ELSEVIER

Modeling of chromosome motility during mitosis

Melissa K Gardner and David J Odde

Chromosome motility is a highly regulated and complex process that ultimately achieves proper segregation of the replicated genome. Recent modeling studies provide a computational framework for investigating how microtubule assembly dynamics, motor protein activity and mitotic spindle mechanical properties are integrated to drive chromosome motility. Among other things, these studies show that metaphase chromosome oscillations can be explained by a range of assumptions, and that non-oscillatory states can be achieved with modest changes to the model parameters. In addition, recent microscopy studies provide new insight into the nature of the coupling between force on the kinetochore and kinetochore–microtubule assembly/disassembly. Together, these studies facilitate advancement toward a unified model that quantitatively predicts chromosome motility.

Addresses
Department of Biomedical Engineering, University of Minnesota, 7-132 Hasselmo Hall, 312 Church Street S.E., Minneapolis, Minnesota 55455, USA

Corresponding author: Odde, David J (oddex002@umn.edu)

Current Opinion in Cell Biology 2006, **18**:639–647

This review comes from a themed issue on
Cell division, growth and death
Edited by Bill Earnshaw and Yuri Lazebnik

0955-0674/$ – see front matter

DOI 10.1016/j.ceb.2006.10.006

Introduction

During mitosis, dynamic microtubules mediate the proper segregation of the replicated genome into each of two daughter cells. These dynamic microtubules are essential components of the mitotic spindle, which is additionally composed of spindle poles, kinetochores and the replicated chromosomes themselves (reviewed in [1–3]).

Microtubules have an inherent polarity, and are generally attached at their minus-end to the poles of the mitotic spindle [4,5]. Microtubule plus-ends are often associated with a kinetochore, a complex protein-based structure that serves as the essential mechanical linkage between dynamic microtubules and chromatin (reviewed in [6,7]). The structure and molecular composition of the kinetochore is now emerging, with the finding that multiple distinct protein complexes cooperate to achieve and maintain chromosome–microtubule coupling and regulate kinetochore-attached microtubule (kMT) plus-end assembly (reviewed in [7,8]).

Microtubules are most dynamic at their plus-ends, with extended periods of polymerization (growth) and depolymerization (shortening) [9,10]. Changes between these two states are stochastic, characterized by 'catastrophe' events (a switch from the growing to the shortening state) and 'rescue' events (a switch from shortening to growth), a process termed dynamic instability [11]. During metaphase, the dynamic instability of kMTs can contribute to the oscillations of chromosomes, a behavior called 'directional instability' [12]. Although microtubule plus-ends are dynamic in a wide range of mitotic spindles, the magnitude of observed chromosome oscillations due to directional instability varies between organisms [13–18] (reviewed in [19]). In general, the dynamic instability behavior of microtubules is thought to be responsible for kinetochore attachment during prophase and prometaphase and for the alignment of kinetochores during metaphase, such that sister chromatids are ultimately segregated into each of two nascent daughter cells during anaphase (reviewed in [20–22]). The congression of sister chromatids to a metaphase plate midway between the two spindle poles is a characteristic hallmark of metaphase, after which correction of segregation errors is relatively limited [23–25]. Therefore, prometaphase, metaphase and anaphase represent key phases in the accurate segregation of chromosomes.

The inherent complexity of the mitotic process, or even a single phase of mitosis such as metaphase, has made it challenging to infer the underlying mechanisms of chromosome motility directly from experimental observation. To manage this complexity, mathematical and computational models have recently been developed to integrate experimental results and provide a physical framework for further investigation of mitotic chromosome motility, in particular its control at the kinetochore. Here we review current theoretical models for kinetochore motility during mitosis. Key common elements of these theoretical models for kinetochore motility include the critical role of kMT dynamic instability, and the importance of forces exerted at the kinetochore in either directly or indirectly regulating kMT dynamic instability. Other elements of spindle dynamics are considered in some of these models, depending on the specific model organism. These elements include the following: microtubule poleward flux (experimental characterization in [14,26–28]); force generation at the kinetochore via microtubule-associated molecular motors (reviewed in [29]); microtubule

depolymerases at the kinetochore (experimental characterization in [30–33]); polar ejection forces (experimental characterization in [16,34,35]); the mechanical properties of the kinetochore; spatial gradients in the parameters of kMT dynamic instability (experimental characterization in [36–39]); and kMT attachment to and detachment from kinetochores (i.e. turnover). Models for each specific organism highlight common features as well as specific differences between particular organisms.

Given the multiple phenomena that mediate mitosis, it is not always obvious how they behave as an integrated system. To manage the inherent complexity and to predict emergent properties, a number of recent studies have used computational modeling. Similar to experimental work, modeling of chromosome motility is now beginning to develop a set of common methods, tools, terminologies and standards. In the work reviewed here, the modeled 'system' has been defined as the mitotic spindle, with special attention paid to the kinetochore–microtubule interface that largely governs chromosome motility during mitosis (other aspects of mitosis modeling are reviewed in [40,41]). In each simulation, the characteristics of the spindle components are defined on the basis of experimental observations (e.g. plus-end directed motors will tend to pull kinetochores in the direction of the kMT plus-end). Beyond the general behaviors of model components, simulation requires quantification (e.g. how quickly and/or with how much force plus-end directed motors pull kinetochores towards the kMT plus-end) and these quantities are defined as model parameters (e.g. stall forces and unloaded velocities), with associated parameter values. Finally, in order to account for cell-to-cell variation as well as for variation in behavior within a single cell, the models reviewed here incorporated stochastic ranges of behavior. These 'Monte Carlo' simulations are accomplished through the use of computer-generated random numbers and calculated probabilities (see Box 1). In addition to these modeling studies, recent kMT structural data obtained using electron microscopy and *in vitro* force measurements obtained from single depolymerizing microtubules impinge on the model assumptions and provide key tests of model predictions.

