Molecular mechanisms for microtubule length regulation by kinesin-8 and XMAP215 proteins

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The cytoskeleton is regulated by a plethora of enzymes that influence the stability and dynamics of cytoskeletal filaments. How microtubules (MTs) are controlled is of particular importance for mitosis, during which dynamic MTs are responsible for proper segregation of chromosomes. Molecular motors of the kinesin-8 protein family have been shown to depolymerize MTs in a length-dependent manner, and recent experimental and theoretical evidence suggests a possible role for kinesin-8 in the dynamic regulation of MTs. However, so far the detailed molecular mechanisms of how these molecular motors interact with the growing MT tip remain elusive. Here we show that two distinct scenarios for the interactions of kinesin-8 with the MT tip lead to qualitatively different MT dynamics, including accurate length control as well as intermittent dynamics. We give a comprehensive analysis of the regimes where length regulation is possible and characterize how the stationary length depends on the biochemical rates and the bulk concentrations of the various proteins. For a neutral scenario, where MTs grow irrespective of whether the MT tip is occupied by a molecular motor, length regulation is possible only for a narrow range of biochemical rates, and, in particular, limited to small polymerization rates. By contrast, for an inhibition scenario, where the presence of a motor at the MT tip inhibits MT growth, the regime where length regulation is possible is extremely broad and includes high growth rates. These results also apply to situations where a polymerizing enzyme like XMAP215 and kinesin-8 mutually exclude each other from the MT tip. Moreover, we characterize the differences in the stochastic length dynamics between the two scenarios. While for the neutral scenario length is tightly controlled, length dynamics is intermittent for the inhibition scenario and exhibits extended periods of MT growth and shrinkage. On a broader perspective, the set of models established in this work quite generally suggest that mutual exclusion of molecules at the ends of cytoskeletal filaments is an important factor for filament dynamics and regulation.

1. Introduction

Microtubules (MTs) are essential constituents of the cytoskeleton of eukaryotic cells. They provide mechanical support and are involved in a wide range of cellular functional modules. For instance, they serve cells to build cilia and flagellae which are slender extensions of the cell used for migration and sensory tasks. In addition, MTs are important during cell division, where they build the mitotic spindle and separate chromosomes. To facilitate this variety of tasks, there have to be mechanisms that control the dynamics of MTs [1]. Such capabilities are crucial for the MT cytoskeleton in order to accomplish such diverse tasks as cell division [2] and migration [3], and further, to
determine cell size and shape [4], and to position the nucleus in the centre of the cell [5,6]. Molecular motors and MT-associated proteins seem to play a crucial role in this regulation process [7,8]: motors move along the MT and interact specifically with the filament at its end. Other associated proteins that influence the dynamics of MTs also bind directly to the MT tip [9]. Biochemical reconstitution experiments have led to considerable insights into the interactions between MTs and associated molecules [10]. In the following, we highlight two specific proteins that are important for the length dynamics of MTs.

Kip3p is an MT depolymerizing molecular motor [11,12] of the kinesin-8 protein family [13]. It binds strongly to the MT lattice and, therefore, exhibits a long run-length [14]. For kinesin-8 the molecular mechanisms that lead to depolymerization are elusive. However for kinesin-13, which is also a depolymerizing molecular motor, it was shown that strong motor binding to the terminal tubulin heterodimer induces depolymerization [15]. Interestingly, Kip3p shows a length-dependent depolymerization activity mediated by the accumulations of motors along the MT, as shown in experiments [11,16] and recent theoretical work [17]. In vitro experiments have unveiled many molecular properties of kinesin-8: the tail of the motor has been shown to be responsible for long residence times on the MT lattice and it influences MT dynamics [18–20] and spindle size [21]; for a brief review of these findings, see [22]. In the mathematical analysis, we will concentrate on the depolymerizing activity of the motor and treat those molecular details effectively in terms of rate constants for movement, depolymerization activity, and attachment/detachment kinetics of the motors.

XMAP215 is an MT-associated protein [23] that has been shown to significantly amplify the growth rate of MTs [24], and to influence the dynamic properties of MTs in the cytosol [25,26]. Recent in vitro experiments investigated the interaction of XMAP215 with single MTs [27] and the interplay with other end-binding proteins, which act as cofactors [28,29]. Specifically, it was found that XMAP215 is a polymerizing enzyme to the MT plus-end; a single XMAP215 is able to polymerize several rounds of tubulin heterodimers to the MT [27]. Similar properties have also been observed for other end-binding proteins (e.g. [30]).

Combining the observations described above suggests that kinesin-8s and XMAP215 may constitute a minimal functional unit able to regulate MT dynamics [25,31] and antagonistically influence MT length. This view is supported by recent experiments on cilia [32] showing that the molecular motor Kif19a, which belongs to the kinesin-8 protein family, regulates the cilia length in a concentration-dependent manner: high motor concentrations lead to short cilia, whereas low motor concentrations lead to long cilia. On a molecular scale, the ability to regulate length is traced back to the observed length-dependent depolymerization speed [7]. In more detail, longer MTs are observed to depolymerize faster than shorter ones. This has been explained as follows [16,17]: molecular motors in the cytosol attach to the MT and subsequently move towards the MT tip. The unidirectional movement of motors towards the MT tip leads to an accumulation of motors and the motor density increases from the minus- to the plus-end of the MT, which finally results in an antennalike steady-state profile of molecular motors. Therefore, there are more motors present at the tip for longer MTs than for shorter ones, which in turn leads to the observed length dependence in the depolymerization speed. In combination with MT polymerization, which is either spontaneous or catalysed by XMAP215, this is a promising starting point to achieve MT regulation [7,33].

