag{7}$$

where N is a sufficiently large number for $[P_N]$ to be so small that the term $k_g[T_t][P_N]$ may be neglected.

A similar reasoning applies for the weighted sum $C_m = \sum i \times P_i$, which only leaves the two terms $k_n n c [T_t]^{nc}$ and $k_g[T_t][C_n]$ for the rate of C_m . The term C_m is the totally consumed amount of tubulin dimers, and its concentration is proportional with experimentally accessible data, such as turbidity.

Catastrophe in this simplified scheme is approximated by a fast single step for each polymer

with rate constant k_c , where polymers of length i depolymerize to yield i hetero dimers of tubulin, now in the state bounded to GDP. By a similar reasoning as above, this may be incorporated into the rate of C_n by adding a term $-k_c[C_n]$, and for C_m by adding $-k_c[C_m]$.

Finally, the tubulin-GDP is activated to tubulin-GTP

with rate constant k_p , making the entire system a far from equilibrium system consuming free energy.

The overall kinetics thus becomes

$$\frac{d[C_m]}{dt} = k_n n c [T_i]^{nc} + k_g [T_i] [C_n] - k_c [C_m] \quad (10)$$

$$\frac{d[C_n]}{dt} = k_n [T_i]^{nc} - k_c [C_n] \quad (11)$$

$$\frac{d[T_i]}{dt} = k_p [T_d] - k_n n c [T_i]^{nc} - k_g [T_i] [C_n] \quad (12)$$

$$\frac{d[T_d]}{dt} = k_c [C_m] - k_p [T_d] \quad (13)$$

This system of rates may be integrated numerically, and with realistic rate constants, the system is capable of accounting for a number of features found in *in vitro* experiments on microtubule assembly [2, 72].

2.3. BINDING OF CATASTROPHE-INDUCED FRAGMENTS TO MICROTUBULE MATRIX

Microtubule assembly *in vitro* has been extensively studied, and among the many features realized in such experiments, the formation of damped concentration oscillations and waves is of particular interest in the current context. When high concentrations of purified ice-cold tubulin dimers are rapidly warmed to 37 °C it is possible to observe a series of damped oscillations [8, 42, 59]. One may at first think that the temperature rise sets off increased nucleation and polymerization, which eventually desynchronize due to random catastrophe of individual microtubules. However, it is possible to obtain more than a dozen such oscillations [8], which is unlikely to be compatible with such a mechanism. Instead several authors have suggested that synchronization is present in microtubule dynamics, in the form of autocatalytic feedback from the released products during catastrophe, such as tubulin-GDP itself or small oligomers or rings of tubulin-GDP. A number of models which incorporate this kind of feedback have been proposed [2, 45, 72], but the nature of this destabilization is still an open question [11].

In analogy with the mechanisms discussed above for prokaryotes, it is suggested here that the destabilization due to small oligomers is due to cooperative binding to the microtubules during catastrophe. Thus extending the model in [2], where the destabilizing term in the kinetics is taken to be linear, a nonlinear such effect is suggested. This simple change has profound consequences for the spatial organization of the microtubules, as we shall see.

The model treated here thus assumes that small oligomers of tubulin-GDP in the form of free oligomers fr may bind cooperatively to the unfolding microtubules. Thus the bound form bfr promotes its own synthesis. We propose the following

model

$$\frac{d[bfr]}{dt} = k_0[C_n][fr] + k_1[fr][bfr]^\gamma - k_t[bfr] \quad (14)$$

$$\frac{d[fr]}{dt} = k_3[T_d]^{nr} - k_b[fr] - k_0[C_n][fr] - k_1[fr][bfr]^\gamma \quad (15)$$

and the bound form increases the catastrophe rate proportional to $H(bfr) = k^*[bfr]^{nh}/(K_h + [bfr]^{nh})$. In Equation (14) the term $k_1[fr][bfr]^\gamma$ describes the cooperative formation of the bound form. The term $k_0[C_n][fr]$ allows for some non-cooperative formation of bfr directly from the unbound form fr and available microtubules. The rate of fr has two equivalent loss terms. fr is additionally allowed to form from T_d and disintegrate to T_d with the terms $k_3[T_d]^{nr}$ and $-k_b[fr]$, respectively.

