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Theoretical analysis of forces generated by mitotic spindle

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Abstract

The mitotic spindle acts as the generator of force that drives mitosis. In prometaphase and metaphase the force is required to align kinetochore/chromosome pairs in the bio-orientated configuration while in anaphase the force is required to drive the separation of kinetochore/chromosome pairs. The forces have been measured experimentally in prometaphase or metaphase of many organisms. For example, in grasshopper spermatocytes, the force on each microtubule (kMT) attached to kinetochores in prometaphase was determined to be about 7 pN. By contrast, in anaphase of grasshopper spermatocytes the force required to halt the motion of a kinetochore/chromosome was determined to be about 700 pN, equivalent to a force of about 50 pN per kMT. A puzzling issue is why in grasshopper spermatocytes the force to stall the kinetochore/chromosome (700 pN) exceeds significantly the normal anaphase force (0.1 ~ 0.7 pN) and why the force of 50 pN per kMT in anaphase is also much larger than 7 pN in prometaphase. Here, based on our proposed model for the spindle in metaphase and anaphase, we study theoretically the forces generated by the spindle, explaining the puzzling issue. Moreover, prediction is provided that the total force to stall all kinetochores in anaphase of grasshopper spermatocytes is about 4-fold larger than the force to stall the single kinetochore or the force on each kinetochore to stall all kinetochores is only about 17% fraction of the force to stall the single kinetochore.

Keywords: Spindle; mitosis; metaphase; anaphase; cell division

Introduction

Mitosis is the process in which a eukaryotic cell nucleus splits in two nuclei. Mitosis proceeds through distinct phases. For example, in metaphase each duplicated chromosome carrying two sister kinetochores that are connected together by cohesin bonds (the linkage) are required to be under the tension to promote the correction of erroneous attachments of microtubules (kMTs) to kinetochores and thus ensure all attachments in the correct bi-oriented configuration (Civelekoglu-Scholey and Scholey, 2010; Lampson and Grishchuk, 2017; Risteski et al., 2021; Barisic and Rajendraprasad, 2021; Sigmund et al., 2024; Wang et al., 2026). The tension is achieved by force generated within the metaphase spindle. In anaphase, the linkage between the sister kinetochores is broken and the force generated by the anaphase spindle is required to separate the sister kinetochores (Asbury, 2017; Scholey et al., 2016; McIntosh, 2021; Vukusic, 2017; Vukusic and Tolic, 2021; Wadsworth, 2021). A critical and interesting issue is how much the force can be generated in the spindle during metaphase and what the magnitude of the force is that drives the separation of a kinetochore/chromosome during anaphase, namely the force required to halt the motion of a kinetochore/chromosome. Many experiments have been presented to measure the forces, as reviewed elsewhere (Civelekoglu-Scholey and Scholey, 2010; Asbury, 2017; Scholey et al., 2016; Caldwell et al., 2024).

For example, Chacón et al. (2014) determined that the force on each kinetochore during metaphase in budding yeast is about 4 to 6 pN. Considering that each kinetochore in budding yeast attaches just a single kMT (Winey et al., 1995), it is indicated that about 4 to 6 pN on each kMT in metaphase of budding yeast (Asbury, 2017). Suzuki et al. (2016) also estimated a force of about 2 to 6 pN per kMT during mitosis in budding yeast. These values are also consistent with the experimental data showing that in budding yeast at about 5 pN the kMT-kinetochore attachment exhibits the maximum lifetime (Akiyoshi et al., 2010) and the force on the kMT at which the maximum frequency occurs is about 5 pN (Mukherjee et al., 2019). Nicklas (1988) determined a force of about 7 pN on each kMT during prometaphase of grasshopper spermatocytes. Alexander and Rieder (1991) estimated a force of about 1 ~ 10 pN per kMT during prometaphase in newt lung cells. Intriguingly, Ye et al. (2016) estimated a force in a wide range of about 12 ~ 61.5 pN per kMT in *Drosophila* metaphase.

More interestingly, Nicklas (1983) measured that the force to halt the motion of a chromosome

during anaphase of grasshopper spermatocytes is as high as about 700 pN. By contrast, viscous drag calculations showed that chromosome motion is normally driven by forces of only 0.1 pN (Nicklas, 1965). Elastic bending of chromosomes showed only 0.7 pN (Marshall et al., 2001). Thus, a puzzling issue is why in anaphase the force to halt the chromosome movement (about 700 pN) exceeds the normal anaphase force needed by as much as more than 1000-fold. In addition, considering about 15 kMTs attached to each kinetochore in the spindle of grasshopper spermatocytes, from the stall force of about 700 pN for one kinetochore/chromosome it was estimated that a force of about 50 pN on each kMT in anaphase (Nicklas, 1983). Thus, it is also puzzling why the force of about 50 pN per kMT in anaphase of grasshopper spermatocytes is much larger than about 7 pN per kMT in prometaphase (Nicklas, 1988).

Despite of the extensive experimental studies, the theoretical and numerical studies of the force generated in the spindle during metaphase and the force required to stall the kinetochore during anaphase are still lacking. In particular, why the force to stall a kinetochore/chromosome in anaphase of grasshopper spermatocytes is much larger than the force on the kinetochore/chromosome in prometaphase is unexplained theoretically or numerically. The purpose of this work is to address the issues, which is critically important to the mechanism of the mitotic movements that underlie kinetochore/chromosome alignment and segregation.

