Supplemental material

Timing of centrosome separation is important for accurate chromosome segregation.

William T. Silkworth*, Isaac K. Nardi*§, Raja Paul†, Alex Mogilner‡, and Daniela Cimini*

* Department of Biological Sciences, Virginia Tech, 1981 Kraft Dr., Blacksburg, VA, 24061, USA
† Indian Association for the Cultivation of Science, Jadavpur, Kolkata 700032, India
‡ Department of Neurobiology, Physiology and Behavior and Department of Mathematics, University of California, Davis, CA 95616, USA
§ Current address: Department of Molecular Cell and Developmental Biology, University of Virginia, School of Medicine, Charlottesville VA 22908, USA

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Corresponding author:
Daniela Cimini
Virginia Tech, Dept. Biological Sciences
1981 Kraft Dr.
Blacksburg, VA, 24061 – USA
Tel. ++1-540-231-3922
Fax ++1-540-231-3414
e-mail: cimini@vt.edu
Development of a mathematical model for simulation of spindle assembly in PtK1 cells.

*Approximate kinetics of spindle assembly.* We recently considered an extremely simplified model (Mogilner and Craig, 2010) of microtubules (MTs) from two poles searching for a single chromosome equidistant from the poles. In the model, the MTs capture one of two kinetochores (KTs) with the rate $k_{on}$ and the pole-KT connection is destabilized with the rate $k_{off}$. Thus, all pole-KT connections are transient, except for the amphitelic connection, which was assumed to be stable. We found, not surprisingly, that numbers of mono-, syn- and mero-telic attachments in this model increased at first, and then slowly decreased to zero, while the number of amphitelic connections increased monotonically to saturation.

Simulations of this model suggested starting with a numerical investigation of an approximate temporal dynamics of the spindle assembly to develop intuition without complications of the explicit spatial chromosome distribution. We will call the assembly of the spindle when the poles are initially very close to each other and the search takes place for 1-2 hours before pole separation **Experiment 1** (Figure S3A); the assembly when the poles are initially very close to each other and the search starts together with pole separation **Experiment 2** (Figure 4C); and finally the assembly when the poles start separating from the initial distance 6-7 μm together with the search **Experiment 3** (Figure 4D).

Experiment 1 informs that after 1-2 hours of search, most KTs are associated with MTs and that at pole separation onset there are almost no unattached, mero- and amphi-telic connections present. If MTs from each pole grow in all directions, then each KT is ‘seen’ from almost the same angle from both poles because in this experiment the poles are so close together.
In this scenario, there is an equal probability to capture any KT from either pole. Thus, in theory, if one KT is captured from one pole, there is an equal chance that the sister KT would be captured from the same as from the other pole. This argument suggests that at early time points following pole separation onset there would be a large number of merotelic and amphitelic attachments in addition to syntelic ones, which was not the case in the experimental results (Figure 4C). One of the most straightforward solutions for this conundrum is that when the centrosomes are very close together, then each searches in its own half-space (Figure 5A), so that each chromosome can only be captured from one of the poles. One possible explanation is that if the poles are close together, MTs from each pole can only cover a half-space because each pole ‘shields’ the other half-space from the other pole.

Furthermore, the data from Experiment 1 indicate clearly that MTs have to detach from the syntelically attached KTs. The respective MT detachment rate cannot be as fast as ~ 1/minute because in that case the attachment rate would have to be of the same order of magnitude, and the spindle would assemble within a couple of minutes, which does not happen. On the other hand, the detachment rate cannot be slower than ~ 1/30 min – in this case, the syntelic attachments would not have time to disassemble in all experiments. Thus, the detachment rate (conversion of syntelic to monotelic attachments) is of the order of one per 10-20 min. This means that during the initial 1-2 hrs, the numbers of syn- and mono-telic attachments equilibrate. Let $N_1$ and $N_2$ be the numbers of mono- and syntelic attachments, respectively, $k_1$ is the rate of monotelic attachment conversion into a syntelic one, and $k_2$ - the rate of syntelic attachment conversion into a monotelic one (Figure S6). Then, $k_1N_1 = k_2N_2$. From the data at $t = 0$ in Experiment 1, $2N_1 \approx N_2$. 


so \( k_1 = 2k_2 \); if \( k_2 = 0.05/\text{min} \), then \( k_1 = 0.1/\text{min} \). The complete spindle assembly time, about 15 min, scales as the logarithms of the KT number (Mogilner and Craig, 2010), so this time is of the order of \((1/k_0)\log 24 \sim 15\) min, where \( k_0 \) is the rate of the capture of one KT. Thus, we can estimate the rate of the capture of one KT as \( k_0 = \log 24/15 = 0.2/\text{min} \).

