Slide-and-Cluster Models for Spindle Assembly

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Summary

Background: Mitotic and meiotic spindles are assemblies of microtubules (MTs) that form during cell division to physically separate sister chromosomes. How the various components of spindles act together to establish and maintain the dynamic bipolar structure of spindles is not understood. Interactions between MTs and motors have been studied both experimentally and theoretically in many contexts, including the self-organization of arrays of MTs by motors and the competition between different classes of motors to move a single load. This work demonstrates how the interplay between two types of motors together with continual nucleation of MTs by chromosomes could organize the MTs into spindles.

Results: We propose a slide-and-cluster model based on four known molecular activities: MT nucleation near chromosomes, the sliding of MTs by a plus-end-directed motor, the clustering of their minus ends by a minus-end-directed motor, and the loss of MTs by dynamic instability. Our model applies to overlapping, nonkinetochore MTs in anastral spindles, and perhaps also to interpolar MTs in astral spindles. We show mathematically that the slide-and-cluster mechanism robustly forms bipolar spindles with sharp poles and a stable steady-state length. This model accounts for several experimental observations that were difficult to explain with existing models. Three new predictions of the model were tested and verified in Xenopus egg extracts.

Conclusions: We show that a simple two-motor model could create stable, bipolar spindles under a wide range of physical parameters. Our model is the first self-contained model for anastral spindle assembly and MT sliding (known as poleward flux). Our experimental results support the slide-and-cluster scenario; most significantly, we find that MT sliding slows near spindle poles, confirming the model’s primary prediction.

Introduction

A mitotic or meiotic spindle must have a bipolar organization to ensure the faithful separation of chromosomes into two equal groups. How a spindle’s many components interact to form a stable but very dynamical structure, and what sets the size of such spindles, is far from fully understood. It is especially difficult to understand how the spindle length is set in cases in which the spindle is very small compared to the cell, as in eggs and egg extracts. Many conceptual models have been put forward to explain spindle assembly and maintenance, including models dominated by microtubule (MT) nucleation and depolymerization at spindle poles [1–4], models with a static spindle matrix providing structural support [5, 6], models involving MT stabilization by the chromosomes or nucleation by a diffusible molecule from the chromosomes [7], and force-balance models with duelng motors [8–11]. This last idea is a theme which we take up and extend, although the behavior and effects of the motors we consider are qualitatively different from those considered previously.

There have been several attempts to analyze dynamical models of spindles, either through simulation or by mathematical analysis [2, 9, 12, 13]. Because it is hard to gain insight from simulations of a complicated system when many of the components are unknown or there are a large number of parameters, some of the most informative theoretical results have been those that focused on the interactions of several simple components. Such approaches allow for broad statements about the capacities of these components. For example, Nedelec showed [13] that no combinations of “homocomplexes,” motor complexes in which both heads walked the same direction, were capable of robustly connecting two asters to make a bipolar spindle. With such simple modeling approaches, features can be added and components combined, enabling the understanding of the roles of each.

In this spirit, we consider a scenario in which MTs nucleate within the spindle, rather than at its poles, and are subsequently rearranged and organized by the actions of motors. This general type of assembly has been shown to occur in artificial spindles that lack point-like MT-nucleating centers [14]. Spindles that assemble like this are termed anastral spindles. In spindles that do have nucleating centers, such as in cultured human cells, organization through the action of point-like nucleating centers at the poles might occur in parallel to anastral-like organizing mechanisms [15].

Concretely, we consider a “slide-and-cluster” spindle-formation mechanism in which MTs are nucleated near the chromosomes, slide outward and cluster, and are lost stochastically. The clustering creates a gradient of MT sliding velocities; if the gradient reaches zero, a pole forms. As a specific example of this mechanism, we introduce a model in which two types of motors act on the MTs. We show that this can produce a robust bipolar structure with well-defined poles.

Ours is the first spindle model that does not refer to unproven entities like a spindle matrix [5, 6] or an inward spring force [2], and it correctly predicts poleward MT sliding, a fixed steady-state length in an open system.
the poles. Both are true in anastral spindles in *Xenopus* egg extracts).

... to the cell in which they assemble, as seen in eggs (and length is set independently, they can be small compared to MTs in a spindle).

... mechanisms intrinsic to the spindle. Because their density increases, and their minus ends can begin to pile up. If the velocity gradient is steep enough, at a certain point, MTs can overtake those that were nucleated earlier, and a sharp, highly clustered pole is formed by the minus ends. Eventually, the increase in minus-end density at the poles is balanced by the loss due to MT depolymerization, and a steady-state is reached. Note that we consider only interpolar MTs. Kinetochore MTs have different dynamics, but experiments show that they are not required for the assembly of stable, bipolar anastral spindles [14] and usually represent a minority of the MTs in a spindle.