Model

Spindle in metaphase

The simplified one-dimensional model for the metaphase spindle in higher eukaryotes can be schematically shown in Fig. 1a (Wang et al., 2025). The kinetochore pairs are located in the spindle center (for illustration, only two kinetochore pairs are shown in Fig. 1a), with each pair being connected by the linkage. As mentioned above, the MTs emanating from spindle poles and being connected to kinetochores are called kMTs (for illustration, only one kMT being connected to each kinetochore is shown in Fig. 1a). The MTs emanating from one spindle pole and forming antiparallel overlaps with respective MTs emanating from the opposite spindle pole are called interpolar MTs (iMTs) (for illustration, only two iMTs emanating from one spindle pole are shown in Fig. 1a). Note that an iMT may also form antiparallel overlap with the adjacent kMT.

For simplicity, only two types of spindle-associated proteins are explicitly considered. One type is the homotetrameric kinesin-5 such as Eg5 motor, which can bind to MTs in the antiparallel overlap regions, with each pair of heads on one end of the common stalk moving toward the MT plus end to drive the relative sliding of the two antiparallel MTs and thus generating force in the spindle (van den Wildenberg et al., 2008; Liu et al., 2021). The other type is the kinesin-13 such as KIF2A motor, which can land on the MT lattice and then by making use of the MT poleward flux and its diffusion along MT can reach the minus end to perform the depolymerase activity (McHugh et al., 2023; Xie, 2024). In addition, to ensure all parallel MTs to slide toward their common spindle pole with similar velocity, NuMA proteins that can crosslink parallel distant MTs are also required (Harborth et al., 1999; Wang et al., 2025).

The dynamics of MT is as follows. The published experimental data showed that in metaphase of higher eukaryotes the MT plus end predominantly grows or pauses, implying that the depolymerization at the plus end can be neglected (Risteski et al., 2021; 2022; 2024). The iMT, which is always under no force on its plus end, can grow with a constant velocity at the plus end. The plus-end polymerization velocity of kMT under zero pulling force on the plus end is denoted by v_{k0} . On the basis of the available experimental evidence that the polymerization velocity increases nearly linearly with the pulling force on the MT plus end (Franck et al., 2007; Gudimchuk et al., 2020; Long et al., 2020), under the pulling force, F , the plus-end polymerization velocity of kMT, v_k , can be simply written as (Wang et al., 2025)

$$v_k = v_{k0} \left(1 + \frac{F}{F_0} \right), \quad (1)$$

where F_0 is the characteristic force. It is noted that in Eq. (1), v_{k0} , which represents the velocity of polymerization at the kMT plus end under $F = 0$, is contributed collectively by the spontaneous polymerization and depolymerization, polymerization catalyzed by polymerase enzymes (Reber et al., 2013; Xie, 2023), the suppression of MT dynamics by kinesin-8 motors (Du et al., 2010; Stumpff et al., 2012) and the depolymerization catalyzed by kinetochore-localized kinesin-13 MCAK motors (Ganem and Compton, 2004; Wordeman et al., 2007; Steblyanko et al., 2020).

Spindle in early anaphase

We propose that the spindle in early anaphase shares the same mechanism of generating force as

that in metaphase, except that in the anaphase spindle the linkage connecting any pair of kinetochores is broken. Specifically, to study the force required to halt the kinetochore movement, we apply a force, $F_{\text{stall-KT}}$, on one kinetochore (Fig. 1b), as done in the experiment of Nicklas (1983).

Results and discussion

Force in metaphase

As showed before (Wang et al, 2025), the model in metaphase (Fig. 1a) can spontaneously lead to the steady state of the spindle, where the plus-end polymerization velocity, MT-sliding velocity and minus-end depolymerization velocity are equal to each other, the system maintains the constant MT length, constant overlap length, constant spindle length, constant pulling force on each kMT to stretch the kinetochore pair, and so on. The steady state is due mainly to the fact that the poleward MT flux results in the length-dependent minus-end depolymerase activity of kinesin-13 motors that can land on the MT lattice and the forces generated by the kinesin-5 motors in the antiparallel overlaps are counter-balanced by the pulling forces on kMTs to stretch the kinetochore pairs. Since this work focuses mainly on the forces, here we do not comment on how the steady state in metaphase can be reached, which has been studied in details elsewhere (Wang et al, 2025).

From Eq. (1), the pulling force F on each kMT in metaphase (Fig. 1a) can be written as

$$F = F_0 \left(\frac{v_k}{v_{k0}} - 1 \right). \quad (2)$$

In metaphase, the total pulling force NF is counter-balanced by the total force arising from the kinesin-5 motors in the antiparallel overlaps, with $2N$ being the number of kMTs connected to all kinetochores in the spindle. The number of kinesin-5 motors can be adjusted spontaneously to make the two total forces be equal to each other, as numerically shown before (Wang et al., 2025). In other words, the number of kinesin-5 motors responds spontaneously to the total force NF .

