

Dynamic stability of spindles controlled by molecular motor kinetics

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Abstract – We analyze the role of the force-dependent kinetics of motor proteins in the stability of antiparallel arrays of polar filaments, such as those in the mitotic spindle. We determine the possible stable structures and show that there exists an instability associated to the collective behavior of motors that leads to the collapse of the structure. The agreement of our results and several experimental observations in eukaryotic cell division suggests an important role of kinesin-5 motors and microtubule bundles in the stability of the mitotic spindle.

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Living cells display several structures that arise from the self-organization of polar filaments and motor proteins [1]. Several *in vitro* studies have shown that mixtures of kinesin motors and microtubules (MTs) can spontaneously develop complex spatio-temporal patterns [2]. These self-organization processes are essential for eukaryotic cell division [3]. During mitosis, motor proteins organize MTs in a bipolar structure, the mitotic spindle, which serves as a scaffold to transmit the necessary forces for chromosome segregation [4]. The mitotic spindle consists of two MT asters that overlap in the central region, with their minus ends located at the aster poles crosslinked by many different motor proteins [3,5]. One particular type of motors, the plus-ended bipolar kinesins (*e.g.*, Eg5 or Klp61F), has been shown to be essential for the spindle stability. A decrease in their concentration below a certain threshold causes the spindle collapse [6,7], and their total inhibition prevents bipolar spindle formation [8]. In addition, Eg5 motors have been shown to drive the MT poleward flux [7] and homolog motors to induce the formation of (interpolar) MT bundles [9].

Bipolar motors are composed of two connected units, each one composed of two motor domains. Both units can move simultaneously and independently on MTs [10].

These motors are able to crosslink MTs [9] and slide them with respect to each other when they are in an antiparallel configuration [10], like in the central region of the spindle (fig. 1a,c). As a result, these motors produce an outward force along the spindle axis and generate a MT flux toward the poles [7]. Typical forces involved in mitosis lay in the nanoNewton range [11]. Since individual motors cannot exert forces larger than a few picoNewtons, their collective action is required to ensure the stability of the mitotic spindle. At metaphase, this dynamic structure reaches a steady state with MTs of nearly constant length undergoing permanent treadmilling [7,12] (usually referred to as MT poleward flux), polymerizing at the + end and depolymerizing at the – end.

The theoretical study of motors and MT mixtures has been recently addressed using continuum coarse-grained descriptions [13–16], which have elucidated their basic self-organizing principles. However, the coupling between force-dependent motor kinetics and local forces in self-organized structures has not been addressed. In this letter, we study the dynamic stability of antiparallel arrays of MTs under the action of longitudinal forces, in the presence of molecular motors able to collectively hold the structure by stochastically crosslinking the filaments. We analyze the effects of the motor kinetics on the stability of the structure, and show that several phenomena observed in eukaryotic cell division appear naturally in our theoretical approach. This suggests that interpolar microtubule

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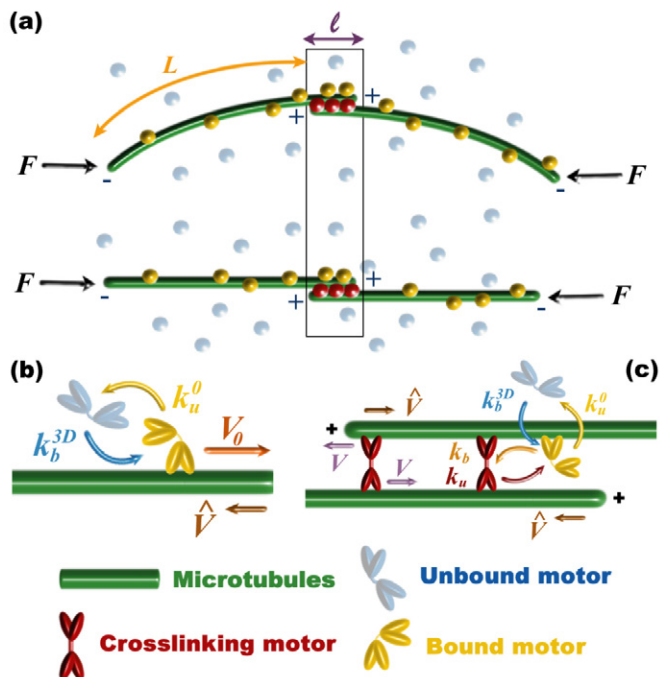


Fig. 1: (a) Antiparallel array of MTs under the action of a longitudinal force F . Buckled and non-buckled arrays are shown. The minus and plus ends of MTs are depicted as $-$ and $+$ respectively. Motors are represented by dots. In the non-overlapping region of length L there are no antiparallel filaments and the motors are not subject to any force. The motors in the overlapping region of length ℓ sustain the structure by crosslinking and sliding antiparallel filaments. (b-c) Kinetic events of a motor in the non-overlapping (b) and overlapping (c) regions. The velocities V and \hat{V} are the crosslinking motor velocity and the MT poleward velocity, respectively.

bundles may, in some conditions, control the stability of the mitotic spindle.