Regardless of the specific details of how MTs are nucleated, lost, moved, or slowed, the slide-and-cluster scenario makes several strong predictions:

First, spindles achieve a steady state in length by mechanisms intrinsic to the spindle. Because their length is set independently, they can be small compared to the cell in which they assemble, as seen in eggs (and egg extracts).

Second, some minus ends will be found throughout the spindle (although the majority can be found at the poles), and nucleation sites must not be exclusively at the poles. Both are true in anastral spindles in *Xenopus* egg extracts [14–16].

Third, MTs at the equator will slide toward the poles with their minus ends leading. Exogenous, stabilized MTs added to *Xenopus* extract spindles do this [14], and all detectable endogenous MTs slide bidirectionally at the equator [17, 18], presumably toward their minus ends. However, critically testing this prediction will require visualizing the dynamics of endogenous minus ends.

Fourth, interpolar MTs will move more slowly near the poles, so the measured rate of MT sliding decreases as a function of distance from the chromosomes (Figure S1 in the Supplemental Data available online). This is in strong contrast to models in which MTs stretch throughout the spindle and flux is produced because of forces localized at the poles [2–4]; such models predict a constant MT velocity throughout the spindle (except possibly in cases in which sliding speed depends on MT length). MTs at the pole will have an average velocity of zero (although they might be observed to jiggle back and forth because of stochastic effects), and any MTs found beyond the pole will have an inward average velocity.

Fifth, because the pole position in the slide-and-cluster scenario is set by the location where the average MT velocity is zero (rather than the position of a nucleating and organizing center), perturbations either to global flux rates or to the positional dependence of MT velocities should affect the pole positions, and thus the spindle length.

Sixth, perturbing the mechanism that produces the MT velocity gradient will also affect the degree to which poles are clustered. Inhibiting the gradient will produce spindles with unfocused or nonexistent poles.

To provide more-detailed predictions of spindle responses to perturbations and other features, we require a more-specific model for how MTs are moved and slowed.

**Two-Motor Model**

We consider a model in which MT motion is controlled by the interactions of two types of simple motors with the MTs. Each is biologically inspired: A “sliding motor” represents the plus-end-directed motor kinesin-5 [19], and a “clustering motor” represents the minus-end-directed dynein-dynactin complex [20] (or perhaps a minus-end-directed kinesin, such as ncd [21]). This model is illustrated conceptually in Figure 2.

When the sliding motor links a pair of antiparallel MTs, it walks toward the plus ends of each, pushing the MTs such that their minus ends slide apart (Figure 2A). This function has been widely hypothesized for kinesin-5 on the basis of its tetrameric structure [11, 21] and is supported by a recent single-molecule study [22]. By contrast, when a sliding motor links a pair of parallel MTs, it simply resists relative motion between the two. The specific effect of each sliding motor depends on the position and velocities of the two MTs it connects, but on average, sliding motors in the spindle contribute an outward component to each MTs velocity (Figure 2B).

A clustering motor moves toward the minus ends of the two MTs that it links. It pauses upon reaching the minus end of one MT while continuing to move along the other. As noted by Nedelec and others [13, 23], a motor with this behavior will slide the parallel MTs such that...
their minus ends move toward each other—i.e., it clusters them (Figure 2C). In a group of parallel MTs, the clustering motors work to pull each MT’s minus end toward the average position of the minus ends in the group. In a spindle, MTs of each orientation have their minus ends spread out predominantly in one half of the spindle, with left-pointing MTs having their minus ends in the right half of the spindle and vice-versa [16]. Minus ends of each orientation thus have an average position somewhere between the chromosomes and the pole toward which they are moving. The clustering motor, by pulling minus ends toward this average position, contributes an outward component to the velocity of the MTs near the chromosomes but an inward component for those near the poles.

At the chromosomes, then, the two motors cooperate to pull MTs outward (Figure 2C). Away from the chromosomes, they compete, and MTs move more slowly. If and where the velocity contributions from the two motor types balance, the MTs stop moving entirely, and their minus ends clump together to form poles (Figures 2B and 2D).

To test whether the intuitive picture in Figures 1 and 2 could indeed give rise to stable bipolar spindles, we explored the behavior of MTs in a highly simplified case. We describe the mathematical basis for this treatment below in the Theory section. These equations can be addressed either numerically, as in the simulations we show, or analytically in certain limits.

Our simplified model spindle is composed of fixed-length MTs that appear at the center of the spindle and disappear in a stochastic fashion with a specified average lifetime. We assume that any MT can interact via motors with any other that it overlaps.