In this work, we elaborate on two possible molecular mechanisms of how molecular motors could interact with the MT tip. We specifically distinguish two scenarios, one where molecular motors prevent the addition of tubulin heterodimers at the MT tip (inhibition scenario), and another neutral scenario where MT growth is possible irrespective of whether the MT tip is occupied by motors or not. These differences in the interaction of motors with the MT tip give rise to a rich dynamics of MT length ranging from accurate length control to intermittent dynamics.

This article is organized as follows. In §2, we introduce a model for the dynamics of molecular motors on an MT. Further, we define different possible molecular scenarios for how kinesin-8s may interact with the MT tip during the depolymerization process, including the case when XMAP215 acts as a polymerase. In §3, we present our main results: we begin with an outline of the theoretical framework, and then employ it to study MT length dynamics. Our analytical calculations are complemented by stochastic simulations. Taken together, this allows us to identify the parameter regimes where length regulation is possible, and to provide a comprehensive analysis on how the ensuing stationary length depends on biochemical rates and protein concentrations. Moreover, we investigate the role of stochastic effects in length regulation, and discuss why there are dramatic differences between the considered scenarios. Finally, we conclude in §4 by discussing our results in terms of their possible biological relevance and their importance for driven diffusive lattice gases.

2. Model definition

To describe an MT, we consider a one-dimensional lattice gas model of finite length [L] [34,35] as illustrated in figure 1. This approximation is valid if the 13 protofilaments of an MT are independent and non-interacting. Motor proteins (kinesin-8), present at a constant bulk concentration c, are assumed to randomly attach to and detach from the MT lattice with rates $\omega_{on}$ and $\omega_{off}$, respectively, defining the binding constant $K = \omega_{on}/\omega_{off}$. Once bound, these motors move towards the plus-end at a constant hopping rate $v$; we fix the time scale by setting $v = 1$ (corresponding to approx. 6.35 steps s$^{-1}$ in the case of Kip3p [16]). As these motors hinder each other sterically, individual binding sites on MTs can at most be occupied once. This lattice gas model is known as the totally asymmetric simple exclusion process (TASEP) with Langmuir kinetics (LK) [36–38].

The right boundary is considered to be dynamic: when a Kip3p motor arrives at the MT plus-end, which is the boundary in our model, it acts as a depolymerase, i.e. it removes the last MT subunit at rate $\delta$ [17]. As we consider stabilized MTs following recent experiments [11,16], we do not include spontaneous depolymerization and MT dynamic instability. In addition, the MT is assumed to polymerize through the attachment of single tubulin heterodimers. Unfortunately, there is insufficient experimental information on the detailed molecular cycle for MT growth in the presence of kinesin-8 motors. We hypothesize the following different but equally plausible mechanisms for MT growth:
(i) The MT only grows at rate $\eta$ if the last site at the plus-end is not occupied by a kinesin-8 motor. Because kinesin-8 inhibits MT growth we call this the inhibition scenario (cf. figure 1).

(ii) The MT grows at rate $\gamma$ independently of whether the tip is occupied or not. This neutral scenario has been considered previously in [33] (cf. figure 1).

(iii) MT polymerization is facilitated by a second protein species, like for instance XMAP215. This enzyme, in the absence of kinesin-8, attaches to and detaches from the MT tip with rates $k_{on}$ and $k_{off}$, respectively. Once bound, XMAP215 prevents kinesin-8 from reaching the tip, and processively polymerizes the MT at rate $\eta_{X}$, i.e. the enzyme immediately binds to the newly formed tip site after polymerization has occurred.

We use the remainder of this section to give a concise summary of the results obtained recently for the neutral scenario [33]: the combined effect of motor attachment in proximity of the minus-end (left boundary) and subsequent movement towards the plus-end (right boundary) leads to an accumulation of motors, which results in an antenna-like steady-state profile [16,17]. At a certain distance from the minus-end, the density profiles saturate to the equilibrium Langmuir density $\rho_{eq} = K/(K + 1)$ [14]. The resulting density profiles in the vicinity of the minus-end are position-dependent, $\rho_{-}(x)$, and can be described by Lambert-W functions [38]. Moving further towards the MT plus-end, the density profile is determined by the interplay of motor current and the boundary conditions at the plus-end, which gives rise to a particular tip density $\rho_{+}(L)$. In a mean-field description [35], this determines the length dynamics

$$\partial_{t}L(t) = -\delta \rho_{+}(L) + \gamma.$$  \hspace{1cm} (2.1)

Steady state is reached at a critical density $\rho_{c}^{\pm} = \gamma/\delta$, where $\partial_{t}L(t) = 0$. Depending on whether the tip density $\rho_{+}(L)$ is smaller or larger than $\rho_{c}^{\pm}$ the MT grows or shrinks.

Because the motor current to the tip depends on the accumulation of motors along the MT, $\rho_{-}(x)$, the tip density depends on the actual length $L(t)$ of the MT. As a consequence a mechanism for MT regulation emerges: on a short MT, when the accumulation of motor density is low, also the tip density is low and the MT grows because the tip density lies below the critical threshold density, $\rho_{+}(L) < \rho_{c}^{+}$. This is in contrast to the case of a long MT where a higher density of motors accumulates along the MT and also the tip density is higher. Once the tip density exceeds the critical threshold value $\rho_{+}(L) > \rho_{c}^{+}$ the MT depolymerizes.

Figure 2 illustrates this mechanism. Shaded areas indicate density profiles for MTs of different length and also schematically account for the fact that the tip density is length-dependent and has a spike-like shape. The dashed line shows a threshold value for the tip density, above and below which the MT shrinks and grows, respectively.