In order to comprehend the basic physical mechanisms controlling the stability of a spindle, we concentrate on a simplified geometry. We consider a pair of antiparallel MTs (or an antiparallel MT bundle) of fixed length, under the action of an inward force F (fig. 1a). In the steady state, there is a region of length ℓ where the antiparallel filament array overlaps (overlapping region). The motors in this region can crosslink antiparallel MTs and slide them in opposite directions, generating an outward force that balances the total applied force F . We assume the antiparallel MT sliding to be the only mechanism generating the poleward MT flux, as suggested experimentally [7]. The MT poleward flux requires a MT treadmilling to maintain a steady state. Consistently with the known activity of certain motor proteins in modifying the MT net growth rate, we assume that the polymerization and depolymerization rates adapt to the sliding velocity, so that MTs keep a constant length. Out of the overlapping region there are two regions of length L (non-overlapping regions; fig. 1a) where motors cannot

apply forces to sustain the spindle. Since we concentrate on the spindle stability and do not address the mechanisms that determine F , L and ℓ , we take them as given parameters; we assume that they vary at time scales longer than those involved in motor dynamics. A generalized and more accurate description should take into account the dynamics of ℓ , F and L .

The motors in the non-overlapping region can be either bound to a MT or freely diffusing in the bulk (fig. 1b). We assume a constant bulk motor density, ρ_{3D} (see footnote¹), and consider the motors in the bulk to attach onto MTs at a rate k_b^{3D} . Once bound to a MT, the dynamics of the motor density, $\rho(s, t)$, can be expressed as [17,18]

$$\partial_t \rho(s, t) + \partial_s J(s, t) = -k_u^0 \rho(s, t) + k_b^{3D} \rho_{3D}, \quad (1)$$

where s is the position along the MT as measured from the MT minus end and $J(s, t)$ is the flux of bound motors. For simplicity, we assume the bound motors to be in a low density phase and write $J(s, t) = \rho(s, t)(V_0 - \hat{V})$ in the laboratory reference frame, with \hat{V} being the velocity of MTs toward the poles and V_0 being the actual velocity of the bound motors with respect to the MT. Motors in this region detach at a rate k_u^0 .

In the central overlapping region, bipolar motors can be either in a crosslinking state or in a bound state. In the former state both motor units are attached to a pair of antiparallel MTs, sliding them in opposite directions and supporting a fraction of the total force F . As a result, the crosslinking motors move with a force-dependent velocity V (fig. 1c). Based on experimental observations [19], we write a linear force-velocity relation, $V = V_0(1 - f_m/f_s)$, where f_m is the load applied on the motor and f_s its stall force. We consider a number n_c of independent crosslinking motors to equally share the total applied force, so that $f_m = F/n_c$. The velocity and kinetic rates become collective quantities through their coupling to the force shared among the total number of crosslinked motors. As the poleward MT movement is driven only by these motors, we identify $\hat{V} = V$ (fig. 1c). Each unit of a motor in the crosslinking state can detach at a force-dependent rate which, consistently with experiments [19] we take of the standard form $k_u(f_m) = k_u^0 \exp(f_m b / K_B T)$ (Kramers theory [20]), where b is a length in the nanometer scale characterizing the activated process and $K_B T$ the thermal energy. After the detachment of one motor unit, the bipolar motor is only bound to one MT and unable to apply force. Such motor can either detach the bound motor unit left at a rate k_u^0 and diffuse into the bulk, or re-attach the unbound motor unit at a rate k_b and become a crosslinking motor again. The motors in the bulk can also attach to the MTs in the overlapping region at a rate k_b^{3D} .

¹This is reasonable for typical spindle lengths ($L_s \sim 5 \mu\text{m}$) as the motor bulk concentration equilibrates over time scales, of order $\sim L_s^2/D \simeq 1 \text{ s}$ ($D \sim 10 \mu\text{m}^2 \text{ s}^{-1}$ being the diffusion constant of the motors in the bulk), shorter than the time scale of the convective motor movement along MTs, of order $\sim L_s/V_0 \simeq 100 \text{ s}$, for typical values of the motor velocity ($V_0 \simeq 33 \text{ nm s}^{-1}$ for Eg5 [10]).

