Generation of Stable Overlaps between Antiparallel Filaments

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During cell division, sister chromatids are segregated by the mitotic spindle, a bipolar assembly of interdigitating antiparallel polar filaments called microtubules. Establishing a stable overlap region is essential for maintenance of bipolarity, but the underlying mechanisms are poorly understood. Using a particle-based stochastic model, we find that the interplay of motors and passive cross-linkers can robustly generate partial overlaps between antiparallel filaments. In this situation, motors reduce the overlap in a length-dependent manner, whereas passive cross-linkers increase it independently of the length. In addition to maintaining structural integrity, passive cross-linkers can thus also have a dynamic role for overlap size regulation.

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Eukaryotic cells store their chromosomes in a nucleus. During nuclear division, called mitosis, the duplicated chromosomes segregate. This process relies on the mitotic spindle, a bipolar structure of interdigitating microtubules [1]. These polar filamentous polymers are physically cross-linked by specific proteins to maintain the spindle’s integrity. Some of these cross-linkers are molecular motors that can generate mechanical stresses from the hydrolysis of ATP, which in turn can result in relative sliding between microtubules. Whereas the gross architecture of spindles is conserved from yeast to human, their detailed internal organization varies largely between species.

In spite of their vital importance, the physical principles underlying formation and size regulation of mitotic spindles are largely unknown. Some works emphasize the inherent ability of microtubules and molecular motors to self-organize into spindle structures by means of their mechanical interactions and microtubule assembly [2–4] as has been studied specifically in vitro [5]. Other authors emphasize the role of external factors in spindle assembly and maintenance, for example, through macroscopic gradients of various regulatory proteins [6–10].

On the scale of individual filaments, notably the regulation of microtubule length by length-dependent depolymerization [11–15] was studied. Less is known about mechanisms regulating the overlap between interdigitating microtubules, which is key for maintaining spindle bipolarity in many organisms. Reduction of the overlap region by cross-linking molecular motors moving directionally can be compensated either by filament growth [16] or by the action of antagonistic motors [17–19]. Both mechanisms, though, turn out to require fine-tuning of parameters. Passive cross-linkers, that is, without motor activity, were long thought to merely provide structural integrity and to effectively increase the friction between sliding microtubules [20]. In vitro experiments, however, indicate a regulatory function for the passive cross-linkers MAP65/PRC1/Ase1 through recruitment of specific motor proteins [21] or through generating friction that depends on the overlap size [22]. Theoretical work shows that these cross-linkers and molecular motors can segregate along microtubules [23]. Passive cross-linkers were also shown to lead to an increase of the overlap between filaments [24,25]. Consequently, passive cross-linkers may play a dynamically much more interesting role for spindle homeostasis than previously assumed.

In this work, we show that passive cross-linkers can oppose the action of molecular motors to slide apart two antiparallel microtubules resulting in the formation of a stable partial overlap. The overlap length can be tuned by varying the relative concentrations of motors and passive cross-linkers. A mean-field analysis shows that the stable overlap results from an effectively length-dependent motor-induced stress and an antagonistic stress induced by the passive cross-linkers. The latter is independent of the overlap length and follows from an asymmetry in cross-linker binding at microtubule ends. Finally, we investigate the effects of steric interactions between motors and passive cross-linkers.

We describe a pair of antiparallel microtubules by two oriented lattices each with $N$ sites and lattice constant $\Delta$; see Fig. 1(a). To indicate the filaments’ polarity, the site $i = 0$ is denoted as the plus and the site $i = N − 1$ as the minus end. Lattice sites can be occupied by two kinds of particles denoted as “$m$” and “$p$.” They, respectively, represent the binding of motors and passive cross-linkers to the microtubules. Since motors and passive cross-linkers have different sizes, they may preferentially occupy different protofilaments on two cross-linked microtubules; hence, for now we neglect steric interaction between $m$- and $p$-particles. Each particle is connected by a harmonic spring to a second particle of the same kind, and together they represent either a motor protein or a passive cross-linker. The partner particle may either be attached to the opposite
lattice or be unbound. Let $M_i$ and $M_j^i$ be the respective probabilities of site $i$ of lattice 1 to be occupied by an $m$-particle with either an unbound partner or a bound partner to site $j$ on lattice 2. Correspondingly, $M_{i,i+1}$ and $M_{i,i+1}^j$ are the probabilities that site $i$ is occupied by an $m$-particle, whereas site $i + 1$ is not. Equivalent quantities for lattice 2 will be indicated by a “hat,” such that $\hat{M}_j$ is the probability for site $i$ of lattice 2 to be occupied by an $m$-particle and so on [26]. These probabilities evolve according to

