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Richard McIntosh's Lecture Part 2:
Experiments to Understand Mitosis

Teaching Tools were prepared by Richard McIntosh.

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1. Keywords and Terms

Mitosis; mitotic spindle; microtubule; microtubule-associated proteins (MAPs); motor enzyme; centrosome; spindle pole; kinetochore; centromere; polymerization, depolymerization; force; tension; dynamic equilibrium; dynamic instability; ultraviolet microbeam; laser microbeam; mutant allele; antibody; phenotype

2. Lecture Notes

Introduction

Experiments that reveal properties of a complex process, like mitosis, are ways to gain a deeper understanding of the machinery that underlies that process, i.e., how it works. Observations and experiments on a range of different organisms can be informative

about the features of mitosis that are basic, but they can also be confusing as a result of natural variability among organisms.

A review-overview of mitosis is given: prophase, prometaphase, metaphase, anaphase, telophase, and what happens in each.

A review of spindle structure is given with an emphasis on spindle symmetry and overall organization. The rest of the lecture will be described below by posing and answering the major questions that are addressed.

Question 1: How do microtubules growing from the spindle's two centrosomes (the spindle poles) interact to form the spindle's 2-fold symmetric structure?

The protein Ase1 is localized where microtubules (MTs) that grow from the two poles interdigitate. When Ase1 is removed from a cell (e.g., fission yeast) by gene deletion, the spindles that form have a strong tendency to break into two parts.

The kinesin-like motor enzyme kinesin-5 is also important for the formation of a bipolar (2-fold symmetric) spindle. Its inactivation by mutation or by drugs that block its enzyme activity rather specifically leads to the formation of monopolar spindles in which the two centrosomes have not separated. The localization of Kinesin-5 is more uniform than that of Ase1, but the enzyme is certainly found in the zone of MT overlap, near the spindle midplane. This motor is a homotetrameric assembly of four kinesin-like heavy chains, arranged in a 2-fold symmetric structure. When working as a motor, it walks towards the plus ends of MTs (which are located distal to the spindle poles), so kinesin-5 is likely to contribute to the forces that push spindle poles apart.

Since Ase1 is not a motor but does bind MTs, we can imagine that its presence as a cross-linker between interdigitating MTs impedes the MT sliding that is favored by kinesin-5. This kind of antagonism between molecular functions will come up again.

Chromosomes themselves are not essential for the formation of a 2-fold symmetric spindle, because they can be removed by micromanipulation, and the spindle still

develops into a 2-fold symmetric structure, so long as the chromosomes are not removed too early in prometaphase.

Question 2: What defines the length of a bipolar spindle?

In the group of unicellular algae called "Diatoms", the spindle is seen by polarization microscopy as a shaft that runs from one spindle pole to the other (though electron microscopy shows that this shaft is actually formed from two sets of interdigitating MTs that overlap near the spindle midplane). When a microbeam of ultraviolet light is used to irradiate one side of this midregion, the remaining spindle collapses by bending with the inside of the bend facing the site of irradiation. This result implies that the poles of the spindle are being pulled inward at metaphase, presumably by the reaction to forces that are pulling the chromosomes toward the spindle poles (recall these forces from the Introductory lecture). Thus, we must think of a metaphase spindle as a mechanical system in which kinesin-5 is forcing the sliding apart of the two interdigitating families of MTs that grew from each spindle pole, Ase1 is resisting this sliding and holding the two MT families together in a bundle, the chromosomes are being pulled toward the spindle poles, and the poles are being pulled in toward the spindle midplane (or "equator", as it is sometimes called).

But there is another motor in the mix: a kinesin-14 is localized in the region of spindle MT overlap, and this kind of motor is minus end-directed. This means that when it cross-links the interdigitating MTs, its motor action will tend to make them INCREASE the extent of their overlap and pull the poles in toward the spindle midplane. Thus, the mechanical equilibrium in the spindle includes multiple, antagonistic actions, much as you might use antagonistic muscles to control a fine motor operation.

Question 3: Are spindle microtubules dynamic, and if so what are their sites of polymerization and depolymerization?