### 3. Motor and microtubule dynamics

Though at first sight the neutral and the inhibition scenario as introduced above appear very similar, there are actually strong qualitative differences in the ensuing length dynamics. Figure 3a,b shows kymographs for the neutral and the inhibition scenario, respectively, as obtained from stochastic simulations employing Gillespie’s algorithm [40]. While in the neutral scenario the overall length of the MT stays approximately constant with only small fluctuations, the length dynamics for the inhibition scenario is intermittent with extended episodes of filament growth and shrinkage, reminiscent of the dynamic instability [41]. Note that for the inhibition scenario there is significant accumulation of motors at the MT tip during periods of depolymerization.

To understand how the system alternates between periods of growth and shrinkage, let us turn to a mathematical description of the dynamics. As already noted in the previous section, the length change of the MT is determined...
Figure 2. Illustration of a linear motor density profile (shaded areas) and the threshold density $\rho_c^s$ (dashed line) for MT regulation. Low tip density $\rho_s(L)$ results in a growing MT, and a high tip density results in a shrinking MT. Note that the density at the tip generally has a spike-like shape [39]. (Online version in colour.)

Figure 3. Kymographs of how molecular motors regulate an MT. In the neutral case (a), the system displays a higher accuracy in length regulation ($\delta = 0.2, \gamma = 0.1316$). This is in contrast to the case of growth inhibition (b) where the system displays intermittent dynamics ($\delta = 0.2, \eta = 0.385$). Attachment and detachment rates are $\omega_{on} = 0.001$ and $\omega_{off} = 0.003$.

by the tip density $\rho_s$, e.g. the probability that the MT tip is occupied by a molecular motor,

$$\partial_t L = \begin{cases} -\delta \rho_s + \gamma & \text{(neutral scenario)}, \\ -\delta \rho_s + \eta(1 - \rho_s) & \text{(inhibition scenario)}. \end{cases} \quad (3.1)$$

Here the first term on the right-hand side stands for depolymerization, and the second term describes polymerization dynamics of the neutral and the inhibition scenario, respectively. Equation (3.1) shows that depending on the magnitude of the tip density, $\rho_s$, the MT either grows or shrinks: for large tip densities, depolymerization is strong and the MT shrinks, while the MT grows for small tip densities (figure 2). The critical tip densities, $\rho_c^s$, where the filament length becomes stationary read

$$\rho_c^s = \begin{cases} \frac{\gamma}{\delta} & \text{(neutral scenario)}, \\ \frac{\eta}{\delta + \eta} & \text{(inhibition scenario)}. \end{cases} \quad (3.2)$$

To make further progress, one needs to determine the actual tip densities employing a mean-field approach for the motor dynamics along the MT [33].

| Table 1. Quantification of model parameters for kinesin-8 and XMAP215. |
|-----------------|-----------------|-----------------|
|                 | kinesin-8        | experiment [16] |
| speed           | $v = 1$          | 6.35 steps s$^{-1}$ |
| attachment      | $\alpha_{on}$   | 24 (nM min $\mu$m)$^{-1}$ |
| detachment      | $\alpha_{off}$  | $4.8 \times 10^{-3}$ s$^{-1}$ |
| depolymerization| $h$             | $n/k$ [17]        |
| tip detachment  | $\beta$         | $0.1 - 0.01$ s$^{-1}$ |
| **MT growth**   |                 |                 |
| neutral         | $\gamma$        | $n/k$            |
| inhibition      | $\eta$          | $n/k$            |
| **XMAP215**     |                 |                 |
| attachment      | $k_{on}$        | $0.1$ (nM s $\mu$m)$^{-1}$ |
| detachment      | $k_{off}$       | $3.8$ s$^{-1}$   |
| polymerization  | $\eta_r$        | $6.6$ dimers s$^{-1}$ |

*aTip dwell times of different kinesin-8 constructs are: 10 – 55 s [18], 20 – 40 s [19], 80 s [16]. In [16], it is shown that dwell times at the tip depend on motor concentration, suggesting cooperative effects of motors at the tip. A theoretical analysis is given in [17].

*bMT growth speeds in the presence of kinesin-8s in vivo are $1.3$ $\mu$m min$^{-1}$ [12], $2$ $\mu$m min$^{-1}$ [4,42]. Rate constants of individual growth events, however, are not available to our knowledge and the complexity of the process [43] renders it difficult to quantify the damping effects of kinesin-8 [44].

3.1. Phase behaviour and tip densities

For biologically relevant parameter ranges, the time scales of the tip dynamics and the motor dynamics are comparable (cf. table 1). Therefore, the motor density profile quickly adapts to changes in the tip density and one can readily assume that the tip density and the bulk density are adiabatically coupled [33]. Moreover, experimental data also show that both the attachment and the detachment rates, $\omega_{on}$ and $\omega_{off}$, are very small [16]. This suggests considering a simplified model where one neglects the attachment and detachment kinetics and assumes that a constant density $\rho_-$ serves as a particle reservoir at the left end of a lattice with fixed size $L$ (figure 4). This allows us to focus on the dynamics at the plus-end and unravel how it depends on the reservoir density $\rho_-$ Owing to the adiabatic coupling between boundary and bulk, the results for the full model can be inferred from the simplified model upon replacing $\rho_-$ by the actual spatially varying profile $\rho_-(x)$.