The simulations showed the self-assembly of spindle-like structures (Figures S2 and S3). After an initial transient, the outward motion of MTs from the center is balanced by clustering, MT loss, and new nucleation. A stable steady state then forms with the MTs mostly moving outward, fastest near the center and slowest near the ends of the spindle. In wide parameter ranges, the spindles form at their ends poles that consist of tight clusters of minus ends at a constant distance from the center. Although the average velocity drops sharply to zero at the poles, individual MTs that are there move stochastically back and forth, with those that move past the pole being pulled back by the clustering motors.

A striking feature of the model is that spindles with poles form for a broad range of physical parameters—no fine tuning of any kind is required. But the properties of the spindles do change as the physical parameters are varied.

First, the basic spindle length scale is set by the minus-end lifetime times the average MT speed, which is roughly the zero-load velocity of the sliding motor (except when the sliding-motor concentration is very low).

Second, the extent to which poles form is determined by the relative influence of the two motor classes. When the sliding motors dominate, well-defined poles do not form (Figures 3A and 3B). MT velocity decreases only gradually throughout the spindle (Figure 3C). By contrast, when the clustering motors dominate (by being faster and either stronger or more abundant than the sliding motor), sharp poles form. These tight peaks in minus-end density correspond to sharp decreases in MT density at the poles. As the dominance of the clustering motors increases, larger fractions of the minus ends are found at the poles, eventually approaching 100% (Figure 3B). Comparing spindles with identical MT lifetimes and average sliding velocities but varying motor balances, those with nearly all minus ends at the poles have about half of the pole-pole separation as do those that barely form poles. Spindles without poles do not have well-defined ends; instead, the MT density far from the chromosomes falls off gradually as MTs are lost. MT velocity decreases gradually before sharply dropping to zero at the poles.

Third, the uniformity of MT density in the spindles varies according to the ratio of the MT length to the spindle length scale. When MTs are short, spindles with poles are sparse in the center but have a high MT density near the poles (Figure 3D). When the MT length is of the order of the spindle length, MTs at the poles reach to the center, and the spindles are comparably dense.
throughout. When MTs are longer than the spindle length, they reach out past the opposite pole. Figure 3 illustrates some of the trends, but the quantitative properties of spindles depend on combinations of the parameters of the model, as discussed in the Theory section. For example, because the clustering motors act on MT ends while the sliding motors act along the lengths of MTs, with other things being equal, the dominance of the clustering motor increases as MTs become shorter.

Gradient of MT Sliding Velocity in Xenopus Extract Spindles
Several experiments on meiotic spindles in Xenopus egg extracts were performed so that predictions of the slide-and-cluster scenario could be tested. We used fluorescent speckle microscopy [24] to observe MT movement and crosscorrelation analysis [18] to quantify the average speckle velocity as a function of position along the spindle-pole axis. We found that speckles moved fastest at the centers of the spindles and slowed...
along the spindle-pole axis for eight untreated Xenopus near the spindle poles (Figure 4A), confirming the most important prediction of the model.

In some but not all spindles, negative average velocities were measured near the ends, suggesting the inward motion of some MTs. Because we used a cutoff to include only points with a high enough MT density to ensure an adequate signal-to-noise ratio for velocity measurement, some of the observed variation between spindles is likely due to slight differences in the cutoff location. An improved measurement technique, perhaps single-speckle tracking, will be necessary to determine what fraction of spindles does in fact have negative average velocities near the spindles’ ends.

Another effect needs to be considered. MTs that bend inward near the pole will appear to have slower motion along the spindle-pole axis even if their speed has not decreased. However, many of the MTs will arrive at the pole close to parallel to the spindle-pole axis, and we estimate that these dominate the measurements. For example, if the MTs arriving at the pole form a cone shape, with the most-angled MTs at 45 degrees from the spindle-pole axis, then the measured sliding velocity would decrease by only about 15%—far too little to explain the drop observed.

The shapes of the observed velocity distributions were similar to those from simulations that used parameters in which clustering dominated over sliding (Figure 3C), with an initial shallow velocity gradient that was followed by a steep drop near the poles. Care is needed in comparing the experimental results with the theory. MTs passing through any single point in the spindle will have minus ends spread over a region whose width is approximately the MT length. Speckle velocity measurements at a point capture the average over all these minus-end locations, rather than the (theoretically simpler) mean velocity of the minus ends at a given point. Near the poles, most MTs will have minus ends at the poles, so the average MT velocity will indeed be small. But in the middle of the spindle, the measured MT velocity reflects the minus-end velocities over a range of positions. This effect, even without any stochasticity, gives rise to a broad distribution of velocities of the MTs found at the same distance from the spindle center.