Evidence that is mentioned but not presented shows that spindle MTs are "labile" in the sense that they turn over rapidly. A more complicated and interesting form of MT dynamics is revealed by "speckle imaging", the use of small amounts of fluorescent tubulin to label MTs. When only a little fluorescent tubulin is added to a cell, the MTs that form have randomly distributed brighter and dimmer spots along their lengths. When these MTs are imaged in with a very sensitive video camera, they are seen to migrate toward both of the spindle poles; all of the spindle MTs seem to be moving

away from the spindle midplane, a motion that has been called “flux”. Yet the spindle at metaphase is not getting longer, suggesting that the MTs are depolymerizing at the poles. There is yet another kinesin-like protein, a kinesin-13, that has been found at the poles, and this motor promotes MT depolymerization. Experiments favor the model that flux includes pushing of MTs from the midplane by the action of kinesin-5 and depolymerization of MTs at the poles by kinesin-13. Thus, the dynamics of spindle MTs are complex and include not only the polymerization and depolymerization of tubulin but also the action of multiple motor enzymes.

Question 4: How might MT flux be related to chromosome motion in mitosis? Do the data support the hypothesis that this is a fundamental and widely used mechanism for chromosome motion?

A plausible model for chromosome motion is that the dynamics of the spindle’s MTs makes a sort of conveyer belt, and anaphase occurs when sister chromatids separate and join the flux of MTs toward the two spindle poles. This may be an explanation for many aspects of mitosis, at least in some cells.

However, biological variability lets us see that this is not the whole story. In fission yeasts there does not appear to be any flux, but there is a motion of the spindle MTs from the midplane toward the poles during anaphase B (spindle elongation), as revealed by experiments in which marks are placed on fluorescent spindle MTs by photobleaching them with a laser microbeam.

Moreover, in some fungi, the motion apart of the spindle poles that occurs during anaphase B is not driven from the zone of MT overlap but is a result of pulling on the spindle poles by astral microtubules that interact with the cell’s cortex. This motion is probably driven by dynein.

Question 5: How do kinetochores form stable attachments with spindle fibers?

To understand chromosome-spindle MT interaction, we must look carefully at the parts of the spindle where this happens and the processes that result from such interactions. Moreover, such work should be done in several organisms. Electron microscopy of both algal and vertebrate cells in prometaphase shows kinetochores interacting with both the walls and the ends of MTs. Descriptions of chromosome attachment by light

microscopy of living cells show that a chromosome starts to move poleward as soon as its kinetochore associates with a MT. This is a minus end-directed motion over the surface of a MT; dynein is localized on kinetochores, so it is plausible that initial chromosome-MT interactions are mediate by this motor enzyme, in at least some cells.

The interaction between a chromosome and its spindle fiber has been probed by micromanipulation. A metaphase chromosome that has been detached from the spindle will soon re-associate with the spindle and go to the metaphase plate. If the detached chromosome forms an **inappropriate** attachment to the spindle, e.g., with both kinetochores associating with MTs from one pole, these connections soon break, and the chromosome reattaches to the spindle, trying again to achieve biorientation. If, however, a maloriented chromosome (one whose kinetochores are both attached to the same pole) is put under tension by the action of a microneedle, it is stable and does not reorient. This suggests that the stability of chromosome-spindle fiber attachment is a result of tension. Dynein may contribute to the generation of this tension, but there are many additional possibilities, as discussed in the next lecture.

The idea that tension enhances stability is important because it allows us to understand how chromosomes that are properly associated with the spindle form stable connections, while those that are improperly associated (e.g., both kinetochores attached on one pole) do not. When sister kinetochores are associated with sister poles, a chromosome is under tension; when sister kinetochores are associate with the same pole, there is little or no tension, so this chromosome will be released and can try again to do the job right. Thus, biologically valuable chromosome attachments to the spindle are "selected", because the others are unstable and break, allowing the chromosome to try again.

Question 6: How does the spindle generate tension on kinetochores?

Dynein is on kinetochores, at least in early mitosis in many cell types, so it is a candidate for tension development. However, the injection of antibodies to dynein does not block chromosome attachment. One can argue that these reagents are simply not effective enough, but using more rigorous experimental approaches poses problems. Dynein does many things in cells, so a deletion mutation is not a good tool for studying the role of dynein in chromosome attachment to the spindle; too many other processes are affected. No good temperature sensitive alleles of dynein are currently available to investigate this issue, so it is still not well resolved. There are drugs and other

experimental treatments that block the action of some kinetochore dynein-associated proteins, but exactly what these reagents do is still open to question.