We make the approximate mathematical analysis of the balance between the force generated by the kinesin-5 motors in the antiparallel overlaps and the pulling force on the kinetochores. We adopt the linear force-velocity form for a kinesin-5 motor containing only one pair of heads, $v_m = v_{m0}(1 - f_m/f_0)$ when $f_m < f_0$ and $v_m = 0$ when $f_m > f_0$, where f_m is the resistance force acting on the stalk of the motor, f_0 is the stall force, v_{m0} is the stepping velocity of the motor under no resistance force and v_m is the stepping velocity of the motor under resistance force f_m . Here, we only

consider the resistance force f_m . From above force-velocity expression, for the stepping velocity v_m , the force on the motor can be written as

$$f_m = f_0 \left(1 - \frac{v_m}{v_{m0}} \right). \quad (3)$$

For the model in metaphase (Fig. 1a), at steady state of the spindle the motor stepping velocity v_m corresponds to the MT-sliding velocity or the MT poleward flux velocity v_k . Denoting by L_O the total length of the antiparallel overlap regions, the number of sites on MTs in the antiparallel overlaps to which the kinesin-5 motors can bind can be calculated as $N_{\text{site}} = L_O/d$, where $d = 8.2$ nm is the distance between two tubulins on a MT. Since the dimeric form of the motor predominantly occupied two tubulins during its processive stepping whereas it initially binds to one tubulin on MT (Chen et al., 2016; Shi et al., 2025), the total number of motors with both pairs of heads bound to MTs in the antiparallel overlaps, N_m , can be approximately calculated by $(N_{\text{site}} - 2N_m)k_{\text{on}} = N_mk_{\text{off}}$, from which we have

$$N_m = \frac{k_{\text{on}} N_{\text{site}}}{(2k_{\text{on}} + k_{\text{off}})} = \frac{k_{\text{on}} L_O}{(2k_{\text{on}} + k_{\text{off}})d}, \quad (4)$$

where k_{on} is the binding rate of kinesin-5 motor to MTs in the antiparallel overlaps and k_{off} is the off rate of kinesin motor from the antiparallel overlaps.

In metaphase, with the motor stepping velocity v_m equal to MT poleward flux velocity v_k , each motor can provide a driving force f_m . Thus, the total driving force provided by N_m motors is $N_m f_m$. As mentioned above, in metaphase we have the relation, $N_m f_m = NF$. Substituting Eqs. (2) – (4) into above relation, we obtain

$$\frac{L_O}{N} = \frac{F_0 (2k_{\text{on}} + k_{\text{off}})d}{f_0 k_{\text{on}}} \frac{\left(\frac{v_k}{v_{k0}} - 1 \right)}{\left(1 - \frac{v_k}{v_{m0}} \right)}, \quad (5)$$

where according to the definition of N , L_O/N corresponds to the antiparallel overlap length per pair of opposite kMTs attached to a pair of kinetochores.

As determined before (Wang et al., 2025), $F_0 = 1.85 \sim 3.2$ pN for human spindle. Thus, we take $F_0 = 2.5$ pN (the average value of $1.85 \sim 3.2$ pN). We take $v_{k0} = 5$ nm/s, because with this value, $F_0 = 2.5$ pN and the published experimental data of $v_k = 10 \sim 20$ nm/s for human spindle (Steblyanko et al., 2020; Risteski et al., 2022) and using Eq. (2) we obtain the pulling force $F = 2.5 \sim 7.5$ pN on one kMT to be consistent with the available experimental results (Chacón et al., 2014; Suzuki et al., 2016; Akiyoshi et al., 2010; Mukherjee et al., 2019; Nicklas, 1988; Alexander and Rieder, 1991) (see

Introduction). The parameter values for kinesin-5 are taken as follows: $k_{\text{on}} = 10^{-3} \text{ site}^{-1}\text{s}^{-1}$, $k_{\text{off}} = 1 \text{ s}^{-1}$, $v_{\text{m0}} = 80 \text{ nm/s}$ and $f_0 = 5 \text{ pN}$, which are taken based on previous studies (Valentine et al., 2006; Xie, 2021; Wang et al., 2025). Using Eq. (5), the calculated results of the antiparallel overlap length per pair of opposite kMTs attached to a pair of kinetochores, L_O/N , versus the MT flux velocity v_k are shown in Fig. 2. From Fig. 2, it is seen that for the kMT flux velocity in the range of $10 \sim 20 \text{ nm/s}$ (Steblyanko et al., 2020; Risteski et al., 2022), L_O/N is about $5 \sim 16 \mu\text{m}$, which is consistent with the published experimental data showing that in human metaphase spindle the antiparallel overlap length is about $6.6 \mu\text{m}$ (Risteski et al., 2022). This implies that in human metaphase spindle the number of kMTs attached to kinetochores is comparable to that of antiparallel overlaps. More interestingly, from Fig. 2 it is seen that L_O/N increases with the increase of v_k . This is consistent with the published experimental data (Risteski et al., 2022).

The mechanism of the kMT flux velocity v_k regulating the antiparallel overlap length can be stated briefly as follows. As Eq. (2) shows, the pulling force F on kMT increases with the increase of v_k . The number of kinesin-5 motors can be adjusted spontaneously to make the total driving force by the kinesin-5 motors equal to NF , resulting in the kinesin-5 number to increase with the increase of F . On the other hand, the number of kinesin-5 motors in the antiparallel overlaps should be proportional to the total length of the antiparallel overlap regions, because the number of sites on MTs in the antiparallel overlaps to which the kinesin-5 motors can bind is proportional to the total length of the overlap regions. Thus, the total length of the antiparallel overlaps or the length of each antiparallel overlap should increase with the increase of the kMT flux velocity v_k . Note that kinesin-14s and PRC1s, which can also be present in the spindle, are not explicitly considered in our model. Since kinesin-14s and PRC1s have a resistance effect on the MT sliding by kinesin-5s, the decrease of v_k caused by the kinesin-14s and PRC1s can be compensated for by the increase of the number of the kinesin-5s. Thus, the inclusion of kinesin-14s and PRC1s does not affect our conclusion.