Figure 4. MT Sliding Slows near Poles in Control Spindles but Not p50 Spindles

(A) The average speed of speckles (μm/min) as a function of position along the spindle-pole axis for eight untreated Xenopus spindles. Magenta lines represent velocity of speckles moving to the left and green lines the velocity of speckles moving to the right. The overlay in last panel shows the MT density, in the same spindle, as a function of position for left-moving (solid line) and right-moving (dashed line) MTs, in arbitrary units.

(B) Speckle speed versus position for eight spindles treated with 0.7 mg/ml p50 to produce unfocused poles. Scale bars represent 20 μm.

Inhibition of Kinesin-5 Causes Spindles to Shorten

The slide-and-cluster model predicts that spindle length closely correlates with average sliding speed. To test this, we assembled Xenopus extract spindles in the presence of different concentrations of the kinesin-5 inhibitor monastrol [28], which has been shown to slow the sliding rate in such spindles [18]. At each monastrol concentration, we found some bipolar structures with chromosomes at the metaphase plate. At higher concentrations, we also found a significant fraction of monoastrers, in which the chromosomes were on the outside of the structure; these structures were not included in the length measurements. We found that the pole-to-pole length of the bipolar structures depended significantly on the concentration of monastrol. When spindle length was plotted as a function of the average sliding speed measured previously for each monastrol concentration [18], the relationship was roughly linear (Figure 5). Spindle length was observed to decrease with lower MT sliding speeds, but the intercept of a linear fit is not zero. Although the data are noisy, this might be evidence of a broad nucleation region near the chromosomes that creates a minimum size for spindles even in the absence of outward sliding.

Monastrol is believed to work by inducing kinesin-5 motors to a low-friction state in which they cannot produce force [29, 30]. This can change their behavior in various ways, the simplest being a lowering of the effective motor concentration. We used the simulations to study how lowering the concentration of the sliding motor might affect both the average flux speed and spindle length. We found that the average flux velocity of MTs in the structures was close to the zero-load velocity of the sliding motor, except at very low concentrations of motor, where the average flux velocity dropped,
approaching zero at zero concentration (data not shown). This was due to the increasingly heavy load on each motor when only a few motors are operating. The pole-to-pole length of the structures dropped accordingly, with the simulations showing a roughly linear relationship between the average flux speed and spindle length, similar to that which we observed experimentally. This might not be the actual mechanism by which monastrol slows the flux, but within the slide-and-cluster scenario, other mechanisms would lead to similar correlations between changes in flux speed and spindle length.

Theory

The basic assumptions of the two-motor model are that MTs are nucleated away from the poles, slide under the combined action of sliding and clustering motors, and disappear because of dynamic instability. Several simplifying assumptions are made: (1) MTs are nucleated at a single point and have a fixed length and a set average lifetime; the ratio of the typical total clustering and sliding forces on a MT, $F_{clust}$ to $F_{slide}$, and the ratio of $v_c$ to $v_s$. We denote the number density of the right-moving MT’s minus ends by $\rho(x)dx$ and the (usually positive) velocities of those with minus ends at $x$ by $v(x)$. For oppositely oriented MTs that move to the left, we denote the minus-end distribution $\bar{\rho}(\eta)d\eta$ and the (usually negative) velocity $\bar{v}(\eta)$. The total force on a MT with minus end at $x$ has several contributions: $F_{net}(x) = F_{slide, par}(x) + F_{slide, anti, par}(x) + F_{clust, par}(x)$. If viscous forces are negligible, then $F_{net}(x)$ must be zero. For now, we ignore stochasticity.

Two parallel MTs with ends at $x$ and $y$ a distance less than $L$ apart will overlap by $L - |y - x|$; thus, the total force from sliding-motor interactions with parallel MTs is

$$F_{slide, par}(x) = \int_{x-L}^{x+L} F_s c_s (L - |y - x|) \frac{v(y) - v(x)}{2v_s} \rho(y) dy.$$  

(1)

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<tr>
<th>Table 1. Motor and MT Parameters in the Model</th>
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<td>Motor Parameters</td>
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<td>$F_s$, $F_c$</td>
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<tr>
<td>Stall forces for sliding</td>
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<td>clustering motors</td>
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<td>$v_s$, $v_c$</td>
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<td>Zero-load velocities for single motor</td>
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<td>$c_s$</td>
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<td>Average number of sliding motors per unit</td>
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<td>length of MT</td>
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<td>$n_c$</td>
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<td>Average number of clustering motors</td>
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<td>per minus-end crosslink</td>
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Similarly, anti-parallel MTs with ends at \( x \) and \( \eta \) will overlap if \( 0 < \eta - x < 2L \), leading to a total sliding-motor force of

\[
F_{\text{slide,anti-par}}(x) = \int_{x - 2L}^{x} F_{c}(L - |L + \eta - x|) \times \left( 1 + \frac{v(\eta) - v(x)}{2v_c} \right) \rho(\eta) d\eta. \tag{2}
\]