The combined kinetics thus becomes Equations (14)–(15) in combination with

$$\frac{d[C_m]}{dt} = k_n n c [T_i]^{nc} + k_g [T_i][C_n] - k_c(1 + H(bfr))[C_m] \quad (16)$$

$$\frac{d[C_n]}{dt} = k_n [T_i]^{nc} - k_c(1 + H(bfr))[C_n] \quad (17)$$

$$\frac{d[T_i]}{dt} = k_p [T_d] - k_n n c [T_i]^{nc} - k_g [T_i][C_n] \quad (18)$$

$$\begin{aligned} \frac{d[T_d]}{dt} = & k_c(1 + H(bfr))[C_m] + nr k_b [fr] + nr k_t [bfr] \\ & - k_p [T_d] - nr k_3 [T_d]^{nr} \end{aligned} \quad (19)$$

With added diffusion, these equations show pattern forming capabilities. For the diffusion coefficients of the small molecules the measured value for free tubulin in sea urchins $D_t = 5.9 \mu\text{m}^2/\text{s}$ has been used [68], and much smaller values $D_{C_n} = D_{bfr} = 0.10 \mu\text{m}^2/\text{s}$ for the bound forms. The parameter values for the microtubule kinetics was adapted from [2] where values for *in vitro* kinetics are given. However, the interesting case is *in vivo* dynamics. Growth and catastrophe rates are substantially increased in mitotic extracts and *in vivo* [88, 38, 67], and we thus increased k_g from 10 to 40, and k_c from 0.0010 to 0.050. The nucleation rate constant was augmented from 2.0×10^{-8} to 6.0×10^{-8} because γ – tubulin, present *in vivo* [33], may act as a more effective nucleation factor as those found in the *in vitro* preparations. Additional constants were $k_p = 0.5$, $K_h = (0.8 \cdot 10^{-3})^{nh}$, $nh = 1.6$, $k^* = 1.33$, $k_0 = 0.875$, $k_1 = 3.2 \cdot 10^4$, $k_3 = 6.5 \cdot 10^{-5}$, $k_4 = 6.5 \cdot 10^{-5}$, $k_t = 5.0 \cdot 10^{-3}$, $k_b = 5.0 \cdot 10^{-5}$, $nc = 2$, $nr = 3$, $\gamma = 2.0$. Units: concentration μM . Time: s. Length: μm . Thus k_g has dimension $\mu\text{M}^{-1}\text{s}^{-1}$, etc.

The total concentration of tubulin hetero dimers $[T_{\text{tot}}] = [C_m] + [T_i] + [T_d] + nr([bfr] + [fr])$ is conserved in the above equations, and we used $[T_{\text{tot}}] = 20$ [17].

3. Results

The model Equations (14)–(19) with added diffusion may be solved numerically. One dimensional space was discretised (100 mesh points) and $\partial^2 y / \partial s^2$ was approximated with $(y_{i+1} - 2y_i + y_{i-1}) / \delta s^2$. The set of 6×100 ordinary differential equations was solved by a stiff solver.

The numerical solution to Equations (14)–(19) is displayed in Figure 2.

From an initial condition of tubulin-GTP $T_t = 20 \mu\text{M}$ microtubules build up and a stationary homogeneous pattern of C_m (e.g. total polymerized tubulin) is seen. This state corresponds to $C_m = 2.5 \mu\text{M}$, $C_n = 2.5 \times 10^{-4} \mu\text{M}$, $T_t = 17.2 \mu\text{M}$ and $T_d = 0.35 \mu\text{M}$. Due to random perturbations, eventually a Turing pattern builds up in the small oligomers fr and bound oligomer bfr , see Figure 3. Due to the much smaller concentrations of the oligomers, the grey scale in this figure is rescaled. The bound oligomer induces increased catastrophe of the microtubules, and the eventual formation of a bipolar spatial distribution in the cell of these oligomers results in an increased catastrophe rate at midcell with corresponding lower C_m here, as seen in the right part of Figure 2. C_m at the cell poles (upper and lower part of Figure 2 eventually) is correspondingly higher.