$$
\frac{d}{dt} M_i = \gamma M_{i+1,i} - \gamma M_{i,i-1} + \gamma(\xi)M_i^0
- \bar{\omega}_{m} M_i + \alpha_m^{\prime} \left( 1 - M_i - \sum_j M_j^i \right) - \sum_j I_j^i, \tag{1}
$$

$$
\frac{d}{dt} M_j^i = \gamma(\xi + \Delta)M_{i+1,j}^i - \gamma(\xi)M_{i,j-1}^i
+ \gamma(\xi + \Delta)\hat{M}_{i+1,j} - \gamma(\xi)\hat{M}_{i,j-1} + I_j^i + \hat{I}_j^i \tag{2}
$$

with $I_j^i = \alpha_m^{\prime}(\xi) M_j^i - \bar{\omega}_{m}^{\prime}(\xi) M_j^i$. Here, $\xi$ denotes the extension of the spring connecting two partner particles. Explicitly, $\xi = (i + j) \Delta - \ell$, where $\ell$ is the overlap length between the two lattices, which equals the distance between the lattices’ plus ends.

The first two terms in Eq. (1) describe hopping at rate $\gamma$ towards the plus end, which is possible if the target site is free. An occupied site is freed at rate $\bar{\omega}_{m}$ by particle detachment and a free site occupied at rate $\alpha_m^{\prime}$. The terms $I_j$ describe the processes of cross-linking and unlinking, with the partner at site $j$ of lattice 2. Equation (2) describes the corresponding processes for an $m$-particle with a bound partner. In this case, the hopping, cross-linking, and detachment rates depend on the spring extension $\xi$. Explicitly, $\gamma(\xi) = \gamma \exp\{k_m \xi / \bar{f}_m\}$, such that hops reducing the spring extension are favored, and $\alpha_m^{\prime}(\xi) = \bar{\omega}_{m}^{\prime} \exp\{-\xi^2 / 2 \sigma^2\} / \sqrt{2 \pi \sigma^2}$. Note that $m$-particles leave lattices at plus ends at the same rate they hop in the bulk: $M_{i,i+1} = M_{0}^{\prime}$ and $M_{0,i-1}^j = M_{0}^{\prime}$. This is another way to undo cross-links, as captured by the third term in Eq. (1).

The corresponding probabilities $P_j$ and $P_j^i$ for the passive cross-linkers evolve similarly. Hops of $p$-particles to an adjacent site occur at rate $\tilde{D}$ if the partner particle is unbound. If both partners are bound, then hopping to the plus and minus ends occurs at rates $D^\pm(\xi) = \tilde{D} \exp\{\pm k_p \xi / \bar{f}_p\}$. In contrast to $m$-particles, $p$-particles cannot hop off the lattices from the sites $i = 0$ and $N - 1$ [22].

To determine the motion of the microtubules, we compute the total force $f_{\text{total}} = \sum_j (f_{m,i} + f_{p,i})$ exerted on them by cross-linking particles. It is given by the forces $f_{m,i} = k_m \sum_j \xi M_j^i$ and $f_{p,i} = k_p \sum_j \xi P_j^i$ generated by motors and passive cross-linkers, respectively. The overlap length then evolves according to $\mu D^{\prime} = 2 f_{\text{total}}$, where $\mu$ is the filaments’ friction coefficient with the surrounding medium. We neglect possible Brownian motion of the filaments. This completes the description of our model.