Finally, the force of clustering-motor interactions between parallel MTs depends on which is further to the right:

\[
F_{\text{clust,par}}(x) = \int_{x - L}^{x + L} F_{c}(x) \left( \text{sign}(y - x) + \frac{v(y) - v(x)}{v_c} \right) \rho(y) dy. \tag{3}
\]

If the MTs are nucleated only at the center of the spindle at rate \( R \), and are lost with an average lifetime \( \tau \), then conservation of MT number yields \( \frac{dN}{dt} = -\frac{N}{\tau} \) [31]. At steady state, \( \frac{dN}{dt} = 0 \), and symmetry implies that \( \rho(\eta) = \rho(-\eta) \) and \( v(\eta) = -v(-\eta) \); the force balance, \( F_{\text{net}}(x) = 0 \), and conservation law then determine \( \rho(x) \) and \( v(x) \). In general, these equations cannot be solved analytically. Even with only three dimensionless parameters, it is not easy to glean useful understanding from exploring the parameter space numerically. But there is an instructive limit in which the behavior simplifies.

If the MT length, \( L \), is much longer than the region over which most of the minus ends are distributed, then most of the parallel MTs overlap each other nearly completely, whereas the overlap between antiparallel MTs is small, of order \( \psi_\tau \). In this limit, the model can be solved explicitly. By introducing the number of minus ends past \( x \), \( N_-(x) \equiv \int_x^L \rho(y) dy \), one finds that in steady state, \( v = -1/(dN/dx) \), and the force balance equation becomes a differential equation for \( N_- \). The steady-state distribution of the minus ends is then entirely determined by a single parameter, \( \psi \equiv \frac{\psi_\tau}{F_{c}/v_c + F_{\text{det}}/v_c} \), the relevant ratio of the effects of the clustering and sliding forces. When \( \psi < 5/5 \), sliding dominates and spindles have no sharp poles. The minus-end distribution has exponential tails, although most are distributed over a region of size about \( \psi \tau \). But the full length of the spindle is longer: It is roughly \( 2L \), twice the length of the MTs. When \( \psi \geq 6/5 \), the clustering motors dominate and the spindles terminate at sharp poles. As \( \psi \) increases, the pole-pole distance decreases, approaching \( 5\tau \), for large \( \phi \), where most of the minus ends are at the poles. Note that \( \psi \) can only be large enough for poles to form if the clustering-motor velocity is substantially larger than the sliding-motor velocity.

This simple limit shows that there are qualitatively different behaviors depending on whether the sliding or the clustering effects dominate. The limit is pathological because it leads to pole-to-pole distances that are much shorter than the MT length and hence than the length of the spindle, but it has been accessed experimentally by inhibiting microtubule catastrophes [25]. Nevertheless, key aspects of the behavior are far more general. This can be seen in the simulations shown in Figure 3, which shows the two types of behavior—with and without a well-defined pole—even when the MT length is comparable to or somewhat shorter than the spindle length. Analysis of the more general model shows that this is expected: A combination of parameters plays a similar role to \( \phi \), whereas the other parameters modify the behavior primarily quantitatively. Sharp poles always form when the clustering motors dominate and contain most of the minus ends when they strongly dominate. Yet the pole-to-pole distance is still set primarily by the sliding motors. When the clustering motors are weak, there is generally a long tail to the distribution of minus ends.

More realistic modifications of the model can be explored, such as an exponential distribution of MT lengths caused by the balance between their growth and shrinkage, as well as the nucleation of MTs over some region of the spindle. These yield similar behavior, although with some differences. For example, if MTs can be nucleated far from the center, then some minus ends will be past the poles and will be pulled inward toward the pole. Stochastic effects can also be added: These have been included in the simulations as discussed in the Experimental Procedures.

Discussion

This work addresses two long-standing questions about meiotic spindles. First, how can MT sliding and MT dynamics be coordinated to produce a structure that is stable over time? Second, how does an anastral spindle assemble; in particular, how do poles form, and what sets their distance from each other? We describe here a general scenario together with a simplified model that addresses both of these questions and makes several new predictions. Spindles form via MT nucleation near the chromosomes then slide outward; slow down, and are lost stochastically. The balance among MT nucleation, loss, and outward motion occurs naturally and determines the spindle length, and well-defined spindle poles are formed.