The distribution of free oligomers (fr) is bipolar as well. It is a standard feature of activator – depletor Turing systems that the fast diffusing component (fr) is low, where the autocatalytic component (bfr) is high and *vice versa*. However, due to its much higher diffusion constant, the amplitude of this pattern is correspondingly lower. fr varies from $0.278 \times 10^{-3} \mu\text{M}$ (poles) to $0.155 \times 10^{-3} \mu\text{M}$ at midcell.

The obtained pattern is geometrically analogous to $\cos(2\pi s/L)$, s being the one-dimensional space coordinate used in this simulation.

The spontaneously created spatial bipolar inhomogeneity in microtubule dynamics displayed in Figure 2 results in a difference in mean microtubule length $\bar{n}$, see Figure 4.

Figure 2. Bipolar pattern formation in microtubule dynamics. The concentration C_m of total tubulin assembled in microtubules is displayed with gray scale over a cell length (vertical coordinate) $L = 50 \mu\text{m}$. At first, a homogeneous (grey) distribution of microtubules is recorded (left part of frame), but in due time, the small random perturbations present spontaneously generate an inhomogeneous bipolar concentration pattern (right part of frame) with high concentration at the bottom and top, low in the middle of the cell.

Figure 3. Bipolar spatial pattern of bound oligomers to microtubules. Initially (left part of frame) a homogeneous build-up (change from black to grey) of attached oligomers is recorded. This eventually undergoes spontaneous symmetry breaking, and a bipolar concentration pattern of bound oligomers (*bfr*) is generated (right part of frame), with high concentration at midcell (white) and correspondingly low concentrations at both ends of the cell. The variation in (*bfr*) concentration is from $0.100 \times 10^{-3} \mu\text{M}$ (black) to $1.29 \times 10^{-3} \mu\text{M}$ (white). This inhomogeneous distribution of bound oligomers induces different catastrophe rates of microtubules, with the result seen in Figure 2.

Figure 4. Concentration of total assembled tubulin C_m (in μM) versus percent cell length. The concentration is highest at the poles of the cell, compare Figure 2. Also shown is concentration of microtubules C_n . As this concentration is of the order $2.0 \times 10^{-4} \mu\text{M}$, the actual values of C_n are multiplied with a factor 1000 here. The fraction C_m/C_n is the mean microtubule length $\bar{n}$, which is 47% higher at the poles, 17% lower at midcell, than the stationary homogeneous value.

We have

$$\bar{n} = \frac{\sum i P_i}{\sum P_i} \equiv \frac{C_m}{C_n} \quad (20)$$

Before the onset of a Turing structure, this evaluates to $\bar{n}_{\text{stat}} = 2.5/(2.5 \times 10^{-4})$ with the numbers taken from the stationary state given above. In Figure 4 the values of C_m and C_n are displayed. An increase in mean microtubule length at the poles is observed, with corresponding lower mean microtubule length at midcell.

Figure 5. Bipolar pattern in 3D spherical cell. If parameters in the model favors the bipolar one-dimensional pattern in Figure 2 in a cell of length $L = 50 \mu m$, a spherical cell with about a 6% larger diameter ($2R \simeq 1.06 \times 50 \mu m$) is predicted to form the 3D pattern shown here in a corresponding simulation in 3D. This pattern has two poles with high-concentration and a low-concentration region in an equatorial band close to the cell surface.

It should be noted that these one-dimensional patterns carry over into analogous patterns in a three-dimensional spherical cell. The analogy to the one-dimensional function $\cos(2\pi s/L)$ in the sphere is depicted in Figure 5.

This pattern is given by the spherical Bessel function $j_2(\kappa_{21}r/R)$ times the spherical harmonic $Y_{20}(\theta, \phi)$ and such patterns have been obtained earlier by the author for simple reaction – diffusion systems in 3D spherical simulations and indeed in a number of related 3D geometries, such as elongated and compressed spheres (prolate and oblate spheroidal coordinates, respectively) [26–28]. In the one-dimensional simulations, parameters in the model are such that the pattern wave length λ is close to the cell length L , and the corresponding wave number κ is close to the so-called critical Turing wave number (see Appendix, Equation (32)). For a pattern of form $\cos(2\pi s/L)$ we have $\kappa = 2\pi/L$. If $\kappa \simeq \kappa_{21}/R = 1.064\pi/R$ in the spherical cell of radius R , the same mechanism is predicted to form the pattern in Figure 5. This amounts to a 6% larger diameter than the cell length treated in the one-dimensional simulation.

