Mechanical stability of bipolar spindle assembly

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Abstract – Assembly and stability of mitotic spindle are governed by the interplay of various intra-cellular forces, e.g. the forces generated by motor proteins by sliding overlapping anti-parallel microtubules (MTs) polymerized from the opposite centrosomes, the interaction of kinetochores with MTs, and the interaction of MTs with the chromosome arms. We study the mechanical behavior and stability of spindle assembly within the framework of a minimal model which includes all these effects. For this model, we derive a closed-form analytical expression for the force acting between the centrosomes as a function of their separation distance and we show that an effective potential can be associated with the interactions at play. We obtain the stability diagram of spindle formation in terms of parameters characterizing the strength of motor sliding, repulsive forces generated by polymerizing MTs, and the forces arising out of the interaction of MTs with kinetochores. The stability diagram helps in quantifying the relative effects of the different interactions and elucidates the role of motor proteins in formation and inhibition of spindle structures during mitotic cell division. We also predict a regime of bistability for a certain parameter range, wherein the spindle structure can be stable for two different finite separation distances between centrosomes. This occurrence of bistability also suggests the mechanical versatility of such self-assembled spindle structures.

The assembly of the mitotic spindle is a key event in cellular division. During mitotic cell division, the two centrosomes within the cell serve as poles and nucleating sites for microtubules (MTs). The polymerizing MTs from the opposite centrosomes overlap, leading to the formation of spindle structure during metaphase [1–4] (see fig. 1). These polymerizing centrosomic MTs interact with the chromosome arms and generate an effective repulsive force between the centrosomes — also called polar ejection forces [5,6]. Some of these MTs also interact with specialized cellular structures of kinetochores forming kinetochore microtubules generating an additional tension between the two centrosomes [7–9]. Finally, MTs from the opposite centrosomes overlap in the spindle midzone. These anti-parallel overlapping MTs are crosslinked by motor proteins such as Eg5 and dynein which attach to the two overlapping MTs and exerts forces which tend to slide the anti-parallel MTs with respect to each other. While kinesin motors exert a force on the overlapping MT filaments tending to increase the centrosome separation distance, dynein motors exert a force which tends to decrease the separation between centrosomes [3,10–12]. Apart from the motor crosslinkers, passive crosslinkers such as Ase1/Map65/PRC1 present in the region of overlapping MTs can generate relative sliding between the MTs [13]. The passive crosslinkers generate a force which tends to increase the overlapping region between the anti-parallel MTs [13,14] and, thus, acting to decrease the separation distance between the centrosomes. Some of the MTs also interact with the cell cortex [15]. When the
cell size is large, the effect of the interactions of MTs with the cell cortex is seen to be relatively less important [3]. Typically when a stable bipolar spindle configuration is attained as a result of these interactions, chromosomes are localized and aligned on a plane whose normal is aligned along the axis of the spindle, and which lies about the midpoint between centrosomes. Even though each of these interaction forces is itself the result of complex phenomena, refs. [3,4] have proposed a simple coarse-grained model which takes into account the combined effect of the interaction between the two centrosomes and the interactions of the individual centrosomes with the chromosomes. Numerical simulations of this model have shown that the relative strength of the different interactions outlined earlier can regulate the stability of the spindle structure, modulate the stable spindle length and determine the organization of the chromosomes within the spindle structure [3,4].

In this letter, we use the minimal model of refs. [3,4] by assuming that a priori chromosomes are homogeneously distributed on a disc or a ring lying in a plane perpendicular to the axis of the spindle at the midpoint of the spindle axis. Such a configuration is typical in both in vivo and in vitro experiments [1,16] and it has been confirmed also in the numerical studies in ref. [3]. This assumption allows us to obtain a closed-form analytical expression for the force acting between the centrosomes as a function of their separation distance. From the expression of the force we derive the criterion for mechanical stability\(^1\), we construct the entire stability diagram of spindle formation which is expressed in terms of parameters which characterize the strength of motor sliding, repulsive forces generated by polymerizing MTs, and the tensions exerted by kinetochores. The stability diagram helps in quantifying the relative effects of the different processes on the stability of the spindle assembly.