Box 1 Stochastic simulation via the 'Monte Carlo' method

The 'Monte Carlo' method is useful for simulating both the mean and standard deviation of an observed experimental observation, thus adding experimentally observed 'noise' to otherwise deterministic computer simulations. For example, simulation of microtubule dynamic instability requires that the microtubule plus-end undergoes both growth and shortening excursions, with abrupt and approximately random switching between these two states. The frequency of switching from growth to shortening is characterized by the catastrophe frequency (kc, units s^{-1}), while the frequency of switching from shortening to growing is characterized by the rescue frequency (kr, units s^{-1}). Therefore, in simulation, the probability of a rescue event for a shortening microtubule is calculated via the expression

$$\mathrm{pr(rescue)} = 1 - e^{-(kr)(\tau)}$$

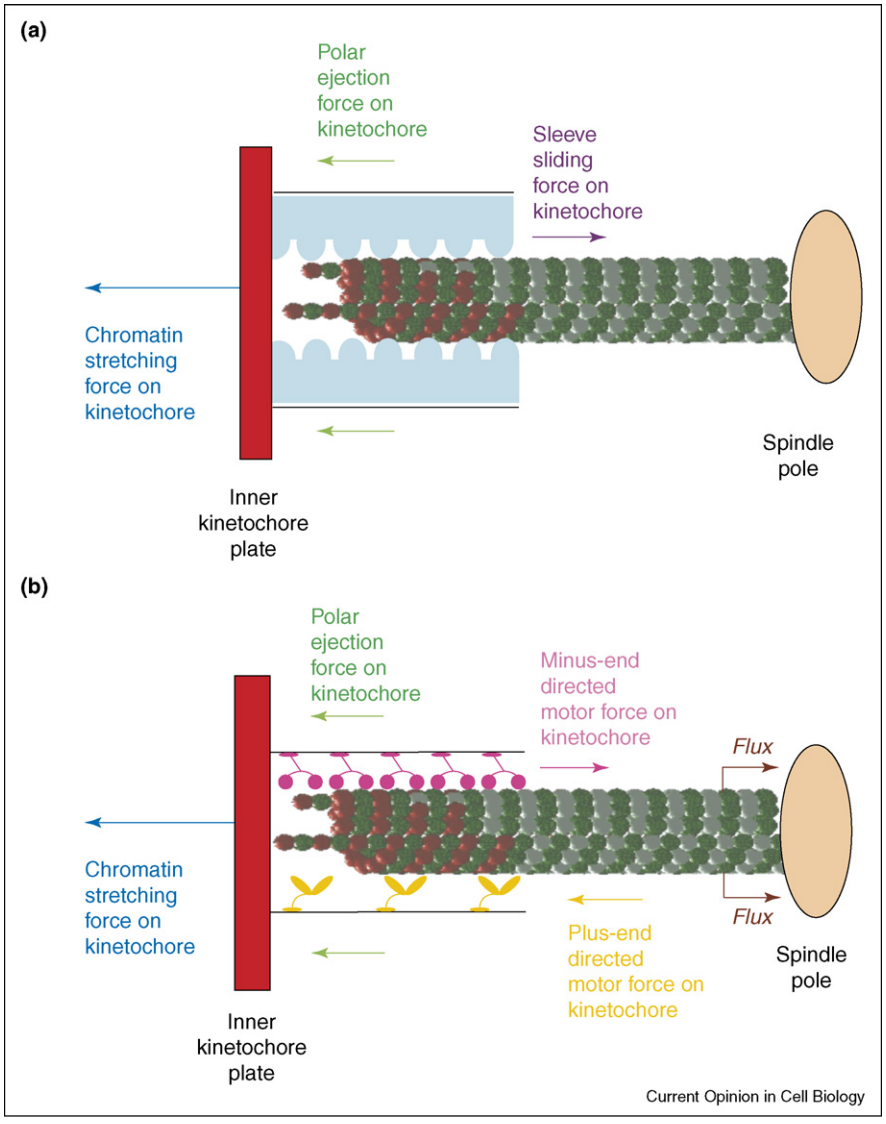
where τ is the simulated time step in seconds. This establishes the probability of a rescue event occurring for a shortening microtubule at any given time step, based on the rescue frequency. Now that a probability is calculated, a computer-generated random number between 0 and 1 can be compared to the probability of the rescue event at every time step. If the random number is less than the probability, the rescue event will occur. For example, if the probability is calculated to be 0.06 and the random number is 0.008, a rescue event would occur during the simulated time step, and the microtubule would now begin to grow. The same method is used to calculate the probability of catastrophe and thus simulate random catastrophe events for growing microtubules. In general, the occurrence of any random event can be simulated with this approach. Further details are given in Gillespie (1977) [93].

A kinetochore motility model for multiple microtubule attachment: PtK1 cells

In 1985, Terrell Hill proposed a model to describe microtubule–kinetochore interactions, providing a starting point for the current modeling efforts [42]. Specifically, this model describes the interaction between a depolymerizing microtubule and a set of binding sites on the kinetochore. Here, the kinetochore is described as a 'sleeve' that surrounds the microtubule plus-end (Figure 1a). Thermal fluctuations allow sliding of the kinetochore sleeve relative to the kMT. Deeper insertion of the kMT plus-end into the sleeve increases the number of favorable wall contacts that the microtubule makes with the kinetochore, and so reduces the free energy of the kinetochore–microtubule interaction (i.e. deeper insertion is energetically favorable; Figure 1a, violet arrow). Conversely, removal of the kMT plus-end from the kinetochore sleeve is energetically unfavorable and hence requires an energy input, which is provided by tension between the kinetochore and the kMT (Figure 1a, blue arrow). Since energy is force multiplied by distance, the force required to detach the kinetochore from the kMT is equal to the energy of wall binding per unit length of microtubule inserted into the sleeve. Hill estimated the detachment force to be ~16 pN (= ~65 sites × 10 pN-nm/site/40 nm) for meiotic grasshopper spermatocytes. Note that in this model the kinetochore remains attached indefinitely to a kMT when the tension is below the detachment force, but that above this force the kMT loses attachment rapidly (within ~1 s or less). This model provides a theoretical basis for how a ~40 nm kinetochore outer plate could in principle be sufficient to provide persistent kMT–kinetochore attachments over a wide range of tension values even while the kMT polymerizes and depolymerizes.