Force to stall a kinetochore in anaphase

In the model, the spindle in early anaphase (Fig. 1b) is identical to that in metaphase (Fig. 1a) except that the linkage connecting any pair of kinetochores is present in metaphase whereas is broken in anaphase. In early anaphase, without the resistance force NF arising from the stretching of all pairs of kinetochores, with N being the number of kMTs connected to all kinetochores, the force from the kinesin-5 motors can drive MT sliding with a velocity larger than the metaphase MT flux velocity v_k , although the MT-sliding velocity can be reduced by the increase of PRC1 amount in

anaphase relative to that in metaphase (Yamashita et al., 2005; Roostalu et al., 2010; Hu et al., 2012; Wang et al., 2024). The kinetochore-kinetochore separation and pole-pole separation can occur coordinately, during which the kinetochore-to-pole motion is achieved mainly by the depolymerization at minus ends, with the initial depolymerization velocity being similar to v_k , because v_k is much larger than v_{k0} . The kinetochore-separation velocity v_{kk} , pole-separation velocity v_{pp} and kinetochore-to-pole motion velocity v_{kp} should satisfy the relation, $v_{kk}/2 = v_{pp}/2 + v_{kp}$, as the available experimental results showed (Yu et al., 2019).

As done in the experiment of Nicklas (1983), we consider a force $F_{\text{stall-KT}}$ on one kinetochore (called manipulated kinetochore) to halt its movement relative to the spindle center (Fig. 1b). In order to stall the kinetochore, it is required that the plus-end polymerization velocity (v_{pol}) of the attached kMT under the force $F_{\text{stall-KT}}$ on the kinetochore, the plus-end polymerization velocity (v_{k0}) of kMT under no force ($F_{\text{stall-KT}} = 0$) on the kinetochore and the velocity (v_{kc}) of the movement of the unmanipulated kinetochore relative to the spindle center under $F_{\text{stall-KT}}$ on the manipulated kinetochore satisfy the relation, $v_{\text{pol}} - v_{k0} = v_{kc}$, where v_{kc} is equal to half of the kinetochore-separation velocity v_{kk} for the unmanipulated kinetochore. Thus, we have $v_{\text{pol}} = v_{k0} + v_{kc}$. Similar to Eq. (2), in order to make the plus-end polymerization velocity of kMT equal to v_{pol} , the pulling force on the kMT can be calculated as $F_{\text{stall-MT}} = F_0 (v_{\text{pol}}/v_{k0} - 1)$. Substituting $v_{\text{pol}} = v_{k0} + v_{kc}$ into above expression, we have the following force $F_{\text{stall-KT}}$ on the kMT that makes its plus end or the attached kinetochore in the fixed position relative to the spindle center

$$F_{\text{stall-MT}} = F_0 \left(\frac{v_{k0} + v_{kc}}{v_{k0}} - 1 \right). \quad (6)$$

With Eqs. (2) and (6) we obtain

$$F_{\text{stall-MT}} = (F + F_0) \frac{v_{kc}}{v_k}, \quad (7)$$

where F corresponds to the pulling force on the plus end of a kMT in metaphase (defined in above section), v_k corresponds to the plus-end polymerization velocity of kMT or the kMT poleward flux velocity in metaphase (defined in above section), and v_{kc} corresponds to the velocity of the movement of the unmanipulated kinetochore relative to the spindle center in anaphase. In Appendix

A, we give the equation for $F_{\text{stall-KT}}$ as the function of the velocity ($v_{\text{kc}0}$) of the movement of the kinetochore relative to the spindle center under no force on any kinetochore in anaphase.

Denoting by N_{kMT} the number of kMTs attached to a kinetochore, the force to stall the manipulated kinetochore can be written as

$$F_{\text{stall-KT}} = N_{\text{kMT}} F_{\text{stall-MT}} . \quad (8)$$

From Eqs. (7) and (8), it is seen that for the spindle of a given organism, with a given N_{kMT} , the force to stall the manipulated kinetochore in anaphase, $F_{\text{stall-KT}}$, is determined by parameters, F , the pulling force on the plus end of a kMT in metaphase, F_0 , the characteristic force in metaphase, and $v_{\text{kc}}/v_{\text{k}}$, the ratio of the velocity of the movement of the unmanipulated kinetochore relative to the spindle center in anaphase, v_{kc} , to the kMT poleward flux velocity in metaphase, v_{k} .

Now, we use Eqs. (7) and (8) to explain the large magnitude of about 700 pN for the force to stall a kinetochore in anaphase of grasshopper spermatocytes. We take F as a variable parameter. As mentioned above, we take $F_0 = 2.5$ pN. From the experimental results in grasshopper spermatocytes measured by Nicklas (1983), it is estimated that the half of kinetochore-separation velocity v_{kk} is about 45 nm/s for the unmanipulated kinetochore/chromosome pairs. Thus, we take $v_{\text{kc}} = 45$ nm/s. Note that Yu et al. (2019) measured that the kinetochore-to-spindle center movement velocity in early human anaphase is also about 45 nm/s. Considering that the kinetochore-to-pole movement is mainly contributed by the minus-end depolymerization with the initial velocity similar to the metaphase kMT flux velocity v_{k} , from the experimental results for the kinetochore-to-pole movement velocity of about 10 nm/s in anaphase of grasshopper spermatocytes (Nicklas, 1983; Chen and Zhang, 2004), we take $v_{\text{k}} = 10$ nm/s. Note that Steblyanko et al. (2020) measured that the poleward kMT flux velocity in human metaphase spindle is also about 10 nm/s. Since about 15 kMTs are connected to one kinetochore in the spindle of grasshopper spermatocytes (Nicklas, 1983), we have $N_{\text{kMT}} = 15$. Using Eqs. (7) and (8), we obtain the results of $F_{\text{stall-KT}}$ versus F , as shown in Fig. 3. From Fig. 3, it is seen that at $F = 7.87$ pN, we have $F_{\text{stall-KT}} = 700$ pN, which is consistent with the experimental value of about 700 pN measured by Nicklas (1983). More interestingly, this value of $F = 7.87$ pN is close to the experimental value of about 7 pN on each kMT during prometaphase of grasshopper spermatocytes determined by Nicklas (1988). This value is also similar to the available experimental value of about 5 pN on each kMT during metaphase in budding yeast (Chacón et al., 2014; Suzuki et

al., 2016; Akiyoshi et al., 2010; Mukherjee et al., 2019) and is also consistent with the estimated force of about 1 ~ 10 pN per kMT during prometaphase in newt lung cells (Alexander and Rieder, 1991).