Our model is qualitatively distinct from proposed mechanisms of spindle maintenance based on centroosomal nucleation of MTs. In those scenarios, the flow of tubulin from the central spindle to the poles is allowed by the exact balance of tubulin added to the MT plus ends in the middle of the spindle and lost from minus ends at the poles, requiring a precise coordination between polymerization, sliding, and depolymerization. Mechanistic proposals for this coordination have been complex and not fully explanatory. In our model, by contrast, tubulin loss occurs primarily at plus ends, which can be anywhere in the spindle. The net outward motion due to MT sliding is balanced simply by the bias toward MT growth created by MT nucleation at the center. No delicate balance between tubulin gain at their plus ends and tubulin loss at their minus ends is needed.

As a concrete illustration, we show that two classes of molecular motors, sliding and clustering, could cause MT sliding and slowdown. Other authors have postulated that antagonism between opposing motors could be important in spindle formation and maintenance. Our work takes this idea a step further: We show that two types of motors can be either antagonistic or cooperative depending on their position within the spindle
and that the result is a robust, self-assembling, bipolar steady state.

Critically, although the relative influence of the two types of motors in our model determines spindle morphology and length, a precise balance is not required. The spindle length is rather insensitive to the exact motor balance because it is set primarily by the minus-end lifetime and the sliding-motor velocity. This might explain why with the partial loss of function, different motors can easily compensate for each other in genetic and biochemical-inhibition experiments [25, 32].

Testing the predictions of the model, we found that MT sliding slows to near zero at the poles by using crosscorrelation velocimetry to reanalyze previously published tubulin speckle image sequences (Figure 4). Slowing near the poles has also been noted in a recent single molecule tubulin imaging study by our group (D. Needleman, personal communication), so it is unlikely to be an artifact of the image-analysis method. This observation confirms the central prediction of the slide-and-cluster model and argues strongly against competing models in which MTs stretch all the way from chromosomes to poles and so must slide at a constant velocity throughout the spindle. The slowing had not been noticed in previous studies, although one can see evidence for it in published kymographs of tubulin speckles (e.g., [33, 18]). We think it was missed because of insufficiently quantitative measurements and because MT density drops sharply near the poles (see last panel of Figure 4). Within the central two-thirds of the spindle, where the MT density is high, the average sliding velocity was approximately constant in many cases, in both experiments and simulations.

Although the observed slowing at the pole is the strongest new evidence for the slide-and-cluster model, by itself it does not prove that this scenario is correct. For example, MTs might slow down at the poles because they run into a static spindle matrix that sets the size of the spindle. Some experiments on extract spindles were interpreted as supporting the existence of a length-governing matrix [25]. The existence of such a matrix is still speculative, and matrix models are problematic in that they do not explain how the length of the matrix itself is specified. The slide-and-cluster model is the first self-contained model for anastral-spindle assembly, and it is based on known molecular activities.

The slide-and-cluster model does not require accumulation of special proteins at the poles. NUMA [34], TPX2 [35, 36], RHAMM [37] and other molecules are known to accumulate at the poles of anastral spindles, where they play a role in promoting minus-end clustering. We suspect these molecules—especially NUMA—cooperate with the clustering motor to focus minus ends and organize discrete poles.

The idea that minus-end-directed motors help move MTs to tightly focus a spindle pole is not new [38, 39, 23, 40], but our model proposes a broader role for these motors: They cluster the minus ends to create the poles by affecting MT motion along the length of the spindle. Key support for the role of motors in the slowing of MTs near the poles was obtained by inhibiting one of the motors. We show that the position-dependent MT slowdown is lost when dynein-dynactin is inhibited by using p50/dynamitin. This supports the idea that, in Xenopus, dynein does indeed act like the clustering motor described in our model. (Ncd might be more important, and perhaps play this role, in other systems.) Our model then predicts that strong dynein inhibition should lead to spindles with very unfocused poles because of a small fraction of minus ends very far from the chromosomes. The distance between the furthest minus ends would increase greatly, and the spindles would thus appear longer, even though the average minus-end distance from the chromosomes would remain roughly constant. This result was observed experimentally [26], although it was interpreted differently, as evidence that dynein carries a minus-end depolymerase to spindle poles. With weaker dynein inhibition, relatively small increases in the distance between furthest ends might be masked by the concurrent reorganization of many minus ends toward the interior of the spindle. This could lead to roughly similar tubulin density profiles in inhibited versus uninhibited spindles, with each having high MT density regions of similar length. This might explain why spindle elongation has not been observed in p50 spindles [39]. (Simultaneous addition of another pole-disrupting factor, Xlp2 tail, does cause elongation [33], perhaps by effectively enhancing the impact of the p50 or stabilizing minus ends.)