More recently, Joglekar and Hunt developed a detailed mechanistic model to describe kinetochore motility during mitosis, based on experimental data collected in potoroo kidney epithelial (PtK1) cells [43••]. This model is based theoretically on the Hill sleeve model, but is

Figure 1



Models for kinetochore motility in organisms with multiple kMT plus-end attachments. **(a)** In a 'Hill-sleeve' model developed by Joglekar & Hunt (2002), a kMT sleeve provides passive resistance to kMT detachment from the kinetochore (violet), while polar ejection forces (green arrows) and stretching of chromatin between sister kinetochores (blue arrow) tend to pull kMTs out of the kinetochore (kMTs shown in green [GDP- tubulin subunits] and red [GTP-tubulin subunits]). The red rectangle represents an inner kinetochore plate that presents a natural barrier limiting kMT growth. **(b)** A force balance model for chromosome motility as developed by Civelekoglu-Scholey and co-workers (2006). Here, plus-end-directed motor forces (orange arrow), stretching of chromatin between sister kinetochores (blue arrow) and polar ejection forces (green arrows) act to pull kMT plus-ends away from the kinetochore, while minus-end-directed motors tethered to the kinetochore (magenta arrow) act to fully insert kMT tips into the kinetochore. Poleward flux of microtubules (brown arrows) is considered to be a 'reeling-in' force in the model, i.e. a depolymerization-driven force at the spindle pole that antagonizes the action of the minus-end-directed motors at the kinetochore. A microtubule depolymerase acts to destabilize the kMT tip as it enters the kinetochore, and the red rectangle represents an inner kinetochore plate that functions as a barrier to limit kMT growth.

more comprehensive, meaning that it can account not only for the attachment of kMT plus-ends to kinetochores, but also for the observed kinetochore oscillations that both mono-oriented kinetochores (where the kinetochore is attached to one pole and the sister kinetochore has no kMTs) and bi-oriented kinetochores (where sister kinetochores are attached to opposite poles) exhibit in PtK1 mitotic spindles.

As in Figure 1a, the Joglekar and Hunt model relies not only on physical interactions between the kinetochore sleeve and the inserted kMT, but also on polar ejection

forces. Polar ejection forces are thought to push chromosomes away from the poles, possibly through kinesin motors associated with chromatin [44–48]. Thus, directional instability in this model results from two antagonistic forces: first, polar ejection forces acting on the arms of the chromosomes, directed towards the center of the spindle (Figure 1a, green arrow), and second, towards-the-pole forces generated by kMT depolymerization and the subsequent tendency of the Hill-sleeve to minimize free energy by fully inserting kMT tips into the sleeve (Figure 1a, violet arrow). Note that the stretching of chromatin between sister kinetochores is not essential for directional instability because mono-oriented kinetochores also exhibit kinetochore oscillations (Figure 1a, blue arrow).

This force balance results in kinetochore oscillations, as consistent kinetochore motion towards the pole relies on maintaining a pool of depolymerizing kMTs at the leading kinetochore. As kMTs continue to depolymerize, the polar ejection force opposes the leading kinetochore movement and thereby promotes the detachment of depolymerizing kMTs from the leading kinetochore. The closer the kinetochore moves towards the pole, the more strongly the polar ejection force opposes its advancement, and eventually the last depolymerizing kMT detaches from the kinetochore as the load exceeds the detachment force. At this point, even a single depolymerizing kMT on the sister kinetochore can establish movement in the opposite direction. This leading kMT at the sister kinetochore is soon joined by other kMTs as they undergo catastrophe, so that the whole complex moves towards the opposite half-spindle. As long as new, growing kMTs are recruited into the vacated sleeves, this model allows directional instability to occur indefinitely.

The Joglekar and Hunt model provided an early example of how a computer can be used to integrate many components of the mitotic spindle to interpret previously published experimental results. Here, the action of many kMTs and two sister kinetochores connected by intervening chromatin (modeled as a spring) were integrated into a comprehensive simulation with relatively simple rules and assumptions. Testing of parameter value ranges can thus be used to predict the sensitivity and importance of different model assumptions and behaviors. For example, force generated at the kinetochore in the model depends strongly on the number of depolymerizing kMTs, rather than on the total number of attached microtubules. This could lead to the predictions that there should be a mixture of polymerizing and depolymerizing kMTs attached to the kinetochore, and that the ratio of polymerizing to depolymerizing kMTs vary as a function of the position of the kinetochore in the spindle. This type of prediction could then be compared to recent EM experiments of the type described below, or more quantitative analysis of the extent of sister kinetochore coordination as measured via light microscopy.

A kinetochore motility model for multiple microtubule attachments: *Drosophila* embryos

Molecular motors are an integral part of the mitotic spindle, and chromosome attachment to depolymerizing kMT plus-ends may be mediated at least in part by the coupling activity of kinetochore-associated microtubule-based motors [49–51] (reviewed by [20]). An attachment mechanism mediated by molecular motors serves as an alternative to the passive 'Hill-sleeve' model.

In a recent model for mitosis in *Drosophila* embryos developed by Civelekoglu-Scholey and co-workers [52••], kMT dynamics are coupled to the action of mitotic motors to regulate plus-end attachment and chromosome motility. In particular, the passive attachment of the Hill sleeve is replaced with attachment via active molecular motors. Like the Joglekar and Hunt model, this model relies on a force balance at the kinetochore to determine the state of tension on each individual microtubule–kinetochore attachment. As shown in Figure 1b, many other elements of the Joglekar and Hunt force balance are included here as well, in particular the polar ejection force acting on the arms of the chromosomes (Figure 1b, green arrows) and the force of chromosome elastic stretching between sister chromosomes (Figure 1b, blue arrows).

However, a number of additional forces are included as well, all of which are ascribed to the activities of molecular motors. First, two forces that act to pull kMT tips away from kinetochores, thus destabilizing their attachments, are included: poleward flux of microtubules, which is considered to be a 'reeling in force' (in other words a depolymerization-driven force at the pole), and which is mediated by KLP10A (Figure 1b, brown arrows); and plus-end-directed motor activity at the kinetochore, which is mediated by CenpE (Figure 1b, orange arrow). Second, forces that act to insert kMTs more fully into the kinetochore, thus stabilizing their attachments, are included in the model. These forces are derived from minus-end-directed motors (dynein) at the kinetochore (Figure 1b; note that a minus-end-directed motor tethered in the kinetochore would pull the microtubule into the kinetochore as the motor walks toward the spindle pole [magenta arrow]). The third addition to the model is a microtubule depolymerase at the kinetochore (KLP59C), which acts to destabilize kMT tips by suppressing rescue frequency when microtubule plus-ends enter the kinetochore.

Importantly, kMT plus-end rescue is promoted by tension on an individual kMT attachment, which was not assumed by Joglekar and Hunt, but was assumed previously for budding yeast (see discussion below)

[24,53,54]. Consequently, tension will tend to prevent a kMT tip from depolymerizing out of the kinetochore, and so will make kMT plus-end residence in the kinetochore longer-lived. This effect is antagonized by the microtubule depolymerase at the kinetochore, as described above.

A key prediction of this model is that the polymerization state of bound kMT plus-ends should be at least partially synchronized, such that poleward kinetochore motion requires that the majority of the kMTs at the leading kinetochore be depolymerizing. Similar to the Joglekar and Hunt model, dynamic kMT attachments (i.e. unstable attachments leading to frequent kMT detachment) form the basis for kinetochore oscillation in this model. In general, if microtubule–kinetochore attachments are unstable, the kinetochore will oscillate, still centered near the spindle equator by the polar ejection force. In contrast, if most of the microtubule–kinetochore attachments are stable, meaning that most of the kinetochore binding sites are occupied by kMT tips, the kinetochore will not oscillate.