We first describe the theoretical framework of the proposed model for the case of a single chromosome. For this case, we also explicitly take into consideration the forces resulting from MTs interacting with cortical dynein motors in the region of the cell cortex. We find that for sufficiently large cells, this interaction does not play an important role in determining the stability of the spindle assembly. Next we extend this model to the case of multiple chromosomes homogeneously distributed on a disc or a ring in the mid-plane between the two centrosomes and we derive the corresponding stability diagram for the spindle structure. Finally we conclude with the discussion on the possible implications of this study in analyzing the mechanical stability of spindles.

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\(^1\)Mechanical equilibrium of the spindle system requires the overall force to vanish. Further, the requirement for stable mechanical equilibrium demands that any slight deviation from the equilibrium position should result in restoring force which tends to bring back the system to its original state of mechanical equilibrium.

**Single chromosome.** — We analyze the stable configurations of the two centrosomes when a single chromosome is located at the mid-plane between two centrosomes located at \(-x\) and \(x\). The net force due to the interactions of the MTs with the chromosome is directly proportional of the number of MTs reaching the chromosome arms. Typically, the number of MTs reaching the chromosome arms at a distance \(x\) is proportional to \(e^{-2x/L}\) [4,17], where \(L\) is mean length of MTs. Accordingly we can write down the expression for the polar ejection force, \(F_{pe} = Ae^{-x/L}\), where \(A\) is the maximum polar ejection force. The sliding force due to the motors is proportional to the overlapping MTs from the opposite centrosomes [3,4]. Thus, for centrosomes separated by a distance \(2r\), the force due to the motor sliding is \(F_m = 2Bxe^{-2x/L}\), where \(B\) is the net force per unit overlap length of the MTs. The sign of \(B\) is positive for net outward forces (when the force due to kinesin motors exceeds the forces of dynein) and it is negative for net inward (attractive) force between centrosomes. The presence of passive crosslinkers would lead to an effective attractive force between the centrosomes [14], so that in our minimal description, it would effectively make a negative contribution to the parameter \(B\). The force due to sub-cellular machinery of kinetochore with MTs is attractive [18,19]. While in general the attractive force due to the interaction between the kinetochore sub-cellular machinery and MTs depends on the distance of the kinetochore from the centrosome [20], for the sake of simplicity of the analysis here it is assumed to be constant [4]. Finally, the interaction of the MTs with the cell cortex is \(F_{cc} = De^{-R_o/L}\), where \(D\) is a measure of the strength of MTs interacting with cortical motors in the cell cortex, and \(R_o\) is the cell radius [3]. Adding all the different contributions, the expression for the net force between the centrosomes as a function of their separation distance reads [4],

\[
F(x) = Ae^{-x/L} + 2Bxe^{-2x/L} - C + De^{-R_o/L}.
\]

We use eq. (1) to determine the mechanically stable steady states. For this we first note that the condition for mechanical equilibrium implies that for a separation distance \(2x = 2x_p\), \(F(x_p) = 0\). Further, for the stable mechanical equilibrium, \(\frac{dF}{dx}\big|_{x_p} < 0\), which simply means that on a slight deviation from equilibrium separation, the restoring forces are such that the system regains its equilibrium configuration. Thus, the conditions \(F(x_p) = 0\) and \(\frac{dF}{dx}\big|_{x_p} = 0\) determine the phase boundary, separating linearly stable and unstable regions. In order to simplify the analysis we express eq. (1) in terms of dimensionless scaled variables \(x_o = 2x/L\), \(F_o = F/A\), \(C_o = C/A\), \(B_o = B/A\) and \(D_o = D/A\). Therefore, on the phase boundary,

\[
F_o(x_o) = e^{-x_o/2} + B_o x_o e^{-x_o} - C_o + D_o e^{-R_o/L} = 0 \quad (2)
\]

\[
\frac{dF_o}{dx_o} = -\frac{1}{2} e^{-x_o/2} - B_o x_o e^{-x_o} + B_o e^{-x_o} = 0. \quad (3)
\]

Using eq. (2) and eq. (3), we obtain the expression for the equilibrium separation between the centrosomes at

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Dashed lines indicate the phase boundary when \( x_o = 0 \), for a range of negative values of \( D_o \), which corresponds to motors exerting a net inward force between the centrosomes, and when \( C_o \) is less than a critical value, there is a region of bistability, where two different solutions of spindles, corresponding to two different finite separation distances between centrosomes, are stable. We restrict our subsequent analysis to the situation in which the typical cell size is large, so that we can effectively ignore the interaction term of MTs with the cell cortex.