Since there is particle conservation, the dynamics of the tip density is given by

$$\partial_t \rho_s = I_b(\rho_+ - \rho_s) - I_{exit}(\rho_s), \quad (3.3)$$

where $I_b$ and $I_{exit} = \delta \rho_+$ denote the bulk current and the loss rate of motors due to depolymerization, respectively. Calculations are conveniently performed in a frame comoving with the MT tip. Then the bulk currents for the neutral (N) and the inhibition (I) scenario read in a mean-field approximation [33]

$$I_b^N = \rho_b(1 - \rho_b) - \eta \rho_b + \delta \rho_+ \rho_b \quad (3.4a)$$

and

$$I_b^I = \rho_b(1 - \rho_b) - \eta \rho_b(1 - \rho_s) + \delta \rho_+ \rho_b \quad (3.4b)$$

...
Here \( \rho_b \) denotes the motor density in the bulk of the MT, and the first term describes the current due to hopping processes accounting for particle exclusion on neighbouring sites. The remainder of the terms indicates polymerization and depolymerization currents, which in a comoving frame simply correspond to simultaneous movement of all particles on the MT lattice to the left and right end, respectively.

The stationary state of the model is determined by a balance of currents, or, in other words, the fixed point of equation (3.3): \( \rho_\pm \delta = \hat{J}(\rho_\pm, \rho_v) \). Solving for the tip density one finds

\[
\rho_\pm = \rho_v(\rho_\pm \delta, \eta) = \frac{\rho_\pm(1 - \eta - \rho_v)}{\delta(1 - \rho_v) - \eta \rho_b},
\]

for the inhibition scenario. The tip density is determined by the bulk particle flux towards the tip and, at the same time, the bulk density depends on the molecular processes at the MT tip. To make progress with the analytical calculations, it is necessary to have some knowledge about the nature of the density profiles and their stability with respect to fluctuations. For exclusion processes, there are in general three distinct phases, each of which corresponds to different bulk densities \( \rho_b \) and ensuing bulk currents [45,46]:

— **IN phase.** In this phase, the particle current that enters the system at the minus-end determines the bulk density. For TASEP, this phase is also called low-density phase.

— **EX phase.** The bulk density is determined by the current of particles that leave the system at the right boundary (TASEP: high-density phase).

— **MC phase.** In this phase, the maximal current (MC) through the system determines the bulk density. It corresponds to a local maximum in the current density relation \( \hat{J}(\rho_\pm) \). In contrast to the other two phases, the bulk density in the MC phase is independent of the boundary conditions.

Moreover, for exclusion processes, there are two possibilities to account for the boundary conditions at the left and right end. Either there is a domain wall (DW) delineating a low-density region, \( \rho_- \), from a high-density region, \( \rho_+ \), or there are boundary layers [47] at one of the MT ends.

### 3.1.1. Density perturbations and domain wall theory

To make progress on the phase diagram, we need to investigate the stabilities of the aforementioned DW and bulk density. To this end, we introduce two important criteria that allow us to analyse the stability of perturbations in exclusion processes known as DW theory and the extremal current principle (ECP) [48–50]. First we consider the stability of a bulk density \( \rho_b \) against a density perturbation. Such a perturbation travels at the collective velocity [48,49]

\[
u_{\text{coll}} = \partial \hat{J}(\rho_\pm, \rho_v) \Big|_{\rho_\pm = \rho_b}.
\]

Since for \( \nu_{\text{coll}} < 0 \) density perturbations move towards the minus-end, they do not affect the tip density and thereby the EX phase remains stable. By contrast, for \( \nu_{\text{coll}} > 0 \), perturbations move towards the plus-end which renders the IN phase stable against density fluctuations. Note that the collective velocity \( \nu_{\text{coll}} = 0 \) in the MC phase (by definition). Second, we consider the stability of DWs. A DW between a left \( \rho^{\text{left}} \) and right density \( \rho^{\text{right}} \) travels at a velocity

\[
u_{\text{DW}} = \frac{\hat{J}(\rho^{\text{left}}, \rho_v) - \hat{J}(\rho^{\text{right}}, \rho_v)}{\rho^{\text{left}} - \rho^{\text{right}}}. \]

Depending on the sign of this velocity the phase corresponding to \( \rho^{\text{left}} \) or \( \rho^{\text{right}} \) is stable [49]. Taken together, \( \nu_{\text{coll}} \) and \( \nu_{\text{DW}} \) lead to analytic results for bulk and tip densities in the various phases (table 2).

### 3.1.2. Phase diagram for the inhibition scenario

With the methods introduced in the previous section, it is a straightforward task to derive the densities and the ensuing...
Table 2. Analytic results for the tip densities $\rho_t$, in the different phases IN/EX/MC and the critical growth rates $\gamma_i$ for the inhibition and neutral scenario, respectively. Note that $\rho^{\text{IN}}_t$ is obtained from the phase boundary of the EX phase as derived in the main text.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>$\rho^{\text{IN}}_t$</th>
<th>$\rho^{\text{EX}}_t$</th>
<th>$\rho^{\text{MC}}_t$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition</td>
<td>$\frac{\beta_p (1-\eta - \rho_\phi)}{\delta (1-\rho_\phi)}$</td>
<td>$\frac{\beta_p (1-\eta - \rho_\phi)}{\delta (1-\rho_\phi)}$</td>
<td>$\eta \gamma + \delta - \frac{1}{\eta}$</td>
</tr>
<tr>
<td>Neutral</td>
<td>$\frac{\beta_p (1-\eta - \rho_\phi)}{\delta (1-\rho_\phi)}$</td>
<td>$1 - \frac{\gamma}{\delta}$</td>
<td>$1 - \frac{\gamma}{\delta}$</td>
</tr>
<tr>
<td>Critical Growth</td>
<td>$\delta \left(1 - \rho_\phi \right)$</td>
<td>$\delta \left(1 - \rho_\phi \right)$</td>
<td>$\delta \left(1 - \rho_\phi \right)$</td>
</tr>
<tr>
<td>Neutral</td>
<td>$\delta \left(1 - \rho_\phi \right)$</td>
<td>$\delta \left(1 - \rho_\phi \right)$</td>
<td>$\delta \left(1 - \rho_\phi \right)$</td>
</tr>
</tbody>
</table>