All three experiments (Figures S3A, 4C-D) show only increase of merotelic and amphitelic connections over time. To account for this, we assumed that amphitelic and merotelic attachments do not disassemble. Although this is a simplification because correction of merotelic attachments is known to occur (Cimini, 2007), mathematical models that predict the observed results but that also include disassembly of merotelic KT attachments are too complex, and were not considered.

Based on these arguments, we screened the parameters and found that if we assume that a monotelic attachment is established with the rate 0.3/min, syntelic one is established with the rate 0.6/min, destabilization rate of the syntelic attachment is 0.3/min, rate of merotelic attachment formation (from mono- or syntelic one) is 0.03/min, and finally if the rate of amphitelic attachment formation from the monotelic one is 0.15/min, we can semi-quantitatively explain the data of all three experiments. In addition to these rates, we also have to assume that the following fraction of chromosomes is visible from both poles as a function of the pole-to-pole distance: 
\[
\frac{f(r) = r^n / (r^n + s^n)}{n = 4, s = 5 \mu m}. \text{ (Here } r \text{ is the pole-to-pole distance in } \mu m) \text{. Based on this function, each pole can access only half of the chromosomes for a pole-to-pole distance of up to } 2 \mu m; \text{ between } 2 \text{ and } 8 \mu m \text{ pole-to-pole distance, the fraction of chromosomes that both poles}
\]
can reach linearly increases so that at 5 μm half of the chromosomes can be captured from both poles; and above 8 μm, almost all chromosomes are visible from both poles (Figure 5D).

Simulation of spindle assembly kinetics using the model described above explains the data of the beginning of experiment 1, and then it semi-quantitatively predicts that as the poles separate in this experiment and more chromosomes can be reached from both poles, the initially high monotelic and syntelic numbers decrease because stable amphitelic and merotelic attachments emerge. In experiments 2 and 3, this simple model predicts correctly that mono- and syntelic connection numbers increase in the first ~ 10 min, and then decrease. However, quantitative fits of the model predictions to the data are impossible for such a simple model. Specifically, very rapid increase of the amphitelic attachment number at the end of the search, as well as rapid increase of the merotelic attachment number at the beginning of the search are hard to capture numerically. In addition, the attachment rates that are constant in this model have to depend on actual location of the chromosomes in space. In order to reverse-engineer the data of the three experiments, we undertook the spatially explicit simulations described below.

Spatially explicit simulations. We considered the nuclear space to be an oblate spheroid with dimensions 19 μm×19 μm × 5 μm (based on experimentally measured dimensions), with 12 chromosomes (24 kinetochores) inside and 2 centrosomes at the periphery. Chromosomes and KTs were cylindrical objects with dimensions given below. During the search, each centrosome nucleated 100 MTs undergoing dynamic instability with the growth and shortening rates shown below. To account for the assumption that the poles search within a certain zone the width of which depends on the pole-to-pole distance, we introduced a spherical region with radius equal to
2 μm around each centrosome such that MTs from one pole invading this region around the other pole immediately catastrophe. This way, the poles search within certain cones in space such that MTs from opposite poles overlap little in space when the pole-to-pole distance is small, but mix more as the poles separate (Figure 5A). Starting with a given initial separation, the centrosomes in the simulations moved apart with a time-dependent velocity. From the data of Experiments 1-3 (Figures S3A, 4C-D), we extracted the pole-to-pole distances as functions of time and implemented these functions in the simulations. There are no spontaneous catastrophe and rescue events: recues prolong MT growth in ‘wrong’ directions, while frequent catastrophes make it hard to capture properly KTs that are too far from poles (Wollman et al., 2005). Instead, the MTs undergo catastrophe upon collisions with the nuclear space boundary. Unlike in (Paul et al., 2009), we did not make the MTs catastrophe upon a collision with the chromosome arm, because this effect is not very important in the geometry considered here. Instead, the assumption was that MTs “slide off” the chromosome arms and continue to grow in a tangential direction.

In the simulations, syntelic MTs dissociate with the constant rate shown below, mero- and amphitelic connections are stable, and monotelic connections do not disassemble but can convert to syntelic or amphitelic ones. Importantly, we do not have fixed rates of making pole-KT connections. Instead, the connections are formed depending on the poles’ and centrosomes’ positions and MT numbers and growth/shortening rates. We found that the following assumptions had to be made to achieve good fit to the data shown in Figures 4C-E:

- Each KT has 2 binding sites for MTs. The first MT interacting with a KT has a binding probability 1, whereas for the second MT the binding rate is reduced to 0.5 (to account for the significant probability of other MTs nucleated or transported on the first attached MT
and saturating the second binding site). Having only two attachment sites is, of course, an over-simplification, because each KT binds 20-25 MTs (McEwen et al., 1997), so we effectively think of MT bundles rather than individual MTs. Simulations with realistic numbers of the binding sites become too involved.