**Multiple chromosomes distributed on a disc.** – When many chromosomes are present, the force acting along the line joining the two centrosomes, eq. (1), can be generalized to

\[
F = 2Bxe^{-2x/L} + \sum_{i=1}^{N} \int_0^{R_i} \int_0^{2\pi} \frac{rd\theta}{\sqrt{r^2 + x^2}} \left( A e^{-R_i/L} - C \right),
\]

where \( R_i = \sqrt{x^2 + r_i^2} \) is the distance between the centrosome and the \( i \)-th chromosome lying on a disc in the mid-plane between the two centrosomes and \( r_i \) is the distance of the chromosome from the center of the disc. Assuming that \( N \) chromosomes are uniformly distributed on the disc of radius \( R_d \), the expression in terms of summation can be converted into an integral expression for the force which reads

\[
F = \frac{N}{\pi R_d^2} \int_0^{R_d} \int_0^{2\pi} \frac{rdrd\theta}{\sqrt{r^2 + x^2}} \left( A e^{-\sqrt{r^2 + x^2}/L} - C \right) + 2Bxe^{-2x/L},
\]

which upon integration yields

\[
F = 2x \left[ Be^{-2x/L} + \frac{NAL}{R_d^2} \left( e^{-x/L} - e^{-\sqrt{x^2 + R^2_d}/L} \right) - \frac{NLC}{R_d^2} \left( \sqrt{x^2 + R^2_d} - x \right) \right].
\]

The net force acting perpendicular to the axis joining the two centrosomes adds up to zero due to the radially symmetric arrangement of chromosomes in the disc configuration. The radius of the disk \( R_d \) is set by the number of chromosomes, \( N \), in the disc and by the chromosomal packing fraction that ultimately depends on the mutual interaction between chromosomes. In order to estimate \( R_d \), we make a crude approximation that each chromosome occupies a surface area \( \pi r_{ch}^2 \) within the disc, where \( r_{ch} = 1 \mu m \) \[3\] is the radius of a single chromosome. Therefore, \( R_d \sim r_{ch} \sqrt{N} \).

In order to determine the stability boundary we first express eq. (8) in terms of dimensionless variables. The choice of scaled dimensionless variables is as follows: \( x_o = x/L \), \( F_o = \frac{FR_d^2}{2N\pi AL} \), \( R_o = R_d/L \), \( C_o = C/A \), \( B_o = \frac{BR_d^2}{N\pi AL} \). Then, in terms of these scaled variables, the expression for the dimensionless scaled force is

\[
F_o(x_o) = x_o \left[ B_o e^{-2x_o} + e^{-x_o} - e^{-\sqrt{x^2 + R^2_o}} \right] - C_o \left( \sqrt{x^2 + R^2_o} - x_o \right).
\]

This expression is of the form \( F(x_o) = x_o g(x_o) \) and thus \( x_o = 0 \) is always a solution for eq. (9). From the condition for stability of this particular solution we obtain

\[
B_o = C_o R_o + e^{-R_t} - 1.
\]
For determining the stability boundary for the case in which \( x_o \neq 0 \), we set \( F'_o(x_p) = 0 \) and \( F_o(x_p) = 0 \), which yields the condition, \( g'(x_p) = 0 \) and \( g(x_p) = 0 \). Using these conditions we obtain

\[
C_o = \frac{e^{-x_o} + \frac{x_o}{\sqrt{x_o^2 + R_o^2}} - 2}{\sqrt{x_o^2 + R_o^2}} e^{-\sqrt{x_o^2 + R_o^2}} \quad \text{(11)}
\]

\[
B_o = e^{-\sqrt{x_o^2 + R_o^2}} + C_o \frac{\sqrt{x_o^2 + R_o^2} - e^{-x_o} - C_o x_o}{e^{-2x_o}} \quad \text{(12)}
\]

These equations are numerically solved to obtain the boundary curves (solid lines) shown in the panels of fig. 3. The stability diagrams in fig. 3 show a variety of scenarios. It exhibits regions where there is bistability with two different stable solutions for bipolar spindle: a region where both bipolar spindle and monopolar configuration \( x = 0 \) are stable, a region with just one solution for stable bipolar spindle and a region where no bipolar spindle is stable and only monopolar configuration is stable. The effect of the increase in \( R_o \) is to decrease the stability of the bipolar spindle configuration. In fact for sufficiently large \( R_o \) the monopolar configuration is stable even for a positive value of \( B_o \) for large enough \( C_o \) (fig. 3(b)). We also find that the region corresponding to the possibility of allowing two different stable spindle solutions vanishes on increasing \( R_o \) (fig. 3).