For example, Civelekoglu-Scholey *et al.* show that an increased dynein level (increased kMT minus-end-directed motor force) has the consequence of pulling kMTs farther into the kinetochore, which initially promotes kMT disassembly. This effect increases the distance between sister kinetochores and thus increases tension on individual kMT attachments. Because increased tension stabilizes attachments by promoting kMT rescue, an increased dynein level ultimately ensures that nearly all of the kinetochore-associated kMT binding sites are occupied. In this case, chromosome oscillations are suppressed as a result of the limited variability in kMT attachments between sister kinetochores (i.e. the forces are balanced). This effect is similar to the motion of merotelically oriented kinetochores as described by Cimini *et al.* (2004), where an imbalance in the number of kMT attachments from opposite spindle poles results in directed motion of a merotelic kinetochore away from the spindle equator and toward the pole that has the larger number of microtubules attached to the kinetochore, which is usually the 'correct' pole [55••]. Cimini *et al.* argued that these merotelic kinetochore motions could be explained if it was assumed that the assembly of a kMT is promoted by tension, which may explain how these merotelic errors are corrected in anaphase.

Thus, it appears that variation in the number of attachments over time is critical to the generation of kinetochore oscillations. The absolute number of attachment sites in the model does not seem to be as important for kinetochore oscillations as modulation of attachment stability via motors and the regulation of rescue frequency with tension. Overall, the model as developed by Civelekoglu-Scholey and co-workers represents a significant advance in scope, as it takes all known force generators in the mitotic spindle into account. Application of this model to experimental results in different organisms will be helpful in dissecting differences in the underlying molecular mechanisms.

A kinetochore motility model for single microtubule attachment: budding yeast

In contrast to organisms with multiple kMT plus-end attachments at the kinetochore, we recently developed a model for kinetochore motility in budding yeast metaphase [56••,57]. Because budding yeast represents a relatively simple system — there is a single kinetochore–kMT attachment [58,59] and little or no detachment of plus-ends from kinetochores during metaphase [60] — the rules governing kMT plus-end dynamics can be deduced directly from observations of kinetochore localization and dynamics [61•].

In previous models of kinetochore motility in organisms with multiple kMT attachments per kinetochore, kMT plus-ends were allowed to polymerize through the outer plate of the kinetochore, but then stop growing as the plus-end impinges on the inner kinetochore plate. In budding yeast metaphase simulations, the kinetochore was assumed to track with the kMT plus-end, and thus there was not a natural barrier to prevent kMT plus-ends from growing indefinitely (other than the opposite pole). Interestingly, though, metaphase in budding yeast is characterized by a bi-lobed configuration, such that kinetochores are clustered away from the spindle equator area into two distinct zones in each half-spindle, about midway between the pole and the equator [18,62–64]. Note that in this configuration the chromosomes themselves are centered in the spindle at the equator, and so can be considered to have undergone metaphase congression. This effect can be modeled in budding yeast by a spatial gradient in kMT catastrophe frequency, such that catastrophe frequency is high near the equator, and relatively low near the pole. An appealing proposition is that a class of kinetochore-associated depolymerases acting to limit the growth of kMT plus-ends into the kinetochore inner plate in organisms with multiple kMT plus-end attachments could be spatially regulated in yeast, acting to prevent plus-end polymerization into the spindle equator area.

In future work, it will be important to model the role of molecular motors in budding yeast kinetochore motility more explicitly [65].

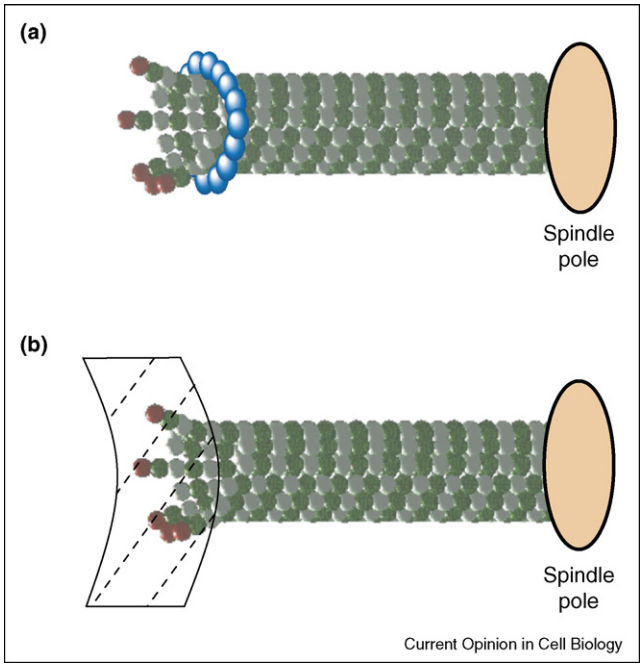
Towards unified cellular and molecular level models: recent studies on force-assembly coupling *in vitro* and *in vivo*

Although modeling efforts combined with experimental evidence have suggested that tension promotes net kMT assembly, an underlying molecular mechanism for this

hypothesis is not yet clear. For this purpose, current cellular-level modeling of mitosis will need to evolve to incorporate *in vitro* experiments, electron microscopy data, and recent advances in molecular-level modeling of microtubule assembly.

For example, significant strides have recently been made in both modeling and measurement of force production by depolymerizing microtubules [49,66••,67•,68–72,73•]. These studies show that even a single depolymerizing microtubule can generate significant forces, and that a simple kinetochore coupler could slow microtubule disassembly. In addition, the discovery that the budding yeast Dam1 kinetochore protein complex forms a ring around microtubules *in vitro* opens up the possibility that there could be a kinetochore-associated coupler in budding yeast that acts to oppose outward bending of GDP-tubulin protofilaments and thus promote kMT rescue (Figure 2a) [74–77]. Further work has now revealed that the Dam1 kinetochore ring complex can move processively at the ends of both polymerizing and depolymerizing microtubules *in vitro*, supporting up to 3 pN of applied tension [78,79••].