The relevant variables being the number of motors sustaining the spindle, we neglect their spatial distribution in the overlapping region. Accordingly, the equations for the average number of crosslinking and bound motors, n_c and n_b , respectively, read

$$\begin{aligned} \frac{dn_c}{dt} &= k_b n_b - 2k_u(n_c)n_c, \\ \frac{dn_b}{dt} &= 2J(L, t) + k_b^{3D} \rho_{3D} \ell + 2k_u(n_c)n_c - (k_u^0 + k_b) n_b, \end{aligned} \quad (2)$$

where $J(L, t)$ is the convective flux of bound motors coming from a non-overlapping region. The value of $J(L, t)$ is determined by the solution of eq. (1). When the motor processivity length, $l_p \equiv V_0/k_u^0$, is smaller than the characteristic spindle length ($l_p \ll L$ (see footnote²)), the flux $J(L, t)$ is determined by a constant bound motor density, $\rho_\infty = k_b^{3D} \rho_{3D}/k_u^0$, fixed by the exchange of motors with the bulk. In this case the dynamics of n_c and n_b are decoupled from the motor density close to the pole.

The existence of antiparallel MT arrays under an external load F is determined by the balance between motor attachment and detachment fluxes, as given by the steady-state solutions, $\{n_c^f, n_b^f\}$, of eqs. (1), (2), which read

$$\begin{aligned} \delta &\equiv \frac{\gamma \rho_\infty \ell}{2\tilde{F}} = \frac{\exp(1/\tilde{n}_c^f) (\tilde{n}_c^f)^2}{2\tilde{l}_p \left[1 - \exp\left(-\tilde{L}\tilde{f}_s\tilde{n}_c^f/\tilde{l}_p\right) \right] / \tilde{f}_s + \tilde{n}_c^f}, \\ \tilde{n}_b^f &= \frac{2 \exp(1/\tilde{n}_c^f) \tilde{n}_c^f}{\gamma}. \end{aligned} \quad (3)$$

We normalize forces by the characteristic detachment force, $K_B T/b$, so that $\tilde{F} \equiv Fb/K_B T$ and $\tilde{f}_s = f_s b/K_B T$. The number of motors is normalized by the dimensionless force, \tilde{F} , leading to $\tilde{n}_c \equiv n_c/\tilde{F}$ and $\tilde{n}_b \equiv n_b/\tilde{F}$. Lengths are normalized by the overlap length, ℓ , as $\tilde{L} = L/\ell$ and $\tilde{l}_p = l_p/\ell$. The asymmetry in motor attachment/detachment events is characterized by $\gamma \equiv k_b/k_u^0$.

There always exists a critical value, δ_m , below which there are no solutions of eq. (3). This situation corresponds to an attachment flux of crosslinking motors that cannot balance their detachment flux, leading to the loss of all crosslinking motors and inducing the spindle collapse. Associated to the critical value δ_m , there is a minimum number of crosslinking motors, \tilde{n}_c^m , necessary to sustain a spindle, whose value is given implicitly by

$$\begin{aligned} 1 + \left[\tilde{n}_c^m \left(1 + \tilde{L}\tilde{n}_c^m \tilde{f}_s/\tilde{l}_p \right) - 1 \right] \exp\left(-\tilde{L}\tilde{f}_s\tilde{n}_c^m/\tilde{l}_p\right) = \\ \tilde{n}_c^m \left[2 + \tilde{f}_s/(2\tilde{l}_p) (\tilde{n}_c^m - 1) \right]. \end{aligned} \quad (4)$$

The actual value of δ_m is obtained by substituting \tilde{n}_c^f in eq. (3) by the solution of eq. (4).

In order to determine the stability of the structures, we perform a linear stability analysis of the solutions of

²Typical values for L are about several microns long for most cell types, and for dimeric Eg5 $l_p < 100$ nm [10,19].