The corresponding plots for scaled force as a function of centrosome separation is plotted in fig. 4 for these four different regions in the stability diagram. We find that for outward motor-MT forces \( B > 0 \), the bipolar spindle is stable as long as \( C \) (corresponding to the kinetochore-MT interaction strength) is not very large.

By integrating the expression of the dimensionless scaled force in eq. (9) it is possible to derive an effective dimensionless potential energy function \( V_o(x_o) \),

\[
V_o = \frac{C_o}{3} \left[ (\sqrt{x_o^2 + R_o^2} - x_o)^2 - x_o^3 \right] - e^{-x_o} (x_o + 1) - e^{-\sqrt{x_o^2 + R_o^2}} \left( \sqrt{x_o^2 + R_o^2} + 1 \right) - \frac{B_o}{4} e^{-2x_o} (2x_o + 1).
\]

Figure 5 shows the potential energy landscape for different strengths of \( B_o \) for fixed values of other parameters. In particular fig. 5 illustrates that although in the bistable regions two different spindle configurations are stable, in general one of the two corresponds to a global minimum in terms of potential energy. The fact that eq. (7) can be derived from an effective potential, \( V_o \) is crucial since the functional form of \( V_o \) provides an insight not only into the stability of the stationary states but also provides a clue about the dependence on the initial configuration of the
system and the long-term evolution of the system in the presence of noise. It also rationalizes many of the observations of the numerical studies in refs. [3,4]. First of all it was seen in refs. [3,4] that the final configuration of the spindle (whether it is bipolar or monopolar) is dependent on the initial separation distance. The potential energy landscape provides an explanation of how the final configuration would be dependent on the zones of attraction of the potential landscape with multiple minima. It was also seen in ref. [3] that for certain situations, the initial configuration eventually relaxed to a fixed configuration after an intermediate metastable configuration. This can understood in terms of the different depths of the potential minima for the two different configurations. Eventually the system would relax to the global minima in the presence of noise in the system. In fact the height of the potential barrier would then determine the time scales of relaxation of the system to its final steady-state configuration.

In order to quantitatively compare the results of the stability diagram obtained here with the numerical studies in ref. [3], we note that they had observed that for parameter values $L = 4 \mu m$, $N = 46$, $C = 10 pN$ and $A = 125 pN$, for $B < 0 \mu m$, the monopolar configuration was stable, whereas for $B > 25 \mu m$, the bipolar spindle configuration was stable. In order to make a comparison, first we estimate the typical value of $R_\theta \equiv R_d/L$. $R_\theta = \sqrt{N}/L \sim 2$. With this specific choice of $R_1$, we find that the bipolar spindle configuration is unstable, while the monopolar configuration is stable for $B = 0$ only for $C_o > 0.4$ (fig. 3(b)), whereas in ref. [3] even for $C_o \equiv C/A = 0.08$ the bipolar spindle was observed to be unstable. We however find that for lower values of $B$, i.e., $(B = -25 \mu m)$, the bipolar spindle is unstable like in ref. [3]. The quantitative discrepancy between the analytical prediction of this model and the simulation results reported in ref. [3] can partially be attributed to our rather crude estimation of the radius of the disc $R_d$. An higher value of $R_d$ would in general result in a lower value of $C$ for which the stability of the bipolar spindle configuration is lost. In general, the parameter range of $A$, $B$ and $C$ along with $L$ and $N$ can be varied over a certain range by altering the physiological conditions within the cell and analogously the scaled variables would span a range of values including $R_d$.

Multiple chromosomes distributed on a ring. – Finally we study the case in which $N$ chromosomes are uniformly distributed on a ring of radius $R_c$. For estimating the radius of the ring $R_c$, we assume that each chromosome occupies $2R_c h \approx 2 \mu m$ length. Therefore, $N$ chromosomes distributed on the perimeter of the ring require $R_c \sim N/\pi \mu m$. Such a configuration has been shown to be stable in ref. [3]. In such a scenario the expression for the scaled force reads

$$F_o(x_o) = x_o \left[ \frac{e^{-\sqrt{x_o^2 + R_o^2}}}{\sqrt{x_o^2 + R_o^2}} - \frac{C_o}{\sqrt{x_o^2 + R_o^2}} - 2B e^{-2x_o} \right].$$

The scaled dimensionless variables for this case are, $x_o = x/L$, $F_o = \frac{F_o}{N/A}$, $R_o = R_c/L$, $C_o = C/A$, $B_o = \frac{B}{N/A}$.