Previous studies showed that in metaphase the kinetochore-kinetochore tension under the pulling force on kMTs is required to correct efficiently the erroneous kMT attachments to kinetochores with the effect of Aurora B activity (Sen et al., 2021; Wang et al., 2026). As both metaphase and anaphase share the same mechanism to generate forces within the spindle (Fig. 1), the large force of about 700 pN to halt the single kinetochore movement in anaphase of grasshopper spermatocytes is due to two factors. One is that in metaphase a pulling force of about 7.87 pN on each kMT is required to stretch the kinetochore pairs so that the erroneous kMT attachments to kinetochore can be corrected efficiently with the effect of Aurora B activity. The other is that the velocity of kinetochore-to-spindle center movement in anaphase is evidently larger than the kMT poleward flux velocity in metaphase.

With above determined $F = 7.87$ pN on each kMT in metaphase of grasshopper spermatocytes, it is interesting to note that the pulling force on a kinetochore that is attached by 15 kMTs in metaphase is about $15F = 118$ pN, which is only about 17% fraction of 700 pN to stall a kinetochore in anaphase. Moreover, consider the application of forces on all kinetochores to halt their movement in anaphase, with the force on each kinetochore being denoted by $F_{\text{stall-each}}$ and thus the total force on all kinetochores being calculated as $F_{\text{stall-all}} = 2N_{\text{chrom}} F_{\text{stall-each}}$, where $N_{\text{chrom}} = 11 \sim 12$ is the number of pairs of chromosomes in grasshopper spermatocytes (Nicklas, 1983). Under this condition, it is expected that if $F_{\text{stall-each}} = 15F = 118$ pN each kinetochore in anaphase experiences the same force along the pole-to-kinetochore direction as in metaphase, thus each kinetochore in the spindle becoming stalled, as in metaphase. Consequently, we predict that $F_{\text{stall-all}} = 2N_{\text{chrom}} \times 15F = 2597 \sim 2833$ pN. This value of $F_{\text{stall-all}}$ to stall all kinetochores is about 4-fold larger than $F_{\text{stall-KT}} = 700$ pN to stall a single kinetochore. It is interesting to note that the force $F_{\text{stall-each}} = 118$ pN on each kinetochore to stall all kinetochores is only about 17% fraction of the force $F_{\text{stall-KT}} = 700$ pN to stall a single kinetochore. Note also that the total force $F_{\text{stall-all}}$ corresponds to the total force generated by the spindle in metaphase.

As seen above, under $F_{\text{stall-KT}}$ the anti-poleward movement of the kinetochore caused by the

increase of the polymerization velocity at plus ends of the attached kMTs can compensate for the poleward movement of the kinetochore in anaphase, halting the kinetochore movement. Based on this mechanism, the experimental data measured by Ferraro-Gideon et al. (2013) using laser traps, which showed that the force to stop kinetochore movement during anaphase in meiotic insect cells and mitotic mammalian cells is much lower than 700 pN measured in grasshopper spermatocytes, can be explained as follows. We argue that the laser illumination can induce the detachment of the kinesin-13 MCAKs bound to the kinetochore, increasing largely the plus-end polymerization velocity of kMTs. Thus, a small increase of the polymerization velocity at plus ends of kMTs connected to the kinetochore can compensate for the poleward movement of the kinetochore. Therefore, under the laser illumination the force $F_{\text{stall-KT}}$ required to stall the kinetochore would be much smaller than under no laser illumination. After the laser illumination is turned off, the experimental data of Ferraro-Gideon et al. (2013) showing that the kinetochore moved with a slower velocity than before the laser illumination can be explained by arguing that only a smaller amount of MCAKs can bind to the kinetochore than before the laser illumination.

Concluding remarks

Based on our proposed model for the spindle in metaphase and the proposal that the spindle in early anaphase shares the same mechanism of generating force as in metaphase, we study analytically the force generated in metaphase and the force (the stall force) applied to one kinetochore in anaphase to stall the kinetochore. The theoretical results in metaphase showing that the length of antiparallel overlap increases with the increase of the kMT poleward flux velocity are consistent with the available experimental data and the underlying mechanism is explained. The theoretical results explain well the intriguing experimental results showing that the measured stall force of about 700 pN for a kinetochore/chromosome in anaphase of grasshopper spermatocytes is significantly larger than needed (< 1 pN) and is also much larger than the pulling force on a kinetochore in metaphase. This is mainly caused by the fact that the velocity of kinetochore-to-spindle center movement in anaphase is evidently larger than the kMT poleward flux velocity in metaphase. To further test the model, it is hoped to test the predicted results that the total force to stall all chromosomes in anaphase of grasshopper spermatocytes is about 4-fold larger than the force to stall the single chromosome or the force on each chromosome to stall all chromosomes is only about 17% fraction of the force to stall the single chromosome.

