Supporting Text

Force-velocity relationship for sliding motors.

We assume that motor heads of the bipolar kinesins are characterized by a linear force -

velocity relation: $f = F_m \left(1 - \dfrac{v}{V_m} \right)$ (Fig. 5), where v is the velocity of the plus-end-

directed motor movement.

Derivation of the motor - generated forces between antiparallel and parallel

microtubules (MTs) in ipMT arrays.

Let us first consider two overlapping antiparallel MTs crosslinked by a single motor (as

in Fig. 3B upper ipMT array), such that the MTs are moving with velocities $V_{sliding, left} = V_l$

and $V_{sliding, right} = V_r$ with their minus ends leading, to the left and right, respectively. In

this configuration, the forces generated by two opposite motor heads are

$f_l = F_m \left(1 - \dfrac{v + V_l}{V_m} \right), f_r = F_m \left(1 - \dfrac{V_r - v}{V_m} \right)$ and have to be equal, because motor heads are

connected by nonmotor domains. Solving this equality, we find that $v = \dfrac{V_r - V_l}{2}$, and

therefore a force $f = F_m \left(1 - \dfrac{V_l + V_r}{2V_m} \right)$ will be generated by each motor sliding apart two

overlapping antiparallel MTs moving with velocities V_l and V_r. We do not analyze the

associated motor transport, because we assume that the motors associate fast with overlap

regions between pairs of MTs as they become available (1); therefore, the motor transport

does not affect the number of engaged motors.

Next, let us consider two parallel MTs crosslinked by a motor [Fig. 3*B*, lower ipMT array, the two MTs at (left)], such that the MTs are moving with velocities V_1 and V_2 (with minus ends leading), respectively. In this configuration, the forces generated by two opposite motor heads are $f = \pm F_m \left(1 - \dfrac{v + V_{1,2}}{V_m} \right)$ and have to be equal, because motor heads are connected by nonmotor domains. Solving this equality, we find that $v = V_m - \dfrac{V_1 + V_2}{2}$, and therefore a force of magnitude $f = F_m \dfrac{V_2 - V_1}{2V_m}$ will be generated by each motor between two parallel crosslinked MTs, slowing down the faster MT and accelerating the slower one. Because the force across each ipMT array is constant, in a given ipMT array the force generated by sliding motors in each overlap is the same, giving rise to Eq. 6, for example, for the lower ipMT array in Fig. 3*B*.

Computer code.

Describing the kinematic equations for each ipMT array together with the force - balance equation (Eq. 7) results in a large system of coupled differential equations.

A computer code was developed to obtain the numerical solutions and generate simulations displayed as movies. An important part of the code is the random number-generated stochastic variations of the model parameters and initial conditions. There are nine ipMT bundles per spindle (Fig. 2*C*), and we assume that each one of these ipMT bundles contains 3-10 ipMT arrays, which in turn are composed of two to four interconnected MTs stretching between the poles (Fig. 3*B*). Therefore, in our code, we

assume that there are typically 30-90 ipMT arrays composed of varying numbers of MTs per spindle. MTs in each ipMT array interact pair wise, and only one among all MTs facing each pole with their minus end is associated with that pole, whereas the other(s) are not. In the ipMT arrays composed of 3 MTs (Fig. 3B, middle ipMT array), the middle MT that is not associated with either one of the poles overlaps and interacts with the two others, which are associated with the poles and do not overlap nor interact directly with each other. In the ipMT arrays with 4 MTs, the configuration is similar to that shown in Fig. 3B, lower ipMT array. The initial position of the MT ends in each ipMT array is computed using random number generators, giving rise to an average antiparallel overlap of $\approx$ 1 μm, and an average parallel overlap of $\approx$ 2.5 μm.

At each computational step, with Δt corresponding to 1 sec, we solve the kinematic equations for each ipMT array and the force - balance equation simultaneously based on the current parallel and antiparallel overlaps between the ipMTs. The force across each ipMT array (f_i in Fig. 3B) is an unknown variable at each time step. Also, the velocities of individual MTs in each ipMT array (v_i in Fig. 3B) are unknown, and they are computed at each time step. The total force generated by sliding motors in the spindle is obtained by summing up the force for all ipMT arrays and this is balanced by the drag force acting on the poles.