Note, however, that this is a stable solution of equation (3.3) only outside of the shaded area indicated in the phase diagram shown in figure 4b. In the EX phase, the bulk density is given by the right boundary, $\rho^{\text{EX}}_b = \rho_\phi$, and equation (3.5) leads to the striking result that the MT tip is always occupied by a molecular motor,

$$\rho^{\text{EX}}_t = 1,$$

in stark contrast to the corresponding result in the neutral scenario (table 2). It implies that an MT always depolymerizes for those parameter regimes where the system is in the EX phase. As in Reese et al. [17], we attribute this behaviour to the slow depolymerization rate in the EX phase, $\delta < \rho_\phi$. It implies that motors leave the tip more slowly than they arrive. Then the MT tip acts as a bottleneck for molecular transport and induces a traffic jam with $\rho^{\text{EX}}_t = 1$ at the plus-end. For the MC phase, the bulk density is given by the maximum of the bulk current $\rho^{\text{MC}}_b$,

$$\rho^{\text{MC}}_b = \frac{\delta - \sqrt{\delta \eta (\delta + \eta - 1)}}{\delta + \eta}. \quad (3.10)$$

Using this bulk density in equation (3.5) gives a constant value for the tip density in the MC phase which is independent of the reservoir density

$$\rho^{\text{MC}}_t = \frac{\delta + \eta (\delta + 1) - 2 \sqrt{\eta \delta (\delta + \eta - 1)}}{(\delta + \eta)^2}. \quad (3.11)$$

Knowing the tip densities, we can now use the DW theory explained above (see §3.1.1) to determine the transition lines between the various phases. The DW velocity gives the direction in which a DW between two densities, one from the left and one from the right, travels. To employ these criteria, we first have to identify the respective densities. Let us start with $\rho^{\text{MC}}_t$: the density at the minus-end is in general determined by the entering current, corresponding to a tip density $\rho^{\text{IN}}_t$ equation (3.8). This tip density, however, is only stable against small perturbations if $u^{\text{coll}} \geq 0$. For parameters where $u^{\text{coll}} < 0$ the density from the left is decreased to $\rho^{\text{left}} = \rho^{\text{MC}}$. This sign-change of the collective velocity defines the phase boundary between the IN and MC phase: $\eta = \delta (\rho_\phi - 1)^2 / (6 - \rho_\phi^2)$. Taken together, the density on the left of the DW is given by $\rho^{\text{left}} = \min\{\rho^{\text{IN}}_\phi, \rho^{\text{MC}}\}$.

Since in that regime the collective velocity is strictly negative we simply have $\rho^{\text{right}} = \rho^{\text{EX}}_t = 1$. Using the above expressions for $\rho^{\text{left}}$ and $\rho^{\text{right}}$ in equation (3.7) gives the remaining phase boundaries: with $\rho^{\text{right}} = \rho^{\text{MC}}$, $\rho^{\text{left}} = 1$ and $p = \rho_\phi$ one obtains $u^{\text{coll}} = \delta - \rho_\phi$, implying that the phase boundary between the IN and EX phase is given by $\delta = \rho_\phi$. The boundary line $\delta + \eta = 1$ signifies that above this line the stationary solution given by equation (3.8) becomes unstable. This instability gives rise to interesting motor dynamics, in particular, a subtle dependence of the ensuing stationary profile on the initial condition. While these effects are certainly worthwhile studying they are irrelevant for our main focus, namely MT regulation, and, hence, we refrain from further analysing this regime here.

Taken together, the above analysis gives the phase diagram shown in figure 4b for two different values of the reservoir density $\rho_\phi$. The general trend is that with decreasing reservoir density the parameter domain where the IN phase is stable expands.

The analytical results obtained from mean-field theory agree nicely with the stochastic simulations (figure 4c) in the case where $L_K$ is neglected. For a depolymerization rate $\eta = 0.3$, concomitant with the phase transition from the IN to the EX phase, the tip density increases upon lowering the depolymerization rate $\delta$ and then continuously saturates at $\rho_\phi = 1$ as the EX phase is reached. By contrast, for $\eta = 0.5$, there is a discontinuous jump in the tip density as one passes from the MC into the EX phase; see discussion above.

The stochastic simulations with $L_K$ show a quite significant increase in the magnitude of the tip density in the MC phase, in particular in the shaded area of the phase diagram, figure 4b. We attribute this to the fact that the Langmuir density in bulk, $\rho^{\text{IN}}_\phi$, acts as a source for kinesin-8 motors which tends to increase the motor density on the MT and at the tip. Although these effects are interesting and worthwhile studying, they are not important for our main concern here, namely regulation of MT length. As discussed previously [33], and elaborated on later in §3.4, MT regulation is possible only if the density profile is determined by the particle current at the minus-end, i.e. if the system in its stationary state is in the IN phase. In that case,
even adding LK in the simulations has only a minor effect on the magnitude of the tip density, and we can safely use the analytical mean-field results to further analyse the stationary MT length.