We performed stochastic simulations using Gillespie’s algorithm; see Figs. 1(b) and 1(c) for parameters obtained in vitro for the motor Eg5 and the passive cross-linker Ase1: $\bar{\omega}_{m} = 1.56 \text{ s}^{-1}$, $\bar{\omega}_{p} = 0.0017 \text{ s}^{-1}$, and $\tilde{D} = 12 \text{ s}^{-1}$ [22,27,28]. For the hopping rate of passive particles, we chose $\tilde{D} = 12 \text{ s}^{-1}$ [29]. The cross-linking rates $\bar{\omega}_{m,p}$ and the spring stiffnesses $k_{m,p}$ are not known experimentally. Throughout this work, we use $\bar{\omega}_{p} = 1.2 \text{ s}^{-1}$, $\bar{\omega}_{m} = 8.4 \text{ s}^{-1}$, and $k_{m,p} = 0.11 \text{ pN/nm}$ [30]. For the characteristic forces, we took the stall force of Eg5, $\bar{f}_m,p = 9 \text{ pN}$ [31], and $\sigma = 8 \text{ nm}$, the lattice constant of a microtubule lattice. Each lattice had 100 sites corresponding to a microtubule length of 800 nm, and the friction coefficient $\mu$ was 0.1 pNs/nm [32]. The rates $\alpha_{m,p}$ depend on the concentrations of proteins in the reservoir and will be used as control parameters. From now on, we scale all rates by $\tilde{\gamma}$, lengths by $\Delta$, and forces by $\bar{f}_m$ and refer only to rescaled values.
completely filled lattice. Exclusion process, which initially increases with the particle current in the totally asymmetric concentration. These observations are reminiscent of the antiparallel microtubules is reduced with increasing motor concentrations above a critical value, we observe full overlap. Between two filaments. Remarkably, also for motor concentrations for which a stable partial overlap is generated. If the diagram in Fig. 2(a) shows that, for a fixed concentration which is in agreement with experiments. The phase overlap fully. This is in line with the reports in Refs. [24,25] that passive cross-linkers alone maximize the overlap between two filaments. Remarkably, also for motor concentrations above a critical value, we observe full overlap. This indicates that the ability of motors to separate antiparallel microtubules is reduced with increasing motor concentration. These observations are reminiscent of the dependence of the particle current in the totally asymmetric exclusion process, which initially increases with the particle concentration and then decreases as it approaches a completely filled lattice [33].

In the steady state, \( M_i = \hat{M}_i, M'_i = \hat{M}'_i \), \( P_i = \hat{P}_i \), and \( P'_i = \hat{P}'_i \), which notably implies \( M_{ij} = M'_i \) and \( P_{ij} = P'_i \). We can get more insight into the generation of stable overlaps by considering the mean-field limit of Eqs. (1) and (2). In this limit, we consider the probabilities \( M_i, M'_i, P_i, \) and \( P'_i \) and use the steady-state relations just given. The mean-field approximation consists in neglecting correlations between neighboring sites, for example, \( M_{ij} = M_i(1 - M_j - \sum_j M'_j) \). The steady-state solutions to the mean-field equations for the parameters of Figs. 1(b) and 1(c) are shown in Fig. 3.

The mean-field analysis reproduces the overlap length of the stochastic simulations; see Fig. 2(b). The probabilities \( M_i, \sum_j M'_j, P_i, \) and \( \sum_j P'_j \) are essentially piecewise constant in the overlap and nonoverlap regions of the filaments [34]; see Figs. 3(a) and 3(c). The corresponding forces \( f_{m,i} \) and \( f_{p,i} \) are constant in the bulk of the overlap region and show pronounced maxima at its boundaries; see Figs. 3(b) and 3(d). The total force exerted by cross-linking motors is dominated by the bulk and tends to decrease the overlap. As the total force originates in the bulk, its magnitude scales with \( \ell \). Consequently, the force decreases as the overlap shrinks. In contrast, for passive cross-linkers, the force vanishes in the bulk. The corresponding total force is determined by the boundary regions and thus is independent of \( \ell \); see Fig. 3(d). The passive cross-linkers’ force tends to increase the overlap. Summing up the forces yields a stable steady-state overlap length.