This leads to a well - posed problem of linear algebra, which is solved at every time step to find the velocities of all MTs comprising each ipMT array, and the velocity of the

spindle poles. The plus and minus ends of individual MTs in each ipMT array are then updated using the newly computed velocities, whereas all plus ends undergo dynamic instability calculated using the scheme described in Sprague *et al.* (2), and the minus ends of those MTs associated with the poles are shortened through depolymerization at prescribed rates (stochastic variations superposed on an average rate V_{depoly}^-). Finally, new overlap lengths are computed for the updated configurations, and all calculations are repeated at the next step. Parameters are listed in Table 2, and the specific values used are given in the figure legends. Because only order of magnitude values of most model parameters are published, in the simulations, we chose the values giving the best fit to the data (Table 2).

Corresponding simulations demonstrate that the average antiparallel overlap of all ipMT arrays in the central spindle changes slowly, whereas the antiparallel overlap between individual ipMT arrays whose ends grow/shrink stochastically due to dynamic instability changes rapidly. On the other hand, the average parallel overlap of all ipMT arrays within the same half-spindle increases very fast, whereas their plus ends also undergo dynamic instability. The increase in the extent of parallel overlap results in stronger shearing/accelerating effects, and thereby equalizes the velocities of all MTs in the same half-spindle, so the initially small differences in tubulin dimer velocities diminish fast in early anaphase B and do not play a significant role in the speckle velocity dispersal.

Estimates on pole - pole separation rate and the sliding motors' operating regime.

Here, to complement the numerical results obtained, we consider the oversimplified

spindle (Fig. 3*A*), for which the corresponding force - balance and kinematic equations

can be solved analytically. The system of three equations (Eqs. 1-3), describe the

dynamics of the spindle poles and ipMTs. In this simplified configuration with N

identical ipMT arrays composed of pairs of MTs emanating from the poles and

overlapping antiparallel over a distance $L(t)$ at the equator, all MTs slide at the common

rate $V_{\text{sliding}}(t)$ and we have (combining Eqs. 1 and 3):

$$kNLF_m\left(1-\frac{V_{\text{sliding}}}{V_m}\right) = \mu\left(V_{\text{sliding}} - V_{\text{depoly}}^-\right), \text{ where } (V_{\text{sliding}} - V_{\text{depoly}}^-) \text{ is the rate at which the}$$

poles diverge from the spindle equator, or equivalently, half the rate of pole separation.

Solving this force - balance equation yields the sliding rate:

$$V_{\text{sliding}} = \frac{\alpha L}{1+\alpha L}V_m + \frac{1}{1+\alpha L}V_{\text{depoly}}^-, \text{ where } \alpha = \frac{kNF_m}{\mu V_m} \approx 10/\mu m \text{ is an important parameter}$$

representing the ratio of the maximal motor generated force to the maximal viscous drag

force per unit overlap length. If we assume that the ipMT overlap remains in the order of

a micron (or few microns), αL is large (a few tens), therefore $\dfrac{\alpha L}{1+\alpha L} \sim 1$ whereas

$\dfrac{1}{1+\alpha L} \sim 0$, and consequently, sliding takes place at a rate almost equal to the "free"

sliding rate: $V_{\text{sliding}} \approx V_m$, indicating that motors operate against an almost negligible

resistance. This estimate, based on the assumption that an overlap in the order of a

micron is maintained throughout anaphase B, illustrates how the sliding rate can remain

near its unloaded velocity in this rigid and idealized spindle geometry. If we also assume

that an overlap in the order of a micron is maintained throughout metaphase/anaphase A, this estimate also predicts that the poleward flux rate in metaphase/anaphase A is the same as half the rate of pole separation in anaphase B, $\approx V_m$, as observed (3). In addition, this estimate also illuminates how the overlap changes during anaphase B as long as it remains larger than or in the order of a micron. The overlap between antiparallel ipMTs decreases due to sliding but increases due to mean net polymerization of the plus ends:

$$\frac{dL}{dt} = 2\left(V_{poly}^+ - V_{sliding}\right).$$ Therefore, as long as the overlap remains larger than or in the

order of a micron, it changes at a rate $\approx 2\,(V_{poly}^+ - V_m)$ during anaphase B. On the other hand, when L becomes small during the course of anaphase B, such that $\alpha L \ll 1$, then

$\dfrac{\alpha L}{1+\alpha L} \sim 0$ whereas $\dfrac{1}{1+\alpha L} \sim 1$, and consequently $V_{sliding} \approx V_{depoly}^- = 0$, indicating that

motors operate near stall, because the depolymerization is turned off at the onset of anaphase B (i.e. $V_{depoly}^- = 0$). The dynamics of the motors' transition from the unloaded regime to the stall regime is seen in Fig. 4E (dotted - dash curve), where both the average depolymerization rate and the net polymerization rate are equal to zero, and pole - pole separation is severely hindered (at $t \approx 20$ sec) when the antiparallel overlap length decreases and fluctuates near zero (due to dynamic instability).