The whole problem is made more complicated by the fact that there are other motors at kinetochores. Some of these are plus end-directed motors, and it is not clear how these could develop tension, but there are kinesin-13s at the kinetochores of some organisms and kinesin-8s at the kinetochores of others. Both these motors promote MT depolymerization, so this might be a source of tension. Moreover, the only kinetochore proteins so far identified that are essential for chromosome-spindle fiber attachment and also universal among organisms are **not motors**. They are simply kinetochore and MT-binding proteins. MT depolymerization is therefore a plausible source of tension generation in mitosis, and this is the subject of the next McIntosh lecture. For now, we must leave this issue as unresolved.

Question 7: How does the spindle get the chromosomes to the spindle midplane?

Experiments with laser microsurgery show that pieces of chromosome that lack a kinetochore are pushed away from the pole by the spindle of a vertebrate cell. This suggests that at least some spindles not only pull on kinetochores, they push on chromosome arms. A “bioriented” chromosome, which is attached by its sister kinetochores to opposite poles, will experience two approximately equal and opposite pulls at its kinetochores, so another force, like a push from the poles that is stronger when the chromosome is near a pole, would result in chromosome motion to the spindle midplane. Such a mechanism probably contributes to the formation of a metaphase spindle in at least some cell types. But not all cells have this pushing mechanism, so there may be other factors that contribute to pre-anaphase chromosome motion.

Question 8: How does the spindle pull its chromosomes to the poles in Anaphase A?

Answers to this question are implied in what is said above, but this issue is the subject of the next McIntosh iBio Seminar and will be left until that lecture.

3. Recommended Reading

Mitchison, T. J. (1989). "Mitosis: basic concepts." Curr Opin Cell Biol 1(1): 67-74.

A good overview, written before we learned too many details of spindle component chemistry, so it focuses on the big issues.

McIntosh, J. R. (2002). "Chromosome-microtubule interactions during mitosis." Annu Rev Cell Dev Biol 18: 193-219.

A review of the issues surrounding kinetochore-MT interaction written in the context of more recent information.

Cleveland, D. W., Y. Mao, et al. (2003). "Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling." Cell 112(4): 407-21.

A review that focuses on the biochemistry of kinetochores and the functions they must accomplish.

Cheeseman, I. M., et al. (2008). "KNL1 and the CENP-H/I/K complex coordinately direct kinetochore assembly in vertebrates." Mol Biol Cell 19(2): 587-94.

A paper showing the importance of one particular kinetochore complex in the process of assembling functional kinetochore-MT interaction.

4. Review Questions

1. Diagram the structure of a kinesin-5 homotetramer. Now place this structure between one pair of MTs that is parallel and another pair that is antiparallel. Now sketch the situation at later times, showing what the action of the motor domain of this enzyme will do to the relative positions of the motor and the MTs.
2. Given what you know about kinesin-5, explain why its inhibition blocks the formation of a bi-polar spindle.

3. The localization of Ase1 and the phenotype of its deletion imply that this protein binds to anti-parallel MTs. What would you expect for the organization of a protein that has this property?
4. Diagram the process of MT flux, showing where tubulin subunits must add and fall off of the spindle MTs during MT movement.
5. Use the above diagram to develop your own model for anaphase B, the elongation of a spindle.
6. Now extend this diagram to include the idea of a pull, exerted on astral MTs by the cell's cortex.
7. Draw a diagram of a cell in which a chromosome is being manipulated, as in the experiment by Nicklas. Recall that the cell's membrane is tough and doesn't break, so the needle is always outside the cell, even when it is pushing on the chromosome.
8. Rieder's experiment that used laser microsurgery to cut a chromosome's arms away from its kinetochores showed the existence of forces that push objects away from the spindle pole. In these same cells, what would you predict for the metaphase position of a chromosome that is associated with only one spindle pole?

5. Answers to Review Questions

1. The answer to this question is shown at the bottom left of the slide that describes kinesin-5 in the spindle.

2. When kinesin-5 interacts with MTs growing from one centrosome, it will simply walk toward the MT plus ends. When it interacts with the MTs from two centrosomes, it will cross-link the MTs wherever they are anti-parallel and cause them to slide apart. This action will push the centrosomes away from one another and would aid in the formation of a bi-polar spindle.

3. To cross-link MTs the protein must have two tubulin binding sites, so it is probably a dimer. To bind specifically to anti-parallel MTs, the two parts of the protein must be associated with sufficient rigidity that the relative orientation of these two binding sites is fixed in an anti-parallel orientation.