3.2. Dynamics of the microtubule length

Figure 5 shows the results of our stochastic simulations with LK for the MT drift velocity, \( v = \partial \delta L \), as a function of the depolymerization and the polymerization rates for both the inhibition and the neutral scenario. There are well-defined boundaries, \( \eta_\text{in}(\delta, \rho_-) \) and \( \eta_\text{in}(\delta, \rho_+) \), separating regimes in which MTs grow and shrink, respectively. Since the tip density, \( \rho_+ \), dictates MT dynamics (see equation (3.1)), those boundaries can be readily calculated upon comparing the tip densities listed in table 2 with the critical tip density, equation (3.2). For the inhibition scenario, we find that for \( \delta < \rho_- \) the critical tip density coincides with the phase boundary of the EX phase

\[
\eta_\text{in} = 1 - \delta, \tag{3.12}
\]

while for \( \delta > \rho_- \) it lies either within the MC or the IN phase:

\[
\eta_\text{in} = \left\{ \begin{array}{ll}
\frac{\delta \rho_- (1 - \rho_+)}{\delta \rho_- (1 - \rho_+)} & \text{for } \rho_- < \frac{1}{2}, \\
\frac{\delta}{\delta - 1/4} & \text{for } \rho_- > \frac{1}{2}
\end{array} \right. \tag{3.13}
\]

(see table 2 for a summary together with the results for the neutral scenario). These analytical results are in perfect accordance with our stochastic simulations (figure 5) with one interesting exception for the inhibition scenario, namely the boundary line of the EX phase for \( \delta < \rho_- \). Since we recover agreement between stochastic simulations and analytical calculations by switching off LK in our stochastic simulations, we can fully attribute this difference to the effect of attachment and detachment of motors in bulk, as discussed in §3.1.2; cf. dotted and dashed lines in figure 5a. Furthermore, the differences between both scenarios are significant, cf. figure 5a,b, respectively. In the inhibition scenario, the regime where MTs shrink—and hence regulation becomes possible—is much broader since kinesin-8 inhibits MT growth when bound to the tip: for small depolymerization rate \( \delta \), motors reside at the MT end for a relatively long time, which dramatically broadens the regime of MT shrinkage.

3.3. Interplay between kinesin-8 and polymerase XMAP215

In this section, we compare the dynamics of the inhibition scenario with a model which explicitly accounts for a second protein, XMAP215, that enzymatically facilitates MT growth (figure 6a and §2). In a case where XMAP215 and kinesin-8 mutually exclude each other at the MT tip, one expects strong similarities between those scenarios. In order to compare with an analytically tractable lattice gas model, we performed the stochastic simulations for the simplified model without LK.1 Figure 6b shows the regimes of MT growth and shrinkage as a function of kinesin-8 and XMAP215 densities for a set of depolymerization rates \( \delta \). The general trend is that the regime where MTs shrink is enlarged with smaller depolymerization rates.

At the mean-field level, the equilibrium density of XMAP215 at the MT tip is given by the product \( \rho_\text{x} = \rho_\text{x}^\text{eq} (1 - \rho_-) \), where \( 1 - \rho_- \) is the probability that kinesin-8 is not bound and \( \rho_\text{x}^\text{eq} \) denotes the Langmuir isotherm for XMAP215 binding

\[
\rho_\text{x}^\text{eq} = \frac{c_k k_{\text{on}}}{c_k k_{\text{on}} + k_{\text{off}}}. \tag{3.14}
\]

Here \( c_k \) is the XMAP215 concentration in solution, and \( k_{\text{on}} \) and \( k_{\text{off}} \) are the attachment and detachment rates of the enzyme to and from the MT tip, respectively. This mean-field approximation neglects that the presence of XMAP215 at the MT tip influences the current of kinesin-8 to the MT end, because it could block the motor particles [51]. Fortunately, as the polymerization rate of XMAP215, \( \eta_\text{x} \), and the walking speed, \( v \), of kinesin-8 are almost the same [16,27] the two molecules rarely interact. This implies that a model explicitly accounting for XMAP215 can be reduced to the inhibition scenario with an effective polymerization rate given by

\[
\eta = \eta_\text{x} \rho_\text{x}^\text{eq}. \tag{3.15}
\]

Indeed, as can be inferred from figure 6b, the predictions of the effective inhibition scenario agree nicely with the numerical simulations. Taken together, this implies that the inhibition scenario may serve as a minimal model to include other MT-associated proteins that antagonize the depolymerization activity of kinesin-8. It remains an open question, however,
growth velocity as a function of kinesin-8 and XMAP215 density. A XMAP215 density of is approximately c \approx 1.5 \text{nM} for a half-filled lattice \rho_s = 0.5 (table 1). (Online version in colour.)

as to what extent our assumption of mutual exclusion between proteins at the MT tip is justified. Because MTs consist of multiple protofilaments, one could think of a multi-lane scenario where kinesin-8 and XMAP215 are simultaneously present at the MT tip. To model this scenario, it would be necessary to rethink the interactions between the different proteins and also between the proteins and the MT.

### 3.4. Microtubule regulation

We now consider the full model for an MT of finite length \textit{L}, where LK leads to an accumulation of kinesin-8 motors along the MT. As discussed in §2, the ensuing antenna-like profile \rho_{\text{-}}(x) can be calculated within the framework of the TASEP/LK model [37,38]; these theoretically predicted profiles have recently been confirmed by \textit{in vitro} experiments [14]. Now length regulation becomes possible if this spatially varying profile translates into a length-dependent velocity \textit{v}(L) of the MT tip [7,33]. This requires that the tip density \rho_{\text{t}}(L) depends on \rho_{\text{e}}(L) (which is the case only for the IN phase (see equation (3.8))). Then the tip density reads

\[
\rho_{\text{t}}(L) = \rho_{\text{e}}^\text{IN}(\rho_{\text{e}}(L), \delta, \eta).
\]

Upon inserting the ensuing length-dependent tip density into equation (3.1), one obtains a length-dependent velocity \textit{v}(L). It is instructive to define an effective potential

\[
\text{U}_{\text{eff}}(L) = - \int_0^L dx \; \textit{v}(x),
\]

whose minimum defines the stationary MT length \textit{L}^*\n
\[
\rho_{\text{e}}(L^*) = \rho_{\text{e}}^\text{IN}(\rho_{\text{e}}(L^*), \delta, \eta) = \rho_e^\text{c},
\]

as illustrated in figure 7a–c. Tight length regulation is restricted to the regime where the critical density \rho_e^\text{c} := \rho_{\text{e}}(L^*) falls well into the linearly increasing antenna profile. The closer \rho_e^\text{c} is to the Langmuir plateau \rho_s, the less well defined is the stationary length; note that the effective spring coefficient

\[
k(L) := \text{U}_{\text{eff}}''(L) = \begin{cases}
\delta \rho_{\text{e}}^\text{c}(L) & \text{(neutral scenario)} \\
(\delta + \eta) \rho_{\text{e}}^\text{c}(L) & \text{(inhibition scenario)}
\end{cases}
\]

is proportional to the slope of the profile, where \text{prime} denotes derivative (see also figure 7c).