How do KLP3A motors regulate the switch from the metaphase/anaphase A steady state to anaphase B?

We propose that KLP3A inhibition interferes with the suppression of flux by influencing the ratio of KLP10A motors to ipMT minus ends at the poles. In control spindles, KLP3A

forms robust ipMT bundles which are "fed" into the poles. In preanaphase spindles these ipMTs are depolymerized at the poles because the ratio of KLP10A to ipMT ends is high, but at anaphase B onset this ratio falls as KLP10A activity is inhibited. In KLP3A-inhibited embryos, ipMT bundles are less robust and the number of ipMT ends is lower, meaning that the actual ratio of residual KLP10A to ipMTs remains high even after anaphase B onset, and thus depolymerization and flux persist.

In the model results shown in Fig. 4, we incorporated the effect of KLP3A inhibition directly as a reduction in the suppression of KLP10A-dependent depolymerization at anaphase B onset. Here, we investigate the above - described mechanism for KLP3A action in our model, by incorporating the effect of KLP3A inhibition in anaphase B through a reduction in the number of ipMT arrays, a corresponding increase in viscous resistance per sliding motor, and a corresponding increase in the ipMT minus end depolymerization rate determined by the ratio of residual KLP10A molecules to ipMT minus ends. To determine the rate of depolymerization in KLP3A - inhibited embryos with reduced numbers of ipMTs, we use a piecewise linear function between the number of active KLP10A motors per minus end and the mean depolymerization rate V_{depoly}^{-} (Fig. 6). Until the number of active KLP10A motors per minus end reaches the critical value, r_c, the mean depolymerization rate is zero, then, as the number of active KLP10A motors per minus end increases, the mean depolymerization rate increases linearly. When the ratio reaches the saturation level, r_s, the mean depolymerization reaches its maximal rate (the pre-anaphase B flux rate), which is limited either by sliding or by the number of motors that can fit onto the minus end, and remains at this maximal value despite further

increases in the number of active KLP10A per minus end. This is the simplest function which represents the mechanism described above. Based on previous studies on another MT depolymerase (4), we assume that, in this linear dependence, the maximal depolymerization rate is attained for $r_s \sim 10$, and $r_c \sim 1$. We further assume that in the control embryo, the ratio of active KLP10A number per minus end is greater than $r_s \sim 10$ during preanaphase B and is reduced to $r_c \sim 1$ at anaphase B onset when KLP10A is downregulated. In our model, we consider decreasing numbers of ipMTs (from $N = 40$-4), representing various degrees of the effect of KLP3A inhibitors. As shown in Fig. 7, the results of these simulations are in good agreement with our experimental data on the flux - anaphase B relationship (Fig. 1*C*). In addition, our model results suggest that the experimental data on KLP3A - inhibited embryos are best accounted for by spindles in which the number of ipMTs has been reduced significantly.

An alternative simple hypothesis that we considered in detail is that KLP3A motors simply change the effective outward force driving the poles apart, either by acting as ipMT sliding motors themselves or by augmenting the force generated by other ipMT sliding motors such as KLP61F (5). However, this alternative model does not explain the slope of the inverse linear relation between flux and spindle elongation (Fig. 1*C*), because a decrease in the outward force and sliding rate after KLP3A inhibition would result in lower rates of spindle elongation but would also decrease the flux rate. This is revealed by computer simulations of a model that assumes that KLP3A inhibition affects the number and efficacy of the force generators; this leads to a decrease in the rate of spindle elongation associated with a decrease in poleward flux rates (data not shown).

Another alternative hypothesis is that KLP3A motors might change the effective outward force driving the poles apart by augmenting the astral pulling forces. This possibility would explain the inverse linear relationship between the spindle elongation and the poleward flux rates, but only if KLP10A - driven depolymerization at the poles persists in anaphase B and the astral pulling rate in control embryos is exactly equal to the ipMT sliding rate (see below). However, this possibility was not further explored, because ipMT sliding by KLP61F, and not astral pulling, is the prominent mechanism during the first half of anaphase B (6) and in addition, the KLP3A motors are localized in the interzone (7) and therefore are unlikely to act on astral MTs.