Figure 2



Molecular-level models for tension-dependent regulation of kMT dynamics. **(a)** The Dam1 protein complex has been observed to form a ring around microtubules *in vitro* (blue, Dam1 protein complex in ring configuration; kMT in green [GDP- tubulin subunits] and red [GTP-tubulin subunits]). In budding yeast, the Dam1 complex could act as a kinetochore-associated coupler that acts to oppose outward bending of GDP-tubulin protofilaments and thus promote kMT rescue. **(b)** As proposed by Vandenbeldt and coworkers [85••], high tension in the outer kinetochore plate could promote rescue of depolymerizing kMTs in organisms with multiple kMTs attached per kinetochore. Here, the outer kinetochore plaque (black) acts to oppose outward bending of GDP-tubulin protofilaments and thus promote kMT rescue (kMT, green, GDP-tubulin subunits; red, GTP-tubulin subunits).

The hypothesis that tension on a mechanical coupler promotes net assembly, or at least suppresses disassembly, is supported by the recent work of Westermann *et al* [79••]. They found that as microtubules depolymerize *in vitro* in the presence of Dam1 complex rings, the rings slide along with the disassembling microtubule tips and accumulate, which slows the microtubule disassembly rate. In this experiment, it may be presumed that the larger the number of rings at the tip, the larger the frictional force associated with their movement. As the rings accumulate they exert an increasing force that resists depolymerization, which is consistent with the observed reduction in shortening rate with increased Dam1 complex accumulation and with the tension-dependent effects on kMT assembly assumed in the *Drosophila* embryo and budding yeast models.

With the inclusion of the molecular-level detail regarding yeast kinetochore structure that is emerging from light and electron microscopy studies [80–83], development of a molecular-level model for kMT dynamics [73•,84•] would ideally be incorporated into cellular-level modeling efforts in order to gain a more complete picture of kinetochore motility during mitosis.

Recent work highlights how quantitative electron microscopy data could be used to inform modeling, especially in cases where there are multiple kMT attachments per kinetochore [85••,86••]. Here, examination of kMT plus-end configurations in PtK1, *Drosophila* S2 and *C. elegans* cells indicates that kMT plus-end dynamics are not tightly coordinated. The mixed polymerization state of kMT plus-ends was predicted by the model of Joglekar and Hunt, and is also consistent with a model where high tension in the outer kinetochore plate promotes rescue of depolymerizing kMTs [85••] (Figure 2b). These discoveries further highlight the importance of integrating quantitative experimental work and molecular level modeling with the current cellular-level modeling approaches.

Conclusions

As kinetochore structure is dissected and new experimental techniques are developed to better understand complex systems such as mitosis, modeling is likely to serve as a useful tool with which to formalize and test specific hypotheses. In addition, close integration between experiment and theory should facilitate the development of novel experiments and correspondingly more quantitative experimental analysis (see [61•]).

Modeling of complex cellular processes is in its early stages, and it is expected that simulations will be continuously updated and improved as new experimental results become available. Interesting possible areas for

development of kinetochore motility models during mitosis include: the integration of spindle pole separation and maintenance models with current models for kMT and kinetochore dynamics [87,88]; chromatin mechanics and how chromatin may interface with the kinetochore [89,90]; and the coupling of kinetochore congression models with checkpoint data to better understand the molecular basis of error correction and aneuploidy [91]. In addition, revised or newly developed models should also account for the effects of both microtubule stabilizing/destabilizing drugs and the molecular perturbation of microtubule-associated proteins on kinetochore motility during mitosis.

Acknowledgements

We thank Drs. Chad Pearson, Alan Hunt, Ajit Joglekar, Gul Civelekoglu-Scholey, and Jon Scholey for helpful comments on the manuscript. MKG is supported by National Institutes of Health NRSA Pre-doctoral Fellowship EB005568 and DJO is supported by National Institutes of Health grant GM071522.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Karsenti E, Vernos I: **The mitotic spindle: a self-made machine**. *Science* 2001, **294**:543-547.
2. Scholey JM, Brust-Mascher I, Mogilner A: **Cell division**. *Nature* 2003, **422**:746-752.
3. Compton DA: **Spindle assembly in animal cells**. *Annu Rev Biochem* 2000, **69**:95-114.
4. McDonald KL, O'Toole ET, Mastronarde DN, McIntosh JR: **Kinetochore microtubules in PTK cells**. *J Cell Biol* 1992, **118**:369-383.
5. Gordon MB, Howard L, Compton DA: **Chromosome movement in mitosis requires microtubule anchorage at spindle poles**. *J Cell Biol* 2001, **152**:425-434.
6. McIntosh JR, Grishchuk EL, West RR: **Chromosome–microtubule interactions during mitosis**. *Annu Rev Cell Dev Biol* 2002, **18**:193-219.
7. Maiato H, DeLuca J, Salmon ED, Earnshaw WC: **The dynamic kinetochore-microtubule interface**. *J Cell Sci* 2004, **117**:5461-5477.
8. Fukagawa T: **Assembly of kinetochores in vertebrate cells**. *Exp Cell Res* 2004, **296**:21-27.
9. Howard J, Hyman AA: **Dynamics and mechanics of the microtubule plus end**. *Nature* 2003, **422**:753-758.
10. Desai A, Mitchison TJ: **Microtubule polymerization dynamics**. *Annu Rev Cell Dev Biol* 1997, **13**:83-117.
11. Mitchison T, Kirschner M: **Dynamic instability of microtubule growth**. *Nature* 1984, **312**:237-242.
12. Skibbens RV, Skeen VP, Salmon ED: **Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: a push-pull mechanism**. *J Cell Biol* 1993, **122**:859-875.
13. Nicklas RB, Kubai DF: **Microtubules, chromosome movement, and reorientation after chromosomes are detached from the spindle by micromanipulation**. *Chromosoma* 1985, **92**:313-324.
14. Maddox P, Straight A, Coughlin P, Mitchison TJ, Salmon ED: **Direct observation of microtubule dynamics at kinetochores in Xenopus extract spindles: implications for spindle mechanics**. *J Cell Biol* 2003, **162**:377-382.
15. Skibbens RV, Salmon ED: **Micromanipulation of chromosomes in mitotic vertebrate tissue cells: tension controls the state of kinetochore movement**. *Exp Cell Res* 1997, **235**:314-324.
16. Rieder CL, Salmon ED: **Motile kinetochores and polar ejection forces dictate chromosome position on the vertebrate mitotic spindle**. *J Cell Biol* 1994, **124**:223-233.
17. Goshima G, Yanagida M: **Time course analysis of precocious separation of sister centromeres in budding yeast: continuously separated or frequently reassociated?** *Genes Cells* 2001, **6**:765-773.
18. Pearson CG, Maddox PS, Salmon ED, Bloom K: **Budding yeast chromosome structure and dynamics during mitosis**. *J Cell Biol* 2001, **152**:1255-1266.
19. Mitchison TJ, Salmon ED: **Mitosis: a history of division**. *Nat Cell Biol* 2001, **3**:E17-E21.
20. Kapoor TM, Compton DA: **Searching for the middle ground: mechanisms of chromosome alignment during mitosis**. *J Cell Biol* 2002, **157**:551-556.
21. Kline-Smith SL, Walczak CE: **Mitotic spindle assembly and chromosome segregation: refocusing on microtubule dynamics**. *Mol Cell* 2004, **15**:317-327.
22. Rieder CL, Salmon ED: **The vertebrate cell kinetochore and its roles during mitosis**. *Trends in Cell Biology* 1998, **8**:310-318.
23. Arana P, Nicklas RB: **Orientation and segregation of a micromanipulated multivalent: familiar principles, divergent outcomes**. *Chromosoma* 1992, **101**:399-412.
24. Nicklas RB: **How cells get the right chromosomes**. *Science* 1997, **275**:632-637.
25. Cimini D, Howell B, Maddox P, Khodjakov A, Degrassi F, Salmon ED: **Merotelic kinetochore orientation is a major mechanism of aneuploidy in mitotic mammalian tissue cells**. *J Cell Biol* 2001, **153**:517-527.
26. LaFountain JR Jr, Cohan CS, Siegel AJ, LaFountain DJ: **Direct visualization of microtubule flux during metaphase and anaphase in crane-fly spermatocytes**. *Mol Biol Cell* 2004, **15**:5724-5732.
27. Mitchison TJ: **Polewards microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence**. *J Cell Biol* 1989, **109**:637-652.
28. Ganem NJ, Compton DA: **Functional roles of poleward microtubule flux during mitosis**. *Cell Cycle* 2006, **5**:481-485.
29. Sharp DJ, Rogers GC, Scholey JM: **Microtubule motors in mitosis**. *Nature* 2000, **407**:41-47.
30. Rogers GC, Rogers SL, Schwimmer TA, Ems-McClung SC, Walczak CE, Vale RD, Scholey JM, Sharp DJ: **Two mitotic kinesins cooperate to drive sister chromatid separation during anaphase**. *Nature* 2004, **427**:364-370.
31. Lan W, Zhang X, Kline-Smith SL, Rosasco SE, Barrett-Wilt GA, Shabanowitz J, Hunt DF, Walczak CE, Stukenberg PT: **Aurora B phosphorylates centromeric MCAK and regulates its localization and microtubule depolymerization activity**. *Curr Biol* 2004, **14**:273-286.
32. Ohi R, Coughlin ML, Lane WS, Mitchison TJ: **An inner centromere protein that stimulates the microtubule depolymerizing activity of a KinI kinesin**. *Dev Cell* 2003, **5**:309-321.
33. Wordeman L, Mitchison TJ: **Identification and partial characterization of mitotic centromere-associated kinesin, a kinesin-related protein that associates with centromeres during mitosis**. *J Cell Biol* 1995, **128**:95-105.
34. Skibbens RV, Rieder CL, Salmon ED: **Kinetochore motility after severing between sister centromeres using laser microsurgery: evidence that kinetochore directional instability and position is regulated by tension**. *J Cell Sci* 1995, **108**:2537-2548.