Appendix A: Calculation of the force to stall a kinetochore from the kinetochore-separation velocity under no force on any kinetochore in anaphase

Consider the effect of $F_{\text{stall-KT}}$ on MT-sliding velocity v_{slide} . Assuming that v_{slide} decreases linearly with the increase of $F_{\text{stall-KT}}$, we have

$$v_{\text{slide}} = -aF_{\text{stall-KT}} + b, \quad (\text{A1})$$

where a and b are constants. Under $F_{\text{stall-KT}} = 0$, v_{kc} becomes v_{kc0} , where v_{kc0} is the half of the velocity of kinetochore separation under no force on any kinetochore in anaphase. Since the MT-sliding velocity is much larger than v_{kc0} , the movement of kinetochore relative to the spindle center in anaphase is mainly contributed by the MT sliding. For approximation, we take $v_{\text{slide}} = v_{\text{kc0}}$ under $F_{\text{stall-KT}} = 0$. Thus, from Eq. (A1) we have $b = v_{\text{kc0}}$. In metaphase, with the kMT flux velocity equal to v_{k} that is equal to the MT-sliding velocity, the force on each kMT is F . This indicates that if $F_{\text{stall-KT}} = 2N_{\text{chrom}}N_{\text{kMT}}F$, where $N_{\text{kMT}} = 15$ is the number of kMTs attached to a kinetochore and $N_{\text{chrom}} = 12$ is the number of pairs of chromosomes, we have $v_{\text{slide}} = v_{\text{k}}$ for the unmanipulated kinetochore in anaphase. Thus, from Eq. (A1) we have $v_{\text{k}} = -2N_{\text{chrom}}N_{\text{kMT}}Fa + v_{\text{kc0}}$, from which we obtain $a = (v_{\text{kc0}} - v_{\text{k}})/(2N_{\text{chrom}}N_{\text{kMT}}F)$. With above-determined a and b , Eq. (A1) can be rewritten as

$$v_{\text{slide}} = -\frac{(v_{\text{kc0}} - v_{\text{k}})}{2N_{\text{chrom}}N_{\text{kMT}}F}F_{\text{stall-KT}} + v_{\text{kc0}}. \quad (\text{A2})$$

Since the movement of kinetochore relative to the spindle center is mainly contributed by the MT sliding, for approximation, we consider that the velocity of the movement of the unmanipulated kinetochore relative to the spindle center under $F_{\text{stall-KT}}$ on the manipulated kinetochore, v_{kc} , is equal to v_{slide} that is described by Eq. (A2), i.e.,

$$v_{\text{kc}} = -\frac{(v_{\text{kc0}} - v_{\text{k}})}{2N_{\text{chrom}}N_{\text{kMT}}F}F_{\text{stall-KT}} + v_{\text{kc0}}. \quad (\text{A3})$$

As it is stated in the main text, in Eq. (7) v_{kc} corresponds to the velocity of the movement of the unmanipulated kinetochore relative to the spindle center under $F_{\text{stall-KT}}$ on the manipulated kinetochore. Thus, from Eqs. (7) and (A3), we obtain

$$F_{\text{stall-MT}} = \frac{v_{\text{kc0}}(F + F_0)}{v_{\text{k}} + \frac{(v_{\text{kc0}} - v_{\text{k}})(F + F_0)}{2N_{\text{chrom}}N_{\text{kMT}}F}}. \quad (\text{A4})$$

With $F_0 = 2.5$ pN, $v_{\text{k}} = 10$ nm/s and $v_{\text{kc0}} = 45$ nm/s, same as those used in Fig. 3, using Eqs. (A4) and (8) the results of $F_{\text{stall-KT}}$ versus F are shown in Fig. A1 (thick dashed line). It is seen that $F_{\text{stall-KT}} = 700$ pN occurs at $F = 8.0$ pN, which is only slightly larger than $F = 7.87$ pN determined in Fig. 3

with $v_{kc} = 45$ nm/s. In fact, by taking $v_{kc0} = 45.6$ nm/s that is only slightly larger than 45 nm/s, we obtain that $F_{\text{stall-KT}} = 700$ pN occurs at $F = 7.87$ pN (see solid line in Fig. A1). These results indicate that to calculate F , for approximation, we can neglect the effect of $F_{\text{stall-KT}}$ on v_{kc} induced by the effect of $F_{\text{stall-KT}}$ on MT-sliding velocity.

Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this article.

Author Contributions

Ping Xie designed research, performed research, analyzed data and wrote the manuscript.

Data Availability

Data sharing no available – the article describes entirely theoretical research.