Fluctuations/ variance in anaphase B rate.

To analyze the variance in the anaphase B rate, let us first consider the simplified situation, in which two ipMT arrays composed of antiparallel MT pairs connect the spindle poles. The poleward fluxes and the mechanical characteristics of the motors sliding the two ipMT arrays differ. The first pair of MTs depolymerize at the poles with rate V_f^1, whereas the second pair depolymerize with rate V_f^2. Two KLP61F motors slide the respective pairs. The forces generated by the motors are $F_i = \zeta\left(V_s^i - V_m^i\right)$, i=1, 2, where V_s^i are the sliding velocities, V_m^i are the free sliding motor rates, and ζ is the slope of the motors' linear force - velocity relation. The poles are separating with rate V_a, and the kinematic constraints on the rates of MT sliding, free motor sliding, and pole separation are: $2\left(V_s^1 - V_f^1\right) = 2\left(V_s^2 - V_f^2\right) = V_a$. In addition, the force - balance equation in the regime

where viscous resistance is negligible compared to motor forces is: $F_1 + F_2 \approx 0$. Solving

these three linear equations demonstrates that the average sliding rate is equal to the

average free sliding motor rate, $\dfrac{V_s^1 + V_s^2}{2} = \dfrac{V_m^1 + V_m^2}{2}$, and that the anaphase B rate is given

by $V_a = 2\left(\dfrac{V_m^1 + V_m^2}{2} - \dfrac{V_f^1 + V_f^2}{2} \right)$. In the general case, when there are many MT pairs

depolymerizing at the minus ends with variable rates and crosslinked by multiple sliding

motors characterized by varying free sliding rates and force - velocity slopes, a similar

analysis assuming independence of the variability of the number of motors per MT and

other stochastic variables shows that the average sliding rate is equal to the average free

sliding motor rate $\langle V_s \rangle = \langle V_m \rangle$, and the anaphase B rate is given by $V_a = 2\left(\langle V_m \rangle - \langle V_f \rangle \right)$.

This characterizes a single spindle and allows us to estimate the variance in the

fluctuations of the pole-pole separation rate as $\mathrm{var}[V_a] = 4\left(\dfrac{\mathrm{var}[V_m]}{N_m} + \dfrac{\mathrm{var}[V_f]}{N_{\mathrm{MT}}} \right)$. Here N_m

is the total number of motors, and N_{MT} is the total number of ipMT arrays in the spindle.

There is no direct data on the variance of the motor sliding rates, but assuming that it is of

the same order as the observed variance of the motor sliding rates in *different* spindles,

then $\mathrm{var}[V_m] \sim 10^{-3} \dfrac{\mu m^2}{\sec^2}$. Using the available estimates of

$\mathrm{var}[V_f] \sim 10^{-3} \dfrac{\mu m^2}{\sec^2}$, $N_m \sim N_{\mathrm{MT}} \sim 100$, we estimate that the standard deviation of the

anaphase B rate of a single spindle is very small, less than $0.01 \dfrac{\mu m}{\sec}$, in agreement with

the law of large numbers (8). Such small fluctuations in the rate of pole separation would

not be detectable in our time - lapse images. Indeed, linear fits to the pole - pole distance as a function of time of individual spindles are excellent.

Variance in speckle rates.

We undertook a quantitative analysis of the variance in flux rates, because such analysis can potentially provide significant insights into the underlying mechanisms (9). Our analysis points to several sources of the observed variance, however, in our current data, the relationship between variance and mean in the flux rates is not convincing enough to make forceful conclusions about the source of the variance.   In our model, we incorporated the factor that was pointed out to be most plausible as a result of the analysis below, and the variance in flux rates in our results (Fig. 4 *C* and *D*) is in very good agreement with the experimental data (Fig. 1 *B* and *C*).

Possible sources of the deviations in flux rates are first, velocity variance due to the motor-dependent "shearing"/"acceleration", a consequence of parallel MTs within ipMTs (refs. 1, 10; Fig. 3*D*); however, our analysis and computer simulations suggest that variance due to this factor is insufficient to explain the large variance observed and that its contribution is insignificant. Secondly, stochastic fluctuations in the elementary rate constants of mechanochemical coupling by the ipMT sliding motors (10-12) are another possible source of variance. Our analysis, including computer simulations, suggests that this factor's contribution is significant, but it cannot by itself explain the large dispersal in the flux rates.