In the slide-and-cluster scenario, the speed of the sliding motors (together with the minus-end lifetime) is the primary determinant of the length scale of the spindle. We show experimentally that spindle length varies roughly linearly with the inferred average MT sliding speed when MT sliding is slowed by the kinesin-5 inhibitor monastrol. This is a new result, not predicted by any previously published models. The simplest interpretation is that monastrol lowers the effective motor concentration to the point at which individual motors were acting under significant load and so were unable to walk at full speed. Previous work showed that a several-fold increase in kinesin-5 concentration from normal levels had no effect on spindle length [2]. Both trends are consistent with the predictions of our model: When the sliding-motor concentration is high enough that the motors move at close to their zero-load velocity, the basic spindle length scale is set by that zero-load velocity. However, high concentrations of sliding motors can also change the balance between sliding and clustering (e.g., as parameterized by $\phi$ in the Theory section) and lead to longer spindles, so quantitative interpretation of these data is problematic. A different study showed no change in spindle length in the presence of a mutant kinesin-5 motor that has a slower in vitro MT gliding velocity [41] but did not measure whether MT sliding was slowed within these spindles. Compelling evidence of a decrease in MT sliding rate while spindle length remains constant would speak against our model.

One assumption of this work has been that MT nucleation happens in a narrow region near the center of the spindle, where the chromosomes are. We can ask what would happen if the zone of nucleation were either broadened to include a region around the chromosomes, or split in space to mimic a situation in which the chromosomes are spread to the outside of a collapsed spindle, as observed to occur with Eg5 inhibition. Simulations and further mathematical analysis show that robust bipolarity persists if the nucleation zone is
broadened, and the resulting spindles are still substantially longer than is the nucleation zone because of the outward sliding. For a split nucleation zone with no sliding-motor activity, provided that the nucleation zones are close enough together that MTs can interact between them, the model predicts that the clustering motor will pull MTs inward and collect their minus ends in the center, resulting in a monopolar spindle. Thus, some sliding motion would be visible in such monopolar spindles (not unlike the NuMA-dependent motion of some sliding motion would be visible in such monopolar spindles). Because the movement is mediated entirely by the clustering motor, the MT velocity distribution should differ greatly from that seen in normal bipolar spindles, and the sliding might or might not be recognizable as poleward flux. An understanding of these monopolar structures will require closer examination of the individual microtubule dynamics, including the pattern of nucleation.

Another approximation was that every MT can interact with every other via motors—the spindle is effectively one dimensional with no structure perpendicular to its axis. In reality, the spatial separation between parallel MTs limits the extent of motor crosslinks. As long as the number of motors linking each MT to others is large, the mean-field approximation we have made should be good. Nevertheless, the stochastic effects would be enhanced because of the cumulative effects of fluctuations between MTs that are far apart. Simulations of the simple model, and more general theoretical considerations, indicate that fluctuations will not qualitatively change the behavior unless they become very large.

We have only considered interpolar MTs, which dominate in *Xenopus* extract spindles. Because the model describes MTs that are free to move throughout the spindle, it is not applicable to kinetochore MTs, many of which seem to reach from the kinetochores all the way to the poles. The fluxing of kinetochore fibers thus might require depolymerization at the poles. This question will have to be addressed in order to describe spindles in other systems, in which kinetochore MTs can comprise up to half of the MTs. This manuscript has also not addressed the possible role of centrosomes or centrosome-nucleated MTs in the spindle. One straightforward way to incorporate these would be to assume that centrosomes, like free minus ends, are moved by the minus-end-directed clustering motor to the spindle pole. By themselves, these would have little effect on spindle length. However, centrosomes that are connected via kinetochore fibers (k fibers) to kinetochores would be expected to affect spindle length; a fuller understanding requires a description of how forces on kinetochore fibers or centrosome-nucleated MTs are transmitted from kinetochores to centrosomes and subsequently to the spindle poles. This will entail modeling the nature of the centrosome-MT interface and is beyond the scope of this work.

A crucial feature of the general slide-and-cluster scenario is the role of the MT-minus-end lifetime. Without knowing this, we cannot use the model to predict the length of *Xenopus* extract spindles. The turnover time of tubulin polymer in metaphase spindles is quite short, on the order of 1 min or less [17], and in this time, MTs grow and shrink away because of dynamic instability. But if they are transported from the center of the spindle to the poles, as presupposed here, minus ends must live significantly longer than this: Their average lifetime must be of order the center-to-pole distance divided by the observed flux speed, or about 8 min in extract spindles. An essential future test will be to track minus ends to investigate their lifetime and whether they are capped or stabilized by a structure that allows MTs to regrow several times. Candidates for such a cap could include gamma tubulin [43, 44] or a suggested protein complex containing HURP, TPX2, Kinesin-5/Eg5, and XMAP215 [45]. If the minus-end-directed motor sticks to ends while continuing to walk on another MT, this might well also involve the same minus-end complex.