35. Marko JF, Poirier MG: **Micromechanics of chromatin and chromosomes**. *Biochem Cell Biol* 2003, **81**:209-220.

36. Kalab P, Weis K, Heald R: **Visualization of a Ran-GTP gradient in interphase and mitotic Xenopus egg extracts**. *Science* 2002, **295**:2452-2456.

37. Budde PP, Kumagai A, Dunphy WG, Heald R: **Regulation of Op18 during spindle assembly in Xenopus egg extracts**. *J Cell Biol* 2001, **153**:149-158.

38. Niethammer P, Bastiaens P, Karsenti E: **Stathmin-tubulin interaction gradients in motile and mitotic cells**. *Science* 2004, **303**:1862-1866.

39. Caudron M, Bunt G, Bastiaens P, Karsenti E: **Spatial coordination of spindle assembly by chromosome-mediated signaling gradients**. *Science* 2005, **309**:1373-1376.

40. Mogilner A, Wollman R, Civelekoglu-Scholey G, Scholey J: **Modeling mitosis**. *Trends Cell Biol* 2006, **16**:88-96.

41. Odde DJ: **Mitotic spindle: disturbing a subtle balance**. *Curr Biol* 2005, **15**:R956-R959.

42. Hill TL: **Theoretical problems related to the attachment of microtubules to kinetochores**. *Proc Natl Acad Sci U S A* 1985, **82**:4404-4408.

43. •• Joglekar AP, Hunt AJ: **A simple, mechanistic model for directional instability during mitotic chromosome movements**. *Biophys J* 2002, **83**:42-58.

The authors describe a simple mechanistic model that explains the origin of the forces that move chromosomes and how these forces could be coordinated to produce kinetochore directional instability as observed in PtK1 cells. The model is based on previous work of T Hill (1985), in which a 'kinetochore sleeve' provides a sliding attachment of the kinetochore to the kMTs via passive linkers diffusing along the microtubule tip.

44. Khodjakov A, Cole RW, McEwen BF, Buttle KF, Rieder CL: **Chromosome fragments possessing only one kinetochore can congress to the spindle equator**. *J Cell Biol* 1997, **136**:229-240.

45. LaFountain JR Jr, Cole RW, Rieder CL: **Polar ejection forces are operative in crane-fly spermatocytes, but their action is limited to the spindle periphery**. *Cell Motil Cytoskeleton* 2002, **51**:16-26.

46. Rieder CL, Davison EA, Jensen LC, Cassimeris L, Salmon ED: **Oscillatory movements of monooriented chromosomes and their position relative to the spindle pole result from the ejection properties of the aster and half-spindle**. *J Cell Biol* 1986, **103**:581-591.

47. Marshall WF, Marko JF, Agard DA, Sedat JW: **Chromosome elasticity and mitotic polar ejection force measured in living Drosophila embryos by four-dimensional microscopy-based motion analysis**. *Curr Biol* 2001, **11**:569-578.

48. Brouhard GJ, Hunt AJ: **Microtubule movements on the arms of mitotic chromosomes: polar ejection forces quantified in vitro**. *Proc Natl Acad Sci U S A* 2005, **102**:13903-13908.

49. Coue M, Lombillo VA, McIntosh JR: **Microtubule depolymerization promotes particle and chromosome movement *in vitro***. *J Cell Biol* 1991, **112**:1165-1175.