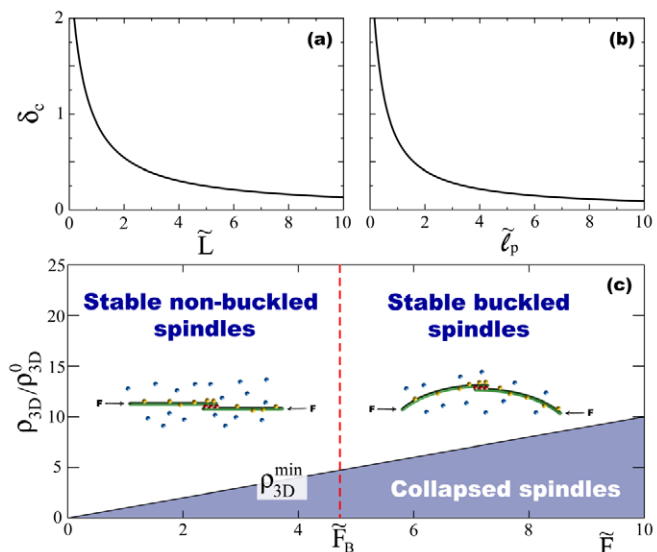


Fig. 2: (a-b) Dependence of the critical value δ_c on \tilde{L} and \tilde{l}_p in the limiting cases where (a) $\tilde{l}_p \gg 1$ and (b) $\tilde{L} \gg 1$ ($\tilde{f}_s = 2$). (c) Possible spindle structures as the bulk motor density ρ_{3D} and the force F are varied ($\rho_{3D}^0 \equiv 2\delta_c k_u^0/k_b^{3D} \ell \gamma$ sets the density units). Above $\rho_{3D}^{\min}(F)$, buckled (straight) stable spindles exist for $F > F_B$ ($F < F_B$). Below $\rho_{3D}^{\min}(F)$ no stable spindles exist.

eq. (3). For $L \gg l_p$ it can be shown that the fluctuations in $\rho(s)$ are negligible and the spindle stability depends only on the dynamics of n_c and n_b (see footnote³). Stable spindles exist above a critical value δ_c . The precise expression for δ_c depends on the ratio between motor attachment/detachment rates at vanishing load. For $\gamma > \gamma_c \equiv 2 \exp(1/\tilde{n}_c^m) (1 - \tilde{n}_c^m)/\tilde{n}_c^m - 1$, the transition from an unstable array to a stable spindle corresponds to a saddle-node bifurcation at $\delta_c = \delta_m$. On the other hand, if $\gamma < \gamma_c$, this transition corresponds to a global bifurcation (saddle connection [21]) at a value $\delta_c > \delta_m$. In the particular case of vanishing processivity length ($l_p = 0$), $\delta_c = \delta_m$ and $\tilde{n}_c^m \geq 1$. Hence a finite processivity widens the parameter range leading to stable spindles. Regardless of the value of γ , the same qualitative scenario is observed as δ is varied and we restrict the following discussion to the regime $\gamma > \gamma_c$ without loss of generality. The threshold value, δ_c , is fixed only by \tilde{L} and \tilde{l}_p/\tilde{f}_s (fig. 2a, b). In the limit $L \gg l_p$, the spindle stability is only determined by the dynamics in the overlapping region, implying that the spindle morphology and its stability are decoupled.

The parameter δ includes the dependence both on the applied force, F , and the bulk motor density, ρ_{3D} , and appears as a convenient parameter to characterize the spindle stability.

The existence of a critical value δ_c implies that for a MT array under the action of a load, F , there exists a minimal motor bulk concentration, ρ_{3D}^{\min} , below which no stable spindles are found. Using the definition of δ (eq. (3)), this

³For $L \ll l_p$ the stability scenarios remain qualitatively unchanged.

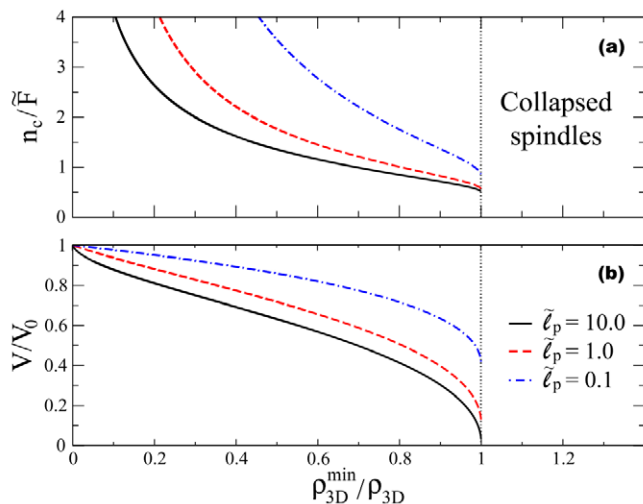


Fig. 3: Steady-state analytical solutions for (a) the number of crosslinking motors and (b) the velocity of the crosslinking motors (sliding velocity), as a function of the bulk density of motors ($\tilde{f}_s = 2$). The different curves represent different values of \tilde{l}_p in the limiting regime $\tilde{L} \gg 1$.