The pattern predicted for the spherical cell has two spontaneously created opposite regions (poles) of high concentrations and a low concentration region, which is now located near the cell surface in a circular band in the equator region. The polar axis is shown vertical, but it is formed in a random direction. It is suggested that such a pattern may play a role in spindle pole generation.

4. Discussion

The interest in microtubule dynamics has recently focused on proteins influencing spindle dynamics. It has been stated that the control processes based on such proteins so far may only have revealed the top of a possible iceberg [81]. The discovery of pattern forming processes involved in prokaryotic cell division has pointed to a possible link of such patterns to spindle dynamics. The present work indicates a connection where the above proteins may have additional roles regulating rates in spindle dynamics to allow for intrinsic dynamical formation of bipolar states in eukaryotic cells.

The model suggested here is based on the assumption that originally, prokaryotes and eukaryotes to some degree shared a common principle for the generation of bipolarity in the cell. This has led to the proposal here that such a link may be cooperative binding of a class of proteins to a matrix, either the cell membrane or the nucleoid (prokaryotes), or unfolding microtubules (eukaryotes).

An eukaryotic relic of the waves seen in prokaryotes may be linked to the observed waves in the actin – myosin system that has been recorded to display cortical waves, though normally suppressed by the microtubule system [3, 60]. The actin ring in the cleavage plane during cytokinesis appears not to be static, but rather forms a highly dynamic structure, dependent on continuous assembly and disassembly [53, 58]. A model for actinbased dynamical structures in the form of pole-to-pole waves has appeared in [19]. Microtubule – actin interactions is discussed in [65]. Actin homologs in prokaryotes are known [51]. MreB and Mbl form spirals along the long axis of the cell, and recently, it was shown that the waves in the MinD,E system are linked to similar spirals winding around the cell [18, 74]. This does not require essential changes to the one-dimensional M-deB model extended here.

Dynamical structures in microtubule dynamics, as discussed here, is based on the unifying principle of cooperative binding of proteins to a matrix, in this case the unfolding microtubule. The nature of the binding protein and the mechanism for increasing the catastrophe rate may be varied from the simple proposal here. Thus it has recently been found that the protein katanin may sever microtubules. Oligomerization on the microtubule wall eventually leads to ring formation and subsequent microtubule disassembly [21], and katanin has its highest concentration close to the spindle poles [62]. Another example is HIV-1 Rev that forms rings binding to microtubule ends [86] and induces depolymerization of the microtubules. This protein shows homology to XKCM1 which also destabilizes microtubules [55]. A system with close analogy to the model proposed here seems to be present in the study of KinI kinesins [52]. These kinesins have two distinct binding sites, one which mediates binding to the microtubule wall and allows the kinesin to move to the end of the microtubule, another which induces cooperative destabilization of the microtubule, possibly by bending single protofilaments [22, 31]. KinI proteins may bind to the same site as the drug Vinblastine that causes extensive peeling of protofilaments [87].

A simple mechanism based on feedback from tubulin-GDP oligomers is used here. Tubulin-GDP undergoes isodesmic polymerization to a number of oligomers, which eventually stabilize in rings [24, 48]. It has been a matter of some discussion to what extent such fragments may form directly from the depolymerization of the microtubules or subsequently forms from tubulin-GDP [5, 43]. Also tubulin-GDP itself may increase the catastrophe rate [6], but also see [83].

A further simplification is the use of total depolymerization to tubulin-GDP during catastrophe that is the possibility of rescue and renewed polymerization before total decomposition is omitted. This has been done to obtain the simplified dynamics

in the summed entities C_m and C_n , following a number of other simplified models. If rescue is included each of the polymers P_i has to be followed and the only simplification is to write their dynamics in terms of partial differential equations [34].