As can be inferred from figure 8a,b, the stochastic simulations agree nicely with the above analytical results for the stationary MT length \textit{L}^* in both scenarios, neutral and inhibition. Previous studies [33] have shown that the variance of the length can be obtained well upon using a van Kampen expansion for the stochastic dynamics of the MT length \textit{L}(t), which assumes that the tip density is adiabatically coupled to the motor density along the MT. This essentially amounts to saying that the MT length performs a random walk in the effective potential \text{U}_{\text{eff}}(L). Such a picture is fully consistent with results obtained from our stochastic simulations; the observed stochastic trajectories resemble those of random walks in confinement (figure 3a). More importantly, the numerically observed value for the probability that the MT tip is occupied, \rho_{\text{e}}(L), agrees well with the mean-field tip density \rho_{\text{e}}(x) (figure 7d). This implies that the stochastic trajectory samples the values of MT length \textit{L}(t) with a statistical weight determined by the effective potential \text{U}_{\text{eff}}(L). Surprisingly, as can be inferred from figure 7c, this is not the case for the inhibition model which immediately invalidates a description of the stochastic dynamics in terms of a continuous random walk in the potential landscape shown in figure 7b. The latter would actually give rise to stochastic trajectories strongly confined to the stationary value \textit{L}^*. By contrast, the actual stochastic trajectories for the inhibition scenario shown in figure 3b rather resemble an intermittent dynamics with abrupt transitions between growing and shrinking states. Even though the magnitude of the length fluctuations resembles MT dynamic instability [41], the microscopic origin of fluctuations in our model differs.

The key to understanding this anomalous dynamics lies in realizing that the stochastic length dynamics in the inhibition scenario is a \textit{dichotomous process} with only two states: while, if the MT tip is empty, the MT grows with a rate \eta, it shrinks with a rate \delta if the MT tip is occupied by a kinesin-8 protein. In other words, depending on whether the MT tip is occupied or not, it is either in a shrinking or a growing state,
Motor attachment and detachment rates are thus samples the effective potential, i.e., $p$ density, and the ensuing probability of tip occupation data were obtained as single trajectories sin-8 protein, it will remain in this state and not depolymerize $23/10^{-1}$ exponential with a typical time of the order of approximately $112/10^{-1}$.

Figure 7. (a,b) Effective potentials for the inhibition and the neutral scenario for the trajectories shown in figure 3. The diagram in (c) illustrates how threshold densities for the tip density $\rho_+(x)$ and minus-end density $\rho_-(x)$ are defined, and how both quantities together set MT length $L^*$. (d,e) Data for the accumulated density, and the ensuing probability of tip occupation $\rho_+(L)$. For the inhibition scenario $\rho_+(L)$ is constant, while in the neutral scenario it is length-dependent and thus samples the effective potential, i.e. $\rho_+(L) = \rho_+(L)$. (Online version in colour.)

Figure 8. Comparison of MT length $L^*$ for the inhibition (solid) and neutral (dashed) scenario with respect to polymerization (a) and depolymerization rates (b). The data were obtained as single trajectories $L(t)$ Data points correspond to the most probable length of the process $L^*$, error bars denote the standard deviation of $L(t)$ Motor attachment and detachment rates are $\alpha_{\text{on}} = 0.001$ and $\alpha_{\text{off}} = 0.003$. In (c) probability distributions of the times during which MTs shrink and grow are shown for parameter values as in figure 3. The exponential tails of the distributions support the view that the inhibition scenario follows dichotomous switching dynamics (see main text for details). (Online version in colour.)

respectively [52]. Consider a configuration where the tip is empty, and hence, the MT is in a growing state (with average speed $\eta$). Then it will remain in this state for some time $\tau_{\text{grow}}$ until the motor closest to the tip actually reaches the tip. Figure 8c shows the probability distribution of $\tau_{\text{grow}}$ for the same parameters as in figure 8b. The distribution is clearly exponential with a typical time of the order of approximately $23/\nu$. On the other hand, if the MT tip is occupied by a kinesin-8 protein, it will remain in this state and not depolymerize the tip for a time of the order of $\delta^{-1}$. During this time, the filament neither grows nor shrinks, and the kinesin-8 protein at the MT tip acts as a strict bottleneck. As a consequence, an extended traffic jam may emerge at the MT tip by motors queuing up behind this bottleneck. These traffic jams can be clearly seen in figure 3b as black clusters. The formation of such clusters is a nucleation process, and the duration of the shrinking state is determined by a subtle interplay between particles gained by stochastic arrival at the left end and depolymerization dynamics. Interestingly, the probability distribution of $\tau_{\text{shrink}}$ shows two typical time scales, and, in particular, a broad exponential tail with a typical time of the order of approximately $112/\nu$. We leave a more detailed investigation of these interesting stochastic effects for future work. The main results we emphasize here are that we have identified two distinct time scales characteristic for prolonged growing and shrinking states. These time scales are macroscopic in the sense that they are much larger than the hopping time of individual motors (which
we have set to 1). This implies that also the typical lengths covered during the growing and the shrinking state are rather large; for the examples shown in figure 8 they are on average approximately 8 and 22 lattice sites during polymerization and depolymerization, respectively. These large length scales explain why the probability to occupy the MT tip as obtained from the stochastic simulations is only weakly dependent on MT length.