minimal motor density reads

$$\rho_{3D}^{\min} = \frac{2k_u^0}{k_b^{3D}\ell} \frac{\delta_c \tilde{F}}{\gamma}. \quad (5)$$

When $\rho_{3D} > \rho_{3D}^{\min}$ the stable spindle may be either straight or buckled depending on the value of the compressive force, F . For forces below (above) the buckling force $F_B = (\pi/2)^2 B/L^2$ of the structure (B being its bending rigidity), the stable spindle is straight (buckled). Note that MT buckling would generate a normal component of the force acting on the motors, which is neglected here. This would, however, not change our qualitative results concerning the existence of a threshold, but it would affect its actual value.

In fig. 2c we plot the structures that can be found as the bulk density of motors and the force applied on the spindle are varied. Indeed, recent experimental observations on the stability of mitotic spindles have shown that the progressive inhibition of Eg5 motors leads to the collapse of the spindle at a finite bulk motor density [7]. Moreover, the total inhibition of homolog motors (Klp61F) has been shown to prevent bipolar spindle formation *in vivo* [8]. The qualitative agreement between these observations and our results suggests an important role of MT bundles and kinesin-5 collective behaviour in the stability of spindles.

Above the threshold density ρ_{3D}^{\min} , there is a finite amount of crosslinking motors n_c collectively holding the spindle. In fig. 3a we plot the stable solutions of eq. (3) as a function of the bulk concentration of motors. Increasing values of ρ_{3D} and ℓ leads to larger motor attachment fluxes, that result in a larger amount of crosslinking motors. For a living cell in native conditions, the MTs in the mitotic spindle are typically buckled [4]. Therefore,

the force applied on the MTs is of order F_B which, for single MTs ($5\mu\text{m}$ in length) is about 1 pN. Using this value for the force F , the number of crosslinking motors leading to a stable antiparallel array turns out to be very small ($\simeq 2$). In this case fluctuations would dominate and, although stable arrays could be transiently formed, their lifetime would be too short (on the time scale of motor detachment). Since the buckling force of a MT bundle can be at least one order of magnitude larger⁴, stable interpolar MT bundles require tenths of crosslinking motors and provide robust spindles with lifetimes over the time scale of the division process. Interpolar MT bundles are indeed observed in several organisms during cell division [9,22,23]. Both the existence of a threshold and the increased spindle stability by bundle formation are independent of the particular value of l_p . However, as discussed above, a larger motor processivity increases the spindle stability.

The speed of the MT flux toward the poles is determined by the MT sliding velocity, \hat{V} , given by the velocity of the crosslinking motors. In fig. 3b we represent this sliding velocity as a function of ρ_{3D} , for different values of \tilde{l}_p . It decreases from its maximal value V_0 as the bulk motor density is decreased, and it is typically finite for the minimal density ρ_{3D}^{\min} at which the spindle collapses, as observed experimentally [7]. At high motor concentrations $\rho_{3D} \gg \rho_{3D}^{\min}$, the crosslinking motors move nearly at their maximal velocity V_0 and the MTs move poleward at this velocity consequently. As the motors in the non-overlapping regions move at velocity V_0 with respect to the MTs in the spindle (fig. 1b), they appear static in the laboratory reference frame, explaining the apparent motor stillness observed experimentally [24]. Our analysis predicts that decreasing the bulk motor density ρ_{3D} would allow the observation of motor movement in the spindle.

The present approach highlights the importance of force-dependent motor kinetics on the self-organization of MTs and motors. In particular, we show that the motor force-dependent kinetics is a key factor in the stability of crosslinked MT bundles under applied load. *In vitro* experiments with simplified systems combining kinesin-5 and MTs in controlled geometries would considerably help understanding the properties of bundles sustained by molecular motors. There are a number of predictions that arise from our analysis which could be quantitatively tested in such experiments. At a qualitative level, there are several experimental observations in eukaryotic cell division, namely the mitotic spindle collapse, the MT poleward flux, the static appearance of motors in the spindle, and the existence of MT bundles, which emerge naturally in our description. Such qualitative agreement suggests that, in some conditions, the loss of stability of interpolar MT bundles may be responsible for the spindle collapse.

⁴ Assuming the bending rigidity of a MT bundle to scale linearly with the number of MTs, the buckling force of an interpolar MT bundle may be of tenths of picoNewtons.

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