For the solution $x_o = 0$, the condition for stability yields

$$B_o = \frac{C_o - e^{-R_c}}{2R_1}$$

that is represented in the panels of fig. 6 as a dash-dotted line. The corresponding equations which determine the phase boundary for the case in which $x_o \neq 0$ are

$$C_o = \frac{e^{-\sqrt{x_o^2 + R_o^2}}}{2} \left[ \frac{x_o}{\sqrt{x_o^2 + R_o^2}} \right] - \frac{e^{-x_o}}{\sqrt{x_o^2 + R_o^2}},$$

$$B_o = \frac{e^{-x_o}}{2\sqrt{x_o^2 + R_o^2}} \left[ e^{-\sqrt{x_o^2 + R_o^2}} - C_o \right].$$

These equations are numerically solved to obtain the boundary curves (solid lines) in fig. 6 separating the stable and unstable region. In particular, fig. 6(a) exhibits regions of bistability for which two different solutions for the bipolar spindle are stable and regions where both bipolar spindle and monopolar configuration are stable. In fig. 6(b) we choose a value of $R_c = 4$, obtained by estimating $N = 46$ and $L = 4$ as done in ref. [3]. We find that for this ring geometry, even for a very low value of $C$, the bipolar spindle becomes unstable as was observed in ref. [3]. Like for the case of chromosomes arranged on the disc, we find that the effect of the increase in $R_c$ is to decrease the stability of the bipolar spindle configuration (fig. 6). As in the previous case, it possible to associate an effective potential energy function $V(x_o)$, as a function of centromere separation distance in the scaled variable $x_o$. This expression reads

$$V_o = C_o \sqrt{x_o^2 + R_c^2} + \frac{B_o}{2} e^{-2x_o} \left( 2x_o + 1 \right) + e^{-\sqrt{x_o^2 + R_c^2}}.$$  

Summary and discussion. – In summary, we have studied and analyzed the mechanical stability of the mitotic spindle structure within the framework of a minimal model proposed in refs. [3,4], which incorporates the interactions between chromosomes with MTs, the sliding forces generated by motor proteins on overlapping MT filaments, and the interactions of the MTs with kinetochores. We have obtained the phase diagram for the case...
in which a single chromosome interacts with the MTs and considered explicitly the effect of the cortical motors interacting with the MTs. We find that for sufficiently large cell radius this interaction does not significantly affect the spindle stability, as reported in ref. [3]. Having assumed that the chromosomes are a priori distributed homogeneously on a disc or a ring in the mid-plane between the two centrosomes, we obtain a closed-form analytic expression for the forces acting between a pair of centrosomes and construct the stability diagram. This stability diagram is expressed in terms of dimensionless parameters which are essentially ratios of the strength of the different interaction forces. The stability diagram allows us to quantify the impact of the different interactions at play in determining the final configuration of the spindle. We find that in general both for the case of chromosomes arranged on a ring and disc, if there is a net outward sliding force by motors and passive crosslinkers (\( B > 0 \)), then the bipolar spindle is stable, whereas for sufficiently high inward sliding forces of motors (\( B < 0 \)), the monopolar configuration is stable. For a sufficiently high value of \( C \), even for outward motor sliding force (\( B > 0 \)), the bipolar spindle configuration can be unstable. Interestingly, when \( B < 0 \), below a threshold value of \( C \), there are regimes of bistability characterized by the coexistence of two different spindle lengths, and also the stability of the bipolar spindle configuration and monopolar configuration. The origin of this bistable behavior can be associated with multiple minima in the effective potential energy function that can be derived from the expression of total interaction force. It also allows us to rationalize the observation in refs. [3,4], where it was observed that the final stable configuration was dependent on the initial separation distance. It would be interesting to investigate whether the prolonged lifetime in the bipolar spindle configuration to the eventual monopolar state observed in ref. [3] can be attributed to the transition from shallow potential minima corresponding to the spindle configuration to deeper potential energy minima associated with the monopolar configuration.

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