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Figures

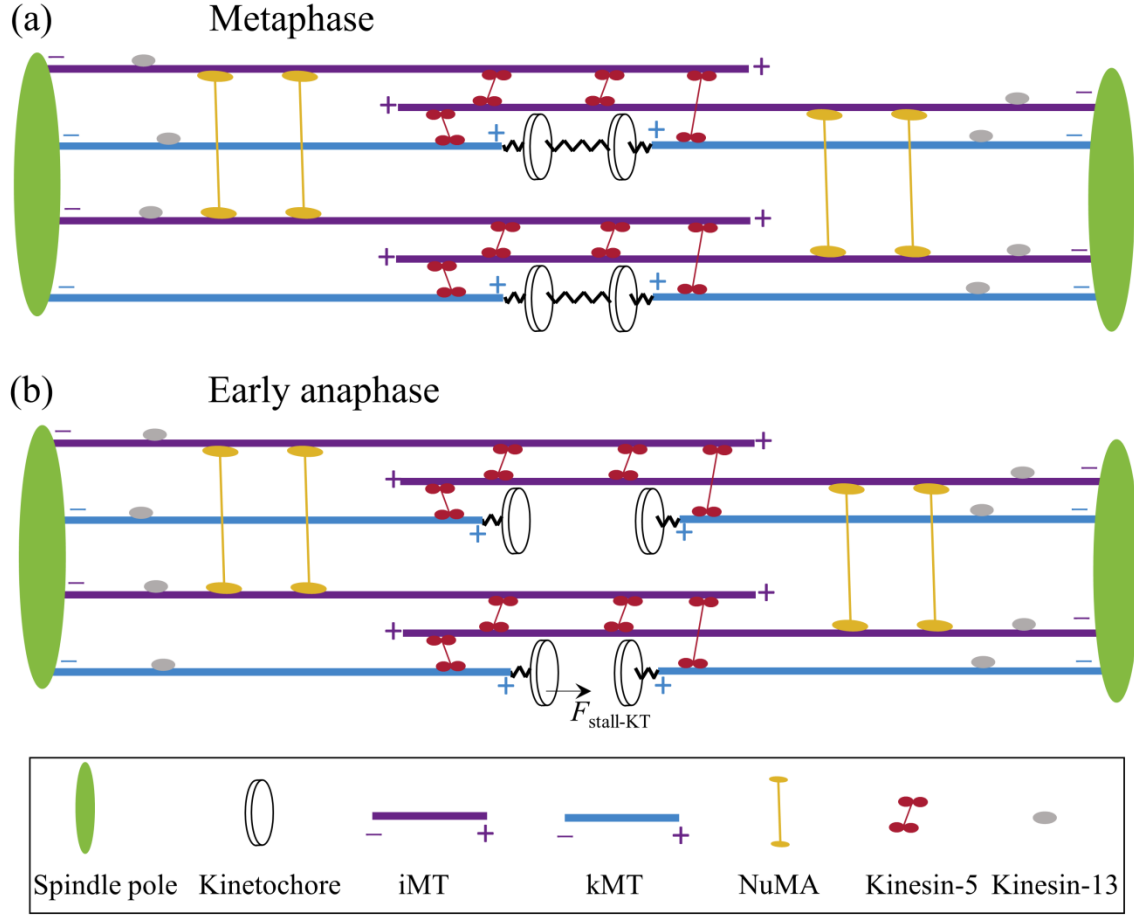


Fig. 1. Schematic of the simplified model for spindle in metaphase (a) and early anaphase (b) (see text for detailed descriptions). The difference between metaphase and early anaphase is that in metaphase (a) the linkage that connects each pair of kinetochores is present whereas in early anaphase (b) all linkages are broken. Specifically, to study the force required to halt the kinetochore movement in early anaphase, we apply a force, $F_{\text{stall-KT}}$, on one kinetochore (b), as done in the experiment of Nicklas (1983).

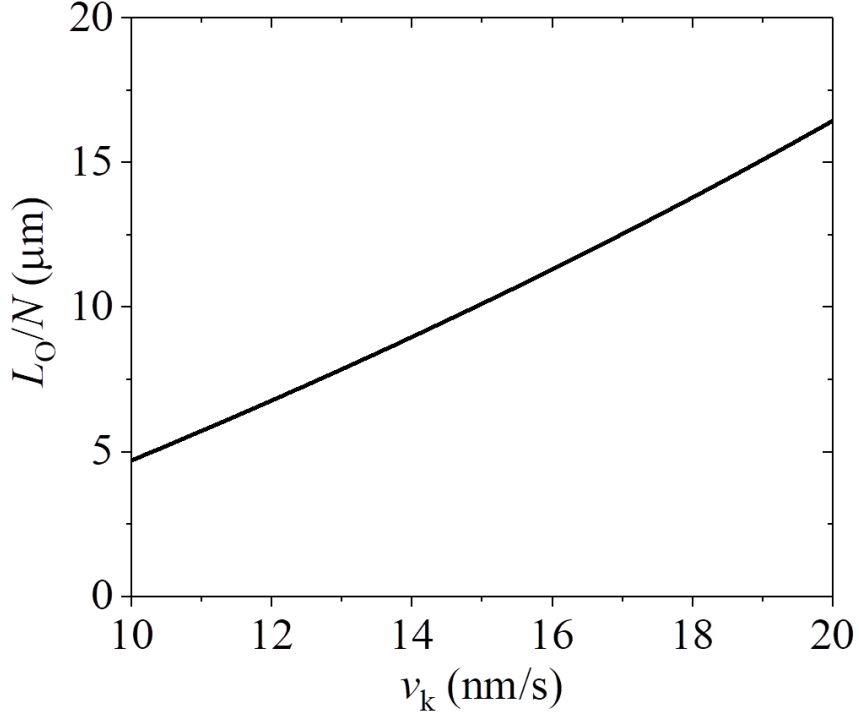


Fig. 2. Results of the antiparallel overlap length per pair of opposite kMTs attached to a pair of kinetochores, L_O/N , versus the kMT flux velocity v_k in metaphase. L_O represents the total length of antiparallel overlaps and $2N$ corresponds to the number of kMTs connected to all kinetochores. The results are calculated using Eq. (5) and with parameter values $F_0 = 2.5$ pN, $v_{k0} = 5$ nm/s, $k_{on} = 10^{-3}$ s⁻¹site⁻¹, $k_{off} = 1$ s⁻¹, $v_{m0} = 80$ nm/s and $f_0 = 5$ pN.