A third source may be variance in the minus end depolymerization rate, which is the most plausible and provides the most significant contribution to the observed variance. The variance in the depolymerization rate may, in turn, be influenced by several factors (13): (*i*) One such factor is simple Poisson fluctuations in the number of subunits disassembled from the MT minus ends at the centrosome. If r is the average number of tubulin rings (tubulin dimers × 13) disassembled per second at the minus end, then the average depolymerization rate is $\langle V_{flux} \rangle = \delta r$, where δ is the size of the tubulin dimer. The variance in displacement is a linear function of time, and the variance of the rate measured over time T is $\sigma_v = \dfrac{Var[X(T)]}{T^2} = \dfrac{\delta^2 r}{T}$ (14). Thus, with the average flux rate over all inhibited and control spindles $\langle V_{flux} \rangle \sim 0.03 \dfrac{\mu m}{sec}$, $\delta = 0.008$ µm, and assuming that a tubulin dimer remains in a speckle on average for a time $T \sim 5$ sec, the flux rate variance due to Poisson fluctuations in the number of subunits dissembled would be $\sim 10^{-4} \dfrac{\mu m^2}{sec^2}$, smaller, but of the same order of magnitude, as observed. (*ii*) Finally, fluctuations in the number of KLP10A motors that depolymerize MT minus ends at the poles could also contribute to the observed variance in flux rates. If each KLP10A motor induces depolymerization with constant rate v_{depoly}, the total number of working KLP10A motors is M and the number of minus ends is N, then the probability that a motor will bind to a given MT is $p=1/N$. Then the average number of motors per minus end is $M/N = n$, and this number fluctuates according to the binomial distribution (8) with variance $Mp(1-p)=M/N(1-(1/N)) \sim n$. Therefore, the average flux rate is $n v_{depoly}$, and its variance is $n v_{depoly}^2$. In this case, the variance to flux ratio is equal to v_{depoly}. The variance in measured

flux velocities ranges from 0 to 0.0013 $\mu m^2/sec^2$, with a mean value $\sigma_v \sim 0.0003$

$\mu m^2/sec^2$. Using this value and $<V_{flux}> \sim 0.03$ $\mu m/sec$, we estimate $v_{depoly} \sim 0.01 \mu m/sec$.

Note, that this analysis predicts that the variance in flux is the increasing linear function

of its mean: $\sigma_v = v_{depoly} \langle V_{flux} \rangle \approx 0.01 \langle V_{flux} \rangle$. We tested this prediction by plotting the

experimentally measured variances in flux as a function of the mean flux rate and found

that the variance and the mean correlate linearly with coefficient ≈ 0.006 of the same

order of magnitude as the predicted coefficient ≈ 0.01. However, the statistical

significance of this linear fit is not high enough to be confident in the numerical value of

the slope, so further work is required to test this prediction and also to evaluate the

influence of unavoidable experimental imprecision, for example, in tracking the positions

of moving speckles that could also contribute to the fluctuations in the observed flux

rates.

In the simulations (Figs. 4-7 and Movies 1-7), we varied both the mean depolymerization

and polymerization (growth and shrinkage) rates and their variances as follows. The

variance of these rates, due to Poisson fluctuations in the number of dimers, is equal to

$\sigma^2 = \delta$(mean rate)$/2\tau$ over time τ. Correspondingly, at each computational time step, we

displaced the minus and plus ends of the MTs by the distance $\Delta x = \Delta t \cdot \sqrt{\sigma_c^2} \cdot randn$,

where $\Delta t = 0.1$ is the time step (corresponding to 1 sec in real time), σ_c^2 is the

corresponding computational value of the variance (0.01-0.1), and randn is a random

number generated by MATLAB (Mathworks, Natick, MA) distributed according to the

standard normal distribution.

To investigate the effect of stochastic fluctuations in the number of motors per unit length, the effective viscous drag coefficient, the maximal motor force, and the free sliding motor velocity on the variance of flux rates, we varied them at each step according to the normal distributions with variances 10, 100 pN sec/µm, 1 pN and 0.03 µm/sec, respectively. Finally, we simulated individual tubulin dimers in different MTs moving poleward with varying rates, assumed that 10 or more fluorescent dimers correspond to a speckle (15), and compared trajectories of dimers and speckles. We found that the statistical behavior of the individual fluorescent dimers and of the speckles were the same.