Taken together, we find that in the neutral scenario MT length is tightly controlled. The variance in MT length is mainly determined by the width of the effective potential, or, equivalently by the effective spring coefficient $k(L) = \delta \rho(L)$. Hence, the slope of the antenna profile is the key determinant of length fluctuations. By contrast, for the inhibition scenario, extended periods of MT growth and shrinkage lead to large length fluctuations as can be seen from the kymographs in figure 3. These large fluctuations result in characteristic exponential tails in the filament's length distributions; the characteristic width of this distribution is shown as error bars in figure 8b. Note also the different dependencies of the two models with respect to the depolymerization rate. While in the neutral scenario the length of the MT is independent of the depolymerization rate $\delta$, it strongly affects MT length in the inhibition scenario.

4. Discussion

We have analysed distinct molecular mechanisms of MT regulation by proteins which are able to catalyse growth and shrinkage of MTs. Specifically, our interest was in the interplay of Kip3p acting as depolymerase when bound to the MT tip and MT growth processes which are either spontaneous or also catalysed by proteins like XMAP215. We investigated two distinct scenarios in a neutral scenario MTs grow independently of whether a kinesin-8 motor is bound to the tip or not. By contrast, in an inhibition scenario the MT only grows if the MT tip is not occupied by a depolymerase. Even though these scenarios were motivated by the MT depolymerizing motor Kip3p, our results can be applied to other molecular motors as well. For example, the human kinesin-8, Kif18a, has been shown to dampen MT dynamics [44] and even to block MT growth entirely [53]. Another example is kinesin-4, which inhibits the growth of MTs in anti-parallel MT overlaps [54].

Experiments with the MT polymerizing enzyme XMAP215 [27] suggest a high binding rate for the MT tip through facilitated diffusion. Then, to a first approximation, one may model XMAP215 as a tip-binding protein which excludes binding of kinesin-8. As we have shown, this tip site exclusion leads to a dynamics which is equivalent to the inhibition scenario.

The results obtained here show how interactions between individual proteins and the MT tip play an important role for MT regulation. There are three main findings: (i) MT regulation is directly affected by motor traffic. It is influenced by the MT growth rate, and attachment and detachment kinetics of motors to and from the MT. Both parameters can be tuned in experiments through the tubulin concentration and the motor and salt concentrations [14], respectively. (ii) Regimes of MT growth and shrinkage critically depend on the probability that a kinesin-8 motor is bound to the MT tip. (iii) Protein–MT interactions at the MT tip are key to distinguish different mechanisms of MT regulation, like for example intermittent dynamics or tight length control.

The parameter regimes where motor traffic constrains MT growth differ dramatically for the two scenarios (cf. figure 5). For the neutral scenario, this parameter regime is relatively small and, in particular, limited to slow growth rates. It is characterized by relatively tight length control [33]. By contrast, for the inhibition scenario, the regime where length regulation is possible is extremely broad and includes high growth rates, however, at the cost of accurate length control: MT dynamics is intermittent with extended periods of MT growth and shrinkage, reminiscent of MT dynamic instability. Therefore, in the view of the regulation of MT length, these findings suggest the inhibition scenario as a mechanism for large length fluctuations, while the neutral scenario provides a mechanism for precise length control. To test these theoretical ideas, we suggest experiments which vary the protein concentration of kinesin-8, tubulin and XMAP215. The specific predictions of our theory will allow one to discern between different molecular mechanisms at the MT tip, simply by analysing how changes in the concentrations affect macroscopic quantities like the MT length and the speed of MT growth and shrinkage.

Besides its biological relevance for MT-related cellular processes, our study also contributes to the field of driven diffusive systems. We not only show how systems with a dynamic length can be treated analytically, but the technique we propose also gives conceptual insights into the determination of boundary-induced phases. This is achieved by extending the ECP [48] to dynamic systems. For instance, we found that a shock forming dynamically at the right boundary (not in bulk) determines whether the system is in the IN or EX phase. In addition, we could identify an unstable region in the phase diagram (between EX and MC phase for the inhibition scenario), where the system not only depends on the boundaries, but also on the initial conditions. This behaviour is to our knowledge not common for driven diffusive systems, and an interesting topic for future studies. Even though the main dynamic behaviour, as MT length, is governed by currents which are determined by the boundaries, also bulk phenomena are important as observed in [14,36,37], especially for lattice length fluctuations. We restricted our analysis to boundary-induced transitions, leaving it as a challenge for the future to capture also the bulk dynamics of motors on the MT.

From a broader perspective, the presented findings support the view that length-dependent disassembly and/or assembly rates due to molecular motor transport are likely to constitute a general mechanism to influence the length of one-dimensional structures in biology regardless of mechanistic details [55]. Specifically, MT tips are crowded spots in the cell, where space limitations for protein binding, inferring mutual exclusion, are relevant factors. Future experimental work needs to study dwell times of molecules at MT tips at the highest possible accuracy, because dwell times encode important information about the underlying molecular interaction networks [56]. Future theoretical studies may include other microscopic scenarios at the tip, as for example an interpolation between the neutral and the inhibition scenario, and the coupling of multiple protofilaments. Similarly, it will be important to learn more about interactions of molecular motors with the MT [57–59] during dynamic instability [60–62] and with networks of MTs [63,64].
References


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