If minus ends are in fact short lived, then a framework similar to slide-and cluster could still hold, e.g., with a broader nucleation zone as described above. In that scenario, however, the determination of spindle length would be dominated by the size of the nucleation zone, rather than by the motor properties and MT lifetime.

We have shown that a simple two-motor model could create stable, bipolar spindles under a wide range of physical parameters. More work will be needed to test how well—if at all—this model or the general scenario which it exemplifies describes real spindles. The clustering behavior postulated here for dynein-dynactin should be tested experimentally, perhaps in an in vitro system. New experimental techniques will be needed to measure the distribution of MT nucleation within the spindle and to track minus ends to see whether they are indeed transported from spindle centers to poles while MTs grow and shrink several times. The ability to follow the dynamics of individual MTs will greatly aid distinguishing between different scenarios.

**Experimental Procedures**

**Spindle-Length Experiments**

Spindle assembly in *Xenopus* extracts with DNA replication (cycled spindles), fixation, and imaging were performed with the general methods described in [46]. Dilutions of monastrol (Sigma-Aldrich) from a 0.2 M solution in dimethyl sulfoxide (DMSO) were added at the time of the initiation of spindle assembly with CSF extract, to final concentrations of 0, 40, 60, 80, 100, 140, and 200 μM. Rhodamine-tubulin as a spindle marker and anti-NUMA IgG labeled with Alexa488 as a pole marker were added as described in [25]. Spindle assembly was allowed to proceed for 90 min, and aliquots were squash fixed. Fluorescent images were captured and analyzed with MATLAB software (Mathworks). Images were thresholded and the location, orientation, and lengths of the spindles determined with the regionprops command and then confirmed visually. At the highest monastrol concentrations, the poles were poorly defined, making the pole-pole length measurements somewhat uncertain. Multipolar spindles and those whose chromosomes were separated into several locations were excluded. The same experiment was performed without fluorescent probes with live spindle imaging by polarization microscopy (methods in [23]), and gave similar results (data not shown).

**Velocity-Distribution Experiments**

Sliding velocity distributions in spindles were collected by the reanalysis of the tubulin speckle-microscopy image sequences described in [18]. The spindles analyzed were the control spindles with no monastrol, with or without p50. To measure the average sliding velocities as a function of position in the spindle, we used the crosscorrelation approach described in the paper but modified it in the following ways: At each position along the spindle pole axis, as described in [16], we measured peaks in the crosscorrelation corresponding to speckle flow toward each pole and then calculated
the average flow velocity in the two directions by finding the positions of the two peaks. To account for possible spindle drift, we selected a subset of positions along the axis where both flow peaks were large and well-defined. We then subtracted a constant value from all velocities such that the leftward velocities and rightward velocities within the selected subset had identical average magnitudes. Where MT densities were very low, noise prohibited an accurate measurement of sliding velocity. We therefore included only leftward velocities for points where the leftward MT density was at least 25% of the median MT density and did similarly for rightward velocities.

We analyzed only spindles that were normal in size and shape (including well-focused poles in the control group), where visual inspection of the crosscorrelation confirmed that the computer had fit the correct peaks at each position along the spindle-pole axis, and where the calculated MT distributions were normal (i.e., similar to those in [16]). This selection produced eight spindles each in the control and p50 groups.

Simulations

Simulations model the motors attaching and falling off MTs at a rate \( k_{\text{on}} \), for both motor types. The stochastic parameters are the mean number of motors “kicks” per MT per lifetime \( \eta \), \( k_{\text{on}} \), and \( c_L \), \( k_{\text{off}} \), and for the stochastic MT nucleation and loss, the average number of MTs, \( N_{\text{MT}} = R \). When these parameters are all large fluctuations are small and the behavior is well approximated by deterministic dynamics using average rates.

In Figures S1 and S2, \( N_{\text{MT}} \) was 300. For the spindles in Figures S1 and S2, \( c_L = 5 \) and \( \eta = 10 \). For the spindles in Figure 3B, \( \eta = 10 \) and \( c_L = 0.5 \) for the short MTs, 5 for the medium MTs and 20 for the long ones. In each case, the on-off rate \( k_{\text{on}} \) was 20 per average minus-end lifetime (implemented by discretizing time in units of \( \frac{1}{k_{\text{on}}} \)).

Supplemental Data

Two figures are available at http://www.current-biology.com/cgi/content/full/17/16/1373/DC1/.

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