Since the concentration of microtubules C_n in the model is about $2 \times 10^{-4} \mu\text{M}$ and thus by implication concentration of bound oligomers bfr is of the same order, the number of molecules in the proposed reaction – diffusion system is quite low in a cell with diameter about $50 \mu\text{m}$, of the order of 4000. It should be noted, though, that the number of molecules of the Turing pattern generating protein MinE in prokaryotes is lower, and a stochastic study of the possibility to have Turing structures with so few molecules has confirmed that this is indeed feasible [23]. Stochastic models for microtubule dynamics have been discussed in [2]. Also see the Landau – Ginzburg model approach [73].

The possibility discussed in this work that spontaneous spatial pattern formation may be an intrinsic part of microtubule dynamics may relate to experiments on microtubule oscillations, in which concentric rings of assembly was observed as well [41, 42]. The present work may further relate to the experimental results described in [75–77]. (Also, see the recent theoretical model developed by Tuszynski's group on spatial self-organization of microtubules in gravitational fields [61]). Striped pattern formation in preparations of pure tubulin *in vitro* was observed, and shown to involve reaction – diffusion processes. However, these patterns are gravity dependent. The stripes contain alternate orientation of microtubules and may involve a component of nematic liquid-crystalline ordering. It has been pointed out in these works that the unraveling microtubule ends leave behind a trail of high tubulin concentration, and by analogy to the present model, a similar trail of oligomers. This may contribute significantly to the effective diffusion coefficient for the oligomers, and thus rapid degradation of extended objects (rather than point entities) as microtubules also affects neighboring microtubules as a possible source of synchronization.

The above remarks point to possible extensions in the form of more detailed models. The main idea studied in this work is that the pattern forming mechanisms so far studied for bacteria may have analogs in eukaryotic cells, and to explore this possibility, extensions of simplified models for microtubule dynamics have been used here.

The analogy proposed is not in the form of waves, as seen in prokaryotes. Rather it is in the form of a dynamical spatial stationary structure, though still maintained by highly related nonlinear reaction–diffusion processes intrinsic to microtubule dynamics itself. The possibility of having spontaneously created bipolar microtubule dynamics may constitute an ancient scaffold on which many extensions may have been built e.g. in the form of dynein-mediated stabilization of asters, or the Ran system for connecting chromatin through microtubules to the centrosomes. Neither of these systems seem to represent the core of bipolarity. Thus in experiments with laser destruction of one or both centrosomes and subsequent cold treatment destruction of polymerized tubulin, bipolar spindles devoid of centrosomes

reformed. Anaphase and cytokinesis appeared normal [1, 37]. The possibility exists that the ability to form a bipolar spindle without centrosomes is present throughout eukaryotes [32]. The Ran system connected to the chromosomes is not present in experiments with *Drosophila* male meiosis without chromosomes, but spindle assembly and cytokinesis were recorded [4].

Such a scaffold role of intrinsic bipolarity in microtubule dynamics itself may also connect to the core mechanism of cytokinesis. Indeed, depolymerizing microtubules are thought to activate *RhoA*, a small GTPase that mediates formation of contractile actin structures [39]. The recent proposal that the division plane is determined by a local minimum of microtubule density [12], or maximal rate of depolymerizing microtubules, is imminently compatible with the structure in Figure 5, which directly yields such a density minimum (or maximal depolymerizing rate) close to the cell surface in the equatorial plane.

The present study thus introduces the possibility of an ancestral role for the presented dynamical structures, and suggests a link between generating bipolarity in prokaryotes and eukaryotes.

Appendix: Linear Stability Theory

The Equations (14)–(19) may be analyzed for stability. The source of spatial pattern formation and waves in prokaryotes and the M-deB model is the formation of a Turing structure in an activator–depletor system of the form Equations (1)–(2), with subsequent destabilization. In the analogy to microtubule dynamics treated here, the search is for the build up of a stable stationary spatial pattern from homogeneous initial conditions.