50. Lombillo VA, Nislow C, Yen TJ, Gelfand VI, McIntosh JR: **Antibodies to the kinesin motor domain and CENP-E inhibit microtubule depolymerization-dependent motion of chromosomes *in vitro***. *J Cell Biol* 1995, **128**:107-115.

51. Hunt AJ, McIntosh JR: **The dynamic behavior of individual microtubules associated with chromosomes in vitro**. *Mol Biol Cell* 1998, **9**:2857-2871.

52. •• Civelekoglu-Scholey G, Sharp DJ, Mogilner A, Scholey JM: **Model of chromosome motility in *Drosophila* embryos: adaptation of a general mechanism for rapid mitosis**. *Biophys J* 2006, **90**:3966-3982.

Here a model is developed to describe *Drosophila* embryo chromosome motility, in which multiple microtubules with high dynamic instability can produce barely detectable metaphase oscillations and rapid poleward motion during anaphase A. This is the first model for kinetochore motility based on a force balance that takes into account all known force generators in the spindle, including molecular motors.

53. Ault JG, Nicklas RB: **Tension, microtubule rearrangements, and the proper distribution of chromosomes in mitosis**. *Chromosoma* 1989, **98**:33-39.

54. King JM, Nicklas RB: **Tension on chromosomes increases the number of kinetochore microtubules but only within limits**. *J Cell Sci* 2000, **113(Pt 21)**:3815-3823.

55. •• Cimini D, Cameron LA, Salmon ED: **Anaphase spindle mechanics prevent mis-segregation of merotelically oriented chromosomes**. *Curr Biol* 2004, **14**:2149-2155.

In this analysis of merotelic kinetochore orientation, the authors describe a model in which the ratio of microtubules attached to the correct versus the incorrect pole could mediate directed motion of improperly attached (merotelic) kinetochores. This work shows that tension-dependent kMT assembly can explain the resolution of merotelic errors in PtK1 cells during anaphase, and motivates current models for chromosome motility that assume tension-dependent kMT assembly.

56. •• Gardner MK, Pearson CG, Sprague BL, Zarzar TR, Bloom K, Salmon ED, Odde DJ: **Tension-dependent regulation of microtubule dynamics at kinetochores can explain metaphase congression in yeast**. *Mol Biol Cell* 2005, **16**:3764-3775.

Here the authors describe congression of kinetochores to a metaphase configuration in budding yeast on the basis of computational simulations that are quantitatively compared to a series of experiments. This paper describes the process of metaphase congression in terms of a spatial gradient in catastrophe frequency at kinetochore–microtubule plus-ends that provides spatial information for the correct positioning of kinetochores, and a tension-dependent rescue frequency of kinetochore–microtubule plus-ends, which allows communication between sister kinetochores and thus localization to the correct half-spindle prior to anaphase.

57. Sprague BL, Pearson CG, Maddox PS, Bloom KS, Salmon ED, Odde DJ: **Mechanisms of microtubule-based kinetochore positioning in the yeast metaphase spindle**. *Biophys J* 2003, **84**:1-18.

58. Winey M, Mamay CL, O'Toole ET, Mastronarde DN, Giddings TH Jr, McDonald KL, McIntosh JR: **Three-dimensional ultrastructural analysis of the *Saccharomyces cerevisiae* mitotic spindle**. *J Cell Biol* 1995, **129**:1601-1615.

59. Peterson JB, Ris H: **Electron-microscopic study of the spindle and chromosome movement in the yeast *Saccharomyces cerevisiae***. *J Cell Sci* 1976, **22**:219-242.

60. Pearson CG, Yeh E, Gardner M, Odde D, Salmon ED, Bloom K: **Stable kinetochore-microtubule attachment constrains centromere positioning in metaphase**. *Curr Biol* 2004, **14**:1962-1967.

61. • Pearson CG, Gardner MK, Paliulis LV, Salmon ED, Odde DJ, Bloom K: **Measuring nanometer scale gradients in spindle microtubule dynamics using model convolution microscopy**. *Mol Biol Cell* 2006, in press.

The authors provide an example of how computational simulation can be applied to develop new experiments and analysis methods. In this work, a spatial gradient in kinetochore microtubule dynamics is observed at 65 nm intervals along the length of the budding yeast mitotic spindle. The gradient corresponds to the quantitative predictions of the model described in [57].

62. He X, Asthana S, Sorger PK: **Transient sister chromatid separation and elastic deformation of chromosomes during mitosis in budding yeast**. *Cell* 2000, **101**:763-775.

63. Goshima G, Yanagida M: **Establishing biorientation occurs with precocious separation of the sister kinetochores, but not the arms, in the early spindle of budding yeast**. *Cell* 2000, **100**:619-633.

64. Tanaka T, Fuchs J, Loidl J, Nasmyth K: **Cohesin ensures bipolar attachment of microtubules to sister centromeres and resists their precocious separation**. *Nat. Cell Biol.* 2000, **2**:492-499.

65. Tytell JD, Sorger PK: **Analysis of kinesin motor function at budding yeast kinetochores**. *J Cell Biol* 2006, **172**:861-874.

66. •• Grishchuk EL, Molodtsov MI, Ataullakhanov FI, McIntosh JR: **Force production by disassembling microtubules**. *Nature* 2005, **438**:384-388.

By conjugating glass microbeads to tubulin polymers, the authors show that a single depolymerizing microtubule can generate about ten times the force that is developed by a motor enzyme, and that even simple microtubule couplers slow polymer disassembly.

67. Molodtsov MI, Ermakova EA, Shnol EE, Grishchuk EL,
• McIntosh JR, Ataullakhanov FI: **A molecular-mechanical model of the microtubule**. *Biophys J* 2005, **88**:3167-3179.
In this paper, the authors develop a molecular-mechanical model in which the structural and biochemical properties of tubulin are used to predict the shape and stability of microtubule plus-ends. This modeling is useful to better understand how the physical configuration of microtubule plus-ends could be related to their behavior *in vivo*.

68. Molodtsov MI, Grishchuk EL, Efremov AK, McIntosh JR, Ataullakhanov FI: **Force production by depolymerizing microtubules: a theoretical study**. *Proc Natl Acad Sci U S A* 2005, **102**:4353-4358.

69. Koshland DE, Mitchison TJ, Kirschner MW: **Polewards chromosome movement driven by microtubule depolymerization *in vitro***. *Nature* 1988, **331**:499-504.

70. Dogterom M, Kerssemakers JW, Romet-Lemonne G, Janson ME: **Force generation by dynamic microtubules**. *Curr Opin Cell Biol* 2005, **17**:67-74.

71. Dogterom M, Yurke B: **Measurement of the force-velocity relation for growing microtubules**. *Science* 1997, **278**:856-860.