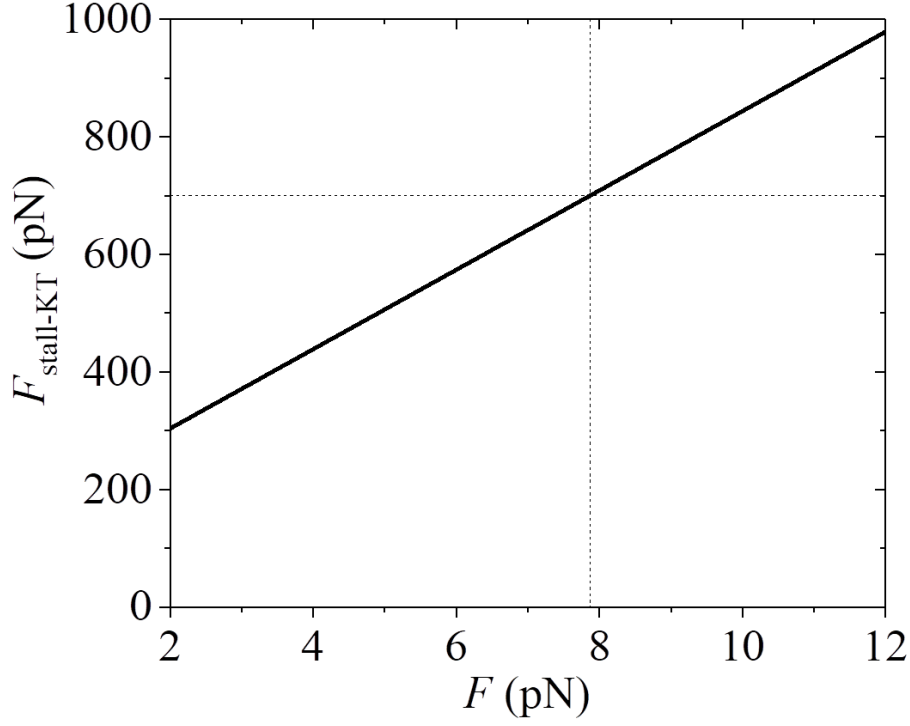


Fig. 3. Results of the force required to stall a single kinetochore, $F_{\text{stall-KT}}$, in early anaphase versus the pulling force on each kMT, F , in metaphase. The results are calculated using Eqs. (7) and (8) and with parameter values for characteristic force $F_0 = 2.5$ pN in metaphase, kMT plus-end polymerization velocity $v_k = 10$ nm/s in metaphase and velocity of the movement of the unmanipulated kinetochore relative to the spindle center, $v_{kc} = 45$ nm/s, in anaphase. The horizontal thin broken line corresponds to $F_{\text{stall-KT}} = 700$ pN and the vertical thin broken line corresponds to $F = 7.87$ pN.

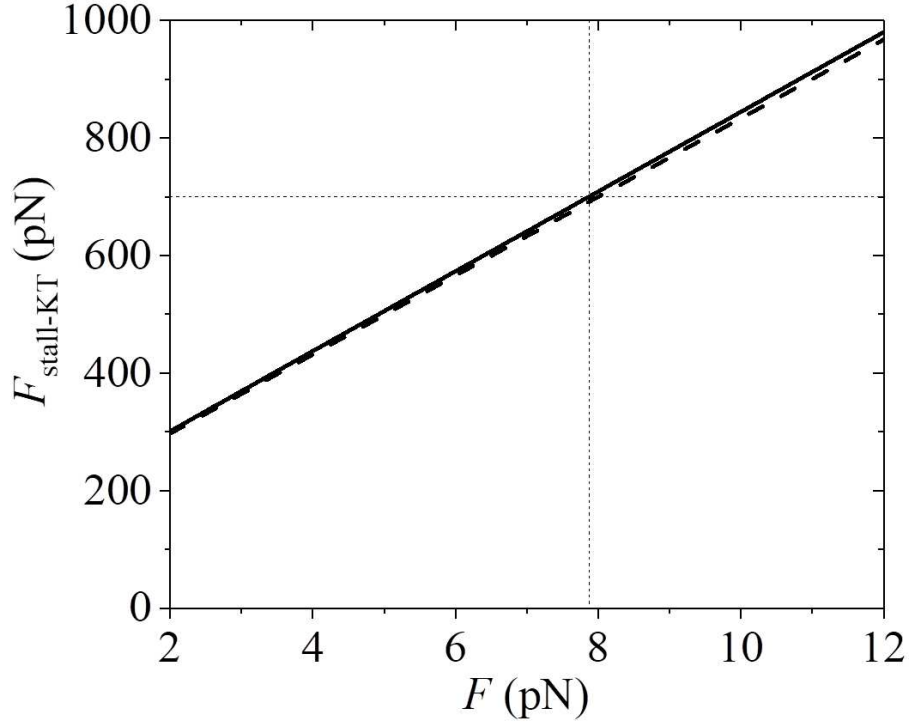


Fig. A1. Results of the force required to stall a single manipulated kinetochore, $F_{\text{stall-KT}}$, in early anaphase versus the pulling force on each kMT, F , in metaphase. The results are calculated using Eqs. (A4) and (8). The thick dashed line corresponds to the results with $F_0 = 2.5$ pN, $v_k = 10$ nm/s and $v_{kc0} = 45$ nm/s. The solid line corresponds to the results with $F_0 = 2.5$ pN, $v_k = 10$ nm/s and $v_{kc0} = 45.6$ nm/s. The horizontal thin broken line corresponds to $F_{\text{stall-KT}} = 700$ pN and the vertical thin broken line corresponds to $F = 7.87$ pN.