- Probability to establish the first, monotelic attachment increases linearly from zero to unity over a few minutes. This assumption can account for the gradual increase in the number of MTs involved in the search and increase in MT dynamicity for cells recovering from the drug treatments. Without this assumption, the fits are still good qualitatively, but quantitatively speaking, the assembly becomes too fast.

- We assume that monotelically attached chromosomes rotate, so that the non-captured KT faces away from the capturing pole (Rieder and Alexander, 1990; Nicklas, 1997). Because the MTs do not penetrate chromosome arms, this effect makes syntelic attachments less frequent than monotelic ones. This also reduces the number of merotelic attachments as the distance between the poles increases, so that the pole without a connection to the chromosome ‘sees’ the captured KT from such angle that the merotelic capture of this KT is less likely.

- Monotelically attached chromosomes move away from the poles to which they are connected, towards the spindle equator (Kapoor et al., 2006; Cai et al., 2009). This bias increases with growing pole-to-pole distance and effectively decreases the probability to establish a syntelic attachment but increases the probability to establish an amphitelic attachment with growing pole-to-pole distance.
Effectively, the rotation and movement of the chromosomes create the following effects:

1) the probability to establish a syntelic attachment decreases with the pole-to-pole distance, \( r \), as \( p(r) = (1 - r/r_{\text{max}}) \); 2) the probability to establish a merotelic attachment decreases with the pole-to-pole distance, as \( p(r) = (1 - r/r_{\text{max}})^2 \); 3) the probability to establish an amphitelic attachment increases with the pole-to-pole distance, \( r \), as \( p(r) = (r/r_{\text{max}}) \). Here \( r_{\text{max}} \) is the model parameter, maximal pole-to-pole separation (12-15 \( \mu \)m in the simulations; results are not sensitive to the exact value).

**Parameters used in the simulations.**

Number of chromosomes = 12; Number of MTs from each pole = 100;
KT length = 0.5 \( \mu \)m; KT radius = 0.44 \( \mu \)m;
Chromosome radius = 0.5 \( \mu \)m; Chromosome length = 2 \( \mu \)m;
MT growth rate = 0.18 \( \mu \)m/s; MT shortening rate = 1 \( \mu \)m/s;
Rate of dissociation of a syntelic MT = 0.5/min.

Stochastic Monte Carlo simulations using the algorithm and parameters described here were performed as described in (Paul et al., 2009). The results are reported in Figures 5B-C and Figure S3B.

We also tested a number of modifications of the model:

1) In order to test a possible effect of plastic KT (Loncarek et al., 2007), we introduced varying random angles between the sister KT and simulated spindle assembly with centrosomes starting at a distance of 0 \( \mu \)m. We found that the results did not fit the experimental data. Specifically, there was a dramatic decrease of amphitelic attachments from \(~80\%\) to less than 40\%
and a respective increase in the number of mis-attachments (syn-, mono- and merotelic attachments). The reasons for that were “easy access” of MTs from both poles to unattached KTs, as well as more difficult access from the unconnected pole to the unattached KT of monotelic chromosomes. Note, that in our simulations, the angles between sister KTs varied but were static; it is entirely possible that a dynamic dependence of these angles on forces and/or attachments of MTs could remEDIATE the errors of assembly. Moreover, in these simulations the angles between sister KTs were set at less than 180° before the establishment of any KT-MT interaction, but it is likely that this would not occur in vivo and that smaller angles would only be a consequence of prior establishment of syntelic attachment. However, our simulation results suggest that even in this case sister pairs that have previously established syntelic attachments would be unlikely to establish other types of attachments (mero- or apmhitelic).

2) To test a possible effect of long K-fibers that could form during extensive pre-search (Khodjakov et al., 2003), we repeated the simulations of Experiment 1 with an effective target size (representing increased target length as a sum of KT and K-fiber lengths) equal to 2 μm. The results are shown in Figure S3C; comparison with Figure S3B illustrates that the increase in the effective target size has very little effect. This is in agreement with the results reported in (Paul et al., 2009) according to which the effective target size does not influence the accuracy of spindle assembly significantly. The K-fiber length is important for the speed of assembly on the scale of minutes, but on the scale of tens of minutes, characteristic for Experiment 1, this effect is not prominent.

3) In the simulations, the chromosome positions were not correlated with the centrosome positions, or with each other. We tried to introduce small unevenness in distributions of the chromosomes, and varied slightly restricted volumes within the oblate spheroid to which positions
of the chromosomes were limited and found that these changes did not affect the predicted time series for the spindle assembly. Greater inhomogeneity and/or restriction of the chromosomes to more confined spaces did perturb the results significantly, but there are no clear indications from our experimental data that such effects take place.