72. Janson ME, de Dood ME, Dogterom M: **Dynamic instability of microtubules is regulated by force**. *J Cell Biol* 2003, **161**:1029-1034.

73. Kerssemakers JW, Laura Munteanu E, Laan L, Noetzel TL,
• Janson ME, Dogterom M: **Assembly dynamics of microtubules at molecular resolution**. *Nature* 2006, in press.
Here the authors use optical tweezers and microtubule growth against a microfabricated barrier to obtain information on the assembly dynamics of microtubules *in vitro*. They find that microtubules exhibit substantial growth and shortening events within growth phases that may be the result of the addition and loss of tubulin oligomers to the growing microtubule plus-end, and that this effect is enhanced by addition of XMAP215.

74. Miranda JJ, De Wulf P, Sorger PK, Harrison SC: **The yeast DASH complex forms closed rings on microtubules**. *Nat Struct Mol Biol* 2005, **12**:138-143.

75. Westermann S, Avila-Sakar A, Wang HW, Niederstrasser H, Wong J, Drubin DG, Nogales E, Barnes G: **Formation of a dynamic kinetochore- microtubule interface through assembly of the Dam1 ring complex**. *Mol Cell* 2005, **17**:277-290.

76. Salmon ED: **Microtubules: a ring for the depolymerization motor**. *Curr Biol* 2005, **15**:R299-R302.

77. McIntosh JR: **Rings around kinetochore microtubules in yeast**. *Nat Struct Mol Biol* 2005, **12**:210-212.

78. Asbury CL, Gestaut DR, Powers AF, Franck AD, Davis TN: **The Dam1 kinetochore complex harnesses microtubule dynamics to produce force and movement**. *Proc Natl Acad Sci U S A* 2006.

79. Westermann S, Wang HW, Avila-Sakar A, Drubin DG, Nogales E,
•• Barnes G: **The Dam1 kinetochore ring complex moves processively on depolymerizing microtubule ends**. *Nature* 2006, **440**:565-569.
Using fluorescence microscopy in an *in vitro* assay, the authors show that the budding yeast Dam1 complex, which forms a ring around microtubules, can diffuse randomly along the microtubule lattice and can move processively for several micrometers at the end of a depolymerizing microtubule. In addition, they show that the accumulation of the Dam1 ring complex slows the microtubule disassembly rate, suggesting that mechanical resistance to ring sliding promotes a shift away from disassembly.

80. He X, Rines DR, Espelin CW, Sorger PK: **Molecular analysis of kinetochore-microtubule attachment in budding yeast**. *Cell* 2001, **106**:195-206.

81. Joglekar AP, Bouck DC, Molk JN, Bloom KS, Salmon ED: **Molecular architecture of a kinetochore-microtubule attachment site**. *Nat Cell Biol* 2006, **8**:581-585.

82. Wei RR, Sorger PK, Harrison SC: **Molecular organization of the Ndc80 complex, an essential kinetochore component**. *Proc Natl Acad Sci U S A* 2005, **102**:5363-5367.

83. Al-Bassam J, van Breugel M, Harrison SC, Hyman A: **Stu2p binds tubulin and undergoes an open-to-closed conformational change**. *J Cell Biol* 2006, **172**:1009-1022.

84. VanBuren V, Cassimeris L, Odde DJ: **Mechanochemical**
• **model of microtubule structure and self-assembly kinetics**. *Biophys J* 2005, **89**:2911-2926.
In this paper, the authors build on a previously published model [92] to incorporate mechanical stress and strain as well as chemical bond energies within the microtubule lattice. This three dimensional mechanochemical model was able to recapitulate experimentally observed microtubule plus-end tip structures, and will be a useful tool for understanding for how microtubule-associated proteins such as XMAP215 could regulate microtubule dynamics.

85. Vandenbeldt KJ, Barnard RM, Hergert PJ, Meng X, Maiato H,
•• McEwen BF: **Kinetochores use a novel mechanism for coordinating the dynamics of individual microtubules**. *Curr Biol* 2006, **16**:1217-1223.
Here, the authors use electron tomography to study the structure of microtubules whose plus-ends are embedded in the kinetochore. They find that most of the plus-ends are in a depolymerizing state, even when the K-fiber exhibits net tubulin incorporation at the plus-end. The authors suggest that tension exerted on the microtubule plus-end by the outer kinetochore plaque could slow microtubule plus-end depolymerization and thus promote stability of attachment between a microtubule plus-end and the kinetochore.

86. O'Toole ET, McDonald KL, Mantler J, McIntosh JR, Hyman AA,
•• Muller-Reichert T: **Morphologically distinct microtubule ends in the mitotic centrosome of *Caenorhabditis elegans***. *J Cell Biol* 2003, **163**:451-456.
Using electron tomography, the authors examine both plus and minus ends of microtubules in *C. elegans* embryonic cells. Although primarily open ends are found on kinetochore-associated microtubules, the authors note that there is a mix of flared and blunt microtubule ends terminating in the kinetochore, with ~78% flared and ~22% blunt.

87. Brust-Mascher I, Civelekoglu-Scholey G, Kwon M, Mogilner A, Scholey JM: **Model for anaphase B: role of three mitotic motors in a switch from poleward flux to spindle elongation**. *Proc Natl Acad Sci U S A* 2004, **101**:15938-15943.

88. Cytrynbaum EN, Sommi P, Brust-Mascher I, Scholey JM, Mogilner A: **Early spindle assembly in *Drosophila* embryos: role of a force balance involving cytoskeletal dynamics and nuclear mechanics**. *Mol Biol Cell* 2005, **16**:4967-4981.

89. Bloom K, Sharma S, Dokholyan NV: **The path of DNA in the kinetochore**. *Curr Biol* 2006, **16**:R276-R278.

90. Mergell B, Everaers R, Schiessel H: **Nucleosome interactions in chromatin: fiber stiffening and hairpin formation**. *Phys Rev E Stat Nonlin Soft Matter Phys* 2004, **70**:011915.

91. Doncic A, Ben-Jacob E, Barkai N: **Evaluating putative mechanisms of the mitotic spindle checkpoint**. *Proc Natl Acad Sci U S A* 2005, **102**:6332-6337.

92. VanBuren V, Odde DJ, Cassimeris L: **Estimates of lateral and longitudinal bond energies within the microtubule lattice**. *Proc Natl Acad Sci USA* 2002, **99**:6035-6040.

93. Gillespie DT: **Exact Stochastic Simulation of Coupled Chemical Reactions**. *J Phys Chem* 1977, **81**:2340-2361.