Investigation of alternative mechanisms for anaphase spindle elongation.
We have addressed the feasibility of several alternative molecular mechanisms for anaphase B spindle elongation by using computer simulations and/or evaluating their compatibility with previous experimental evidence.

1. Polymerization Ratchet
Could the outward force be generated by polymerizing MTs at the midzone rather than by the sliding action of KLP61F motors? The corresponding maximal force, namely hundreds of pN [a few pN per plus end (5)] would be sufficient, but the "free sliding" rate would be the polymerization rate, and such a model would predict that the anaphase B

rate is proportional to the polymerization rate. However, because it is unclear what these MTs push against, we did not simulate this possibility.

2. Sliding against a spindle matrix

A second possibility is that the outward force is generated by KLP61F motors acting on ipMTs associated with a spindle matrix rather than by sliding antiparallel ipMTs (16). We have investigated this mechanism in a force - balance model. Under the assumption that KLP61F motors slide MTs against a hypothetical fixed spindle matrix, the forces, and thus the sliding velocities of MTs, depend on each one's overlap with the spindle matrix, and not on the antiparallel overlap between MTs. The associated force - balance equations involving MTs' sliding velocities are similar to the ones described in our model. We have investigated the case of a fixed spindle matrix extending between the spindle poles, as well as a matrix within a limited region in the central spindle (Movie 6). In this model, a separate force - velocity relationship exists for each pole. MTs and motors in each half spindle develop a force and velocity depending on the number of MTs, matrix - MT overlaps, and viscous resistance of the corresponding pole, which may well result in large fluctuations in pole - pole elongation rate. Thus, when the ipMTs slide against a fixed spindle matrix, as opposed to against one another, each pole would move independently at velocities related to their own molecular and biophysical parameters, which could potentially vary to a large extent (e.g. if the movement of one of the poles is hindered, the rate of pole - pole separation could be reduced by half) and lead to large fluctuations in anaphase B velocity, which are not observed. However, in a symmetric configuration (as in Movie 6) the poles move apart steadily and linearly, and

our simulations show that this model could, in principle, explain all the data (in particular the data in Fig. 1*C*), but it would not explain why KLP61F has evolved a bipolar structure.

3. Astral MTs pulling the poles

Finally, could motors at the cortex, for example dynein, generate the outward force by pulling on astral MTs? To investigate this mechanism, we simulated anaphase B spindle elongation by astral MT pulling, and we could recover the flux/anaphase B data in Fig. 1*C*, under the following assumptions. (*i*) The rate of pulling of dynein-like motors on astral MTs is exactly equal to the sliding rate of the KLP61F motors in control embryos. (*ii*) KLP10A continues to depolymerize MT minus ends that are pushed into the poles by KLP61F-driven sliding throughout anaphase. (*iii*) MTs that do not have an antiparallel overlap are pulled apart with the poles, and KLP10A is inactive on these MTs. We compute the sliding velocities of ipMTs similarly as in the other mechanisms, and anaphase spindle elongation in this case starts when dynein-like motors on astral MTs start pulling the poles apart (Movie 7). If we assume that KLP3A affects the pulling activity of dynein-like motors, and its inhibition leads to a reduction in the pulling rate, this model is able to explain the inverse relationship between the flux and anaphase B rates shown in Fig. 1*C*. However, assumption (*i*), particularly, seems unlikely, and this model cannot explain how inhibition of KLP61F on ipMTs has the observed effect of abolishing anaphase B (6).

Additional note on KLP10A driven depolymerization rate and the shortening rate of ipMT minus ends at the poles.

We assume that KLP10A motors remove tubulin subunits from the minus ends of ipMTs that are pushed by ipMT - MT sliding during preanaphase B. Fluorescence Speckle Microscopy of 10 – μm - long metaphase - anaphase A spindles reveals that tubulin speckles move away from the equator and towards the pole at ≈ 0.05 μm/sec (Table 1). If we assume that the velocity of the speckles reflects the ipMT sliding rate, to maintain a steady - state pole - pole spacing during preanaphase B, a shortening rate of ipMT minus ends must occur at V_{depoly}^{-} = 0.05 μm/sec. This would imply that KLP10A motors must remove ≈ 80 (= 0.05 μm/sec × 1,000 × 8nm/ 13) tubulin subunits per second per MT at the poles. These estimates are in reasonable agreement with biochemical studies on KLP61F and KLP10A-related motors (4, 17).

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