Our mean-field analysis suggests that, close to the steady-state value, the total force of passive cross-linkers and molecular motors on the filaments varies essentially linearly with the overlap length. Consequently, in the case of a stable partial overlap, fluctuations around the steady-state value should be Gaussian. The stochastic simulations confirm this expectation; see Fig. 1(c).

At the level of individual particles, what causes the total net force of the passive cross-linkers? Consider a passive cross-linker in the bulk. The distribution of the spring extensions will be symmetric around zero due to the diffusion of bound cross-linker particles and because the cross-linking rate is symmetric with respect to \( \xi \). At the boundary, however, this distribution is asymmetric, because binding sites are absent beyond the filament’s end. This leads to a net force. The same effect exists for motors. However, usually the bulk forces will mask it. Only in the case where the motor density is so high that motor movements are essentially blocked by steric effects does the end effect dominate. This asymmetry explains the maximal overlaps observed for very low and very high motor densities observed in Fig. 2.
So far, we have not considered steric interactions between motors and passive particles. Extending our model to include steric interactions, we find that stable partial overlaps can be generated under these conditions, too; see Fig. 4(a). Interestingly, for a similar average overlap length, the distribution is broader in the presence of steric interactions; see Fig. 4(c). Up to a critical value of the occupancy rate \( \omega_m^\ell \), the phase diagram looks similar to the case without interspecies steric interactions; see Fig. 4(d). However, steric interactions increase the range of occupancy rates for which a stable partial overlap is generated; see Fig. 4(d). Above the critical rate, we observe a phase with fluctuations that are of the order of the filament length; see Fig. 4(b). More work is necessary to characterize this phase in detail and to identify the origin of this behavior. The mean-field approach is not able to capture the effects in the presence of steric interactions, suggesting that correlations might play a more significant role in this case [23].

In summary, we have shown that motors and passive cross-linkers together can robustly generate stable overlaps between antiparallel microtubules. This is in line with \textit{in vitro} experiments [21,22,25]. In agreement with these studies, we find an accumulation of passive cross-linkers in the overlap region. Accumulation of motors in the overlap region is less pronounced, which is different from observations for the motor Xklp1 [21]. The passive cross-linker PRC1 used in that study, however, was found to recruit Xklp1 to the overlap region. Similar to Ref. [25], we find a positive correlation between the concentration of passive cross-linkers and the overlap length. Previous theoretical analysis restricted attention to passive cross-linkers [24,25]. Including explicitly the dynamics of molecular motors, we analyzed the fluctuations around the steady-state overlap length. Furthermore, we studied the effects of interspecies steric interactions. We found them to increase fluctuations and to extend the range of motor occupancy rates for which a stable partial overlap is generated. For high rates, these steric interactions furthermore generate a new phase, where length regulation is lost.

Microtubules typically exhibit dynamic instability or treadmilling behavior during spindle organization. We will address the impact of microtubule dynamics on stable overlaps in our forthcoming work. Eventually, it will be interesting to explore the consequences of these collective effects for the organization of ensembles of microtubules and their possible impact on spindle formation.

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[26] Note that $M_i = M'_j$.
[29] The actual rate is $D = 860 \text{s}^{-1}$ [27]. To save computation time, we chose a lower value after checking that the results are qualitatively not affected by this reduction.
[30] The actual spring stiffness of Eg5 is 1 pN/nm [31]. We chose a lower value for numerical stability of the simulations.
[32] This value corresponds to a viscosity of the surrounding fluid of 70 Pa s. We checked that for the viscosity of water, $10^{-3}$ Pa s, for which the simulations take much longer, the distribution of the overlap length does not change.
[34] Note that occupation numbers in the stochastic simulations fluctuate too strongly to allow for a meaningful comparison with the mean-field probabilities.