4) We simulated the scenario in which the tendency for monotelic chromosomes to move away from the capturing pole was delayed by a few minutes. Within the delay period, a few amphitelic chromosomes appear, so the monotelic chromosomes can move along respective k-fibers. Such delay did not change the results significantly.

5) We allowed MTs to search the whole space. The results (Figure S5A-B) compare poorly to the experimental data. However, when we at the same time allowed amphitelic and merotelic attachments to disassemble as a function of pole-to-pole distance – faster at small distances and slower at greater distances, the model predicted time series that better reproduce, despite residual discrepancies (see main text for details), the experimental data (Figure S5C-D). The rationale for such assumption is the possible effect of tension on the sister KTs exerted by attached MTs. Such tension depends on the angle between KTs connecting respective chromosomes to opposite poles, and therefore on the pole-to-pole distance. Specifically, we scaled the disassembly with probability of MT detachment equal to $P = 0.5 \times (1-r/r_d)$ where $r$ is the pole-to-pole distance, and $r_d = 3.5 \mu m$ is the maximal distance at which this effect takes place.
**Supplemental Figures**

**Figure S1.** Examples of cells with incomplete centrosome separation at NEB. Examples of the three centrosome configurations [Top-bottom (A); side-side (B); top-side (C)] depicted in Figures 1A-Bii-iv in PtK1 cells expressing GFP-γ-tubulin. For each example, images of the cell prior to NEB (Pre-NEB) are shown in the top row and images of the cell upon NEB (Upon-NEB) are shown in the bottom row. Images of chromosomes and centrosomes at a single focal plane are shown in the first and second column, respectively. Maximum intensity projections in XY accompanied by YZ and XZ views are shown in the third column. The far-right column displays the overlay of phase contrast and fluorescence images at a single focal plane upon NEB. Scale bars, 5 μm.
Figure S2. Pole-to-pole distance in PtK1 cells with complete vs. incomplete centrosome separation at NEB. Pole-to-pole measurements were obtained both upon NEB and at the end of prometaphase for all PtK1 cells imaged during the experiments summarized in figures 1 and 2.
Figure S3. The size of the KT target does not affect the numbers and types of KT attachments in cells recovering from a 2-hour STLC treatment. (A) Frequencies of different types of KT attachments in cells washed-out of STLC after a 2-hr treatment. In these cells, the spindle poles gradually separated after persisting in very close proximity for 2 hrs and establishing KT attachment (spindle poles moving apart from 0 μm, with a 2-hr period of pre-search). N represents the number of cells analyzed. The numbers in square brackets represent the average pole-to-pole distance (in μm) at each time-point. (B) Computer simulation of spindle bipolarization with spindle poles starting at a distance of 0 μm after a 2-hr pre-search period (as in the experiment in A). (C) Computer simulation of spindle bipolarization under conditions identical to those used in B, except for the KT target size, which was set to 2 μm, as opposed to 0.5 μm in B.
**Figure S4.** *Frequencies of different types of KT attachments in PtK1 cells with an initial pole-to-pole distance of 2-3 µm.* Cells were first treated with STLC for 2 hrs, then washed out and re-incubated in STLC-free media for 15 min (to allow spindle poles to move apart to an average distance of 2-3 µm), then incubated in NOC for 30 min, and finally washed out of the NOC and fixed at subsequent time-points. N represents the number of cells analyzed. The numbers in square brackets represent the average pole-to-pole distance (in µm) at each time-point.
Figure S5. Computer simulations of spindle assembly without shielding effect can reproduce experimental results only if merotelic and amphitelic KT attachments disassemble at very high rates at small pole-to-pole distances. (A-B) Time-course simulations of spindle assembly in the absence of shielding effect between the two centrosomes (whole-space search). (A) Simulation starting with a spindle pole distance of 0 μm without pre-search (as in figure 4C). (B) Simulation starting with a spindle pole distance of 6 μm without pre-search (as in figure 4D). Whereas the simulation results at the initial distance of 6 μm fit the experimental results, the simulation results for an initial distance of 0 μm are very different from the experimental results. (C-D) Time-course simulations of spindle assembly with whole-space search and rapid disassembly of merotelic and amphitelic attachments at small pole-to-pole distances. (C) Simulation starting with a spindle pole distance of 0 μm without pre-search (as in figure 4C). (D) Simulation starting with a spindle pole distance of 6 μm without pre-search (as in figure 4D). At the initial pole-to-pole distance of 6 μm the simulation results fit well the experimental results, but that is not the case for an initial pole-to-pole distance of 0 μm.
Figure S6. Possible pathways of establishment of KT attachment during simulation of approximate kinetics of spindle assembly. All the KTs are unattached (bottom left) at the beginning of the simulation. Merotelic and correct amphitelic attachments can be achieved through different pathways.
Supplemental References

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