A necessary condition for this is differential diffusion. The system may thus be integrated numerically at first with all diffusion constants equal, to obtain the homogeneous steady state, notably C_n^* and T_d^* , as these enter the rates for the presumptive Turing system, Equations (14)–(15). With these obtained, we set $\epsilon = k_0[C_n]^*$ and $k = k_3[T_d^*]^{nr}$. The system Equations (14)–(15) is required to be stable in the stationary state in the absence of differential diffusion. The subsequent introduction of differential diffusion, notably for bfr and fr , may lead to a Turing type instability.

For the stationary state we find

$$bfr^* = k/k_t \quad (21)$$

$$fr^* = k/[k_1(k/k_t)^2 + \epsilon] \quad (22)$$

For the Jacobian J evaluated at the stationary state we define

$$a = J_{11} = 2\left(\frac{k_1}{k_t}\right)\left(\frac{k^2}{k_1(k/k_t)^2 + \epsilon}\right) - k_t \quad (23)$$

$$b = J_{12} = k_1(k/k_t)^2 + \epsilon \quad (24)$$

$$c = J_{21} = -2\left(\frac{k_1}{k_t}\right)\left(\frac{k^2}{k_1(k/k_t)^2 + \epsilon}\right) \quad (25)$$

$$d = J_{22} = -k_1(k/k_t)^2 - \epsilon \quad (26)$$

The conditions for Turing pattern formation is treated in many sources. An account close to the notation used here is given in Hunding and Engelhardt [30]. For an activator–depletor type Turing system, it is required that a and b has the same sign. Since $b > 0$ we must ensure $a > 0$. For small ϵ , $a \rightarrow k_t$, but larger ϵ diminishes a . Thus ϵ (*i. e.* k_0) has to be sufficiently small.

The trace $Tr = a + d$ is evaluated to

$$Tr = 2\left(\frac{k_1}{k_t}\right)\left(\frac{k^2}{k_1(k/k_t)^2 + \epsilon}\right) \quad (27)$$

$$-\left(k_t + k_1\left(\frac{k}{k_t}\right)^2 + \epsilon\right) \quad (28)$$

and the determinand is

$$\det = ad - bc = k_t[k_1(k/k_t)^2 + \epsilon] \quad (29)$$

For the stationary state to be stable in the absence of differential diffusion it is required that $\det > 0$, which is automatically fulfilled, and $Tr < 0$ which for small enough ϵ approximates to $k_t^3 < k_1 k^2$.

In the presence of differential diffusion, $D_{bfr} \ll D_{fr}$, standard Turing theory [30] requires

$$\frac{D_-}{D_+} \equiv \frac{D_{fr}}{D_{bfr}} > \frac{\det - bc + 2\sqrt{-bc \det}}{a^2} \quad (30)$$

For $\epsilon \ll k_1(k/k_t)^2 = w$ this yields

$$\frac{D_-}{D_+} > \frac{k_1 k^2}{k_t^3}(3 + 2\sqrt{2}) \quad (31)$$

If this inequality is fulfilled, Turing patterns build up with a spatial wave number κ close to the so-called critical Turing wave number κ_c which is given by

$$\kappa_c^2 = \frac{aD_- + dD_+}{2D_+D_-} \rightarrow \frac{a}{2D_+} \quad (32)$$

and thus a spatial wave length

$$\lambda = 2\pi/\kappa_c \simeq 2\sqrt{2}\pi\sqrt{D_+/k_t} \quad (33)$$

where we have used $a \rightarrow k_t$ for $\epsilon \ll w$.

For a bipolar pattern to emerge, λ should be roughly equal to the length of the cell. Since $D_+ \ll D_-$ and D_- has been measured for sea urchin eggs, this places restrictions on k_t . From the trace condition $k_t^3 < k_1 k^2$ this subsequently restricts the last constants as well. For related simple models, it is often found though that the Turing structure is stable even for a slightly positive trace, in which case an increased amplitude of the stable spatial pattern may be obtained. The same may be achieved with a higher spread in diffusion coefficients.

After the onset of a spatial pattern, the assumption that C_n is homogeneous is no longer valid, as $H(bfr)$ enters its rate, whereas it is still a reasonable approximation for T_d due to its comparatively large diffusion constant. The evolution of the total system after the onset of the Turing instability is thus determined numerically.

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