4. The diagram should include two spindle poles, each with associated MTs, and these two MT families should interdigitate. Flux means that each family of MTs is moving toward the centrosome from which it grew, and to allow this movement, tubulin must add at the pole-distal MT end and come off from the pole-proximal end.

5. All that is required is to stop the process of MT depolymerization at the spindle pole, so each MT continues to elongate and slide away from the midzone, helping to push the spindle poles apart.

6. Now the diagram should look much like the review slide shown in the talk.

7. The trick here is to represent the spindle and one of its chromosomes with the cell's membrane being deformed so far by the needle that it pushes on the chromosome. The membrane is still separating the needle from the chromosome.

8. The kinetochore(s) of this chromosome is(are) presumably being pulled in toward the pole that it (they) faces, but the arms of this chromosome are being pushed away from the pole. The "metaphase" position should be the place (the distance from the pole) at which these two forces are equal, so the chromosome is in a position of mechanical equilibrium.

6. Discussion Questions

1. It has been shown by Nicklas that stable connections between kinetochores and spindle microtubules are formed only when this junction is under tension. What properties could to attribute to the kinetochore-MT junction so it would become stable only when under tension?

2. The spindle generates forces at multiple places (kinetochores, poles, zones where MTs from the two ends of the spindle interdigitate, etc.), and the structure of MTs allows them to resist tension, compression, and bending stresses. Draw your own diagram of the spindle and put into it all of the forces you can think of that are relevant to understanding the mechanics of spindle-mediated chromosome motion.

3. Recent evidence shows that chromosomes can initiate MT polymerization in association with either their arms or their kinetochores. What impact do you expect these features of at least some spindles to have on spindle structure and/or function?

4. The flux of spindle MTs suggests that these polymers are polymerizing and depolymerizing at different places. Where are these events occurring, and how would you test your answer experimentally?
5. What spindle components do you think are responsible for MT flux? How would you test your ideas?
6. What do you suppose would happen if a single kinetochore became attached to two poles? How might the cell deal with such a problem?
7. Diagram a spindle in late prometaphase in which one chromosome has not yet arrived at the spindle midplane. Consider what must go on at that chromosome for it to move to join the other chromosomes. Add to your diagram all the polymerization and depolymerization events that must occur and what forces must act on the chromosome to get it where it belongs. Identify all the ways you can think of to develop the necessary forces.
8. The speed of chromosome motion is generally quite slow (micrometers per minute). Calculate the amount of force necessary to overcome the viscous drag on a chromosome at such speeds. Each motor enzyme can generate around 5×10^{-12} N, so how many motors of this kind would be required to move a chromosome? Does the answer surprise you in any way? What do you think your result suggests about force generation in mitosis?

7. Answers to Discussion Questions

1. One approach to this question is to give the molecules that connect a kinetochore to a microtubule the properties you want. For example, a motor enzyme that is stably attached to the kinetochore might have motile (and therefore labile) connections with the wall of the microtubule, particularly if it is not a “processive” motor (i.e., one that stays on the MT for long periods). If the motor is trying to walk along the microtubule, but it is prevented from doing so by tension acting in the opposite direction, the motor will stall. If the motor has to change its shape to go through a full cycle of force-producing motor activity, the tension would prevent this shape change and hold it in some intermediate stage. If the release of the motor from the microtubule (to continue with its motility cycle) requires that it finish its shape changes, then tension would lock the motor in this intermediate state, and it would

not be able to release, providing a bond with the kinetochore that is stabilized by tension.

2. The start for an answer to this question is found in the slide from Pollard et al. presented around the middle of this lecture in which they provide their own answer to this question. Beyond that, one can add the forces that are generated by MT polymerization and depolymerization, forces added by motors that influence MT polymerization, bending forces that throw the MTs of many spindles into arc-shaped fibers, forces that result from the viscous drag on moving chromosomes, and forces that may be generated by the interaction of any spindle component (fibers, motors, chromosomes) with a “matrix”, i.e. a not yet understood structural material that pervades at least some spindles.

3. This situation poses many questions: What is the polarity of the MTs that form on chromosome arms and/or kinetochores? What kind of mechanical attachment is there between the chromosomes and these MTs? Do the MTs exert forces on the chromosomes or simply swim or diffuse away? Are there factors that impose order on these MTs, so they take on a global pattern, or are they simply random? Little is actually known about the answers to these structural and mechanical questions. Information about such MTs can be found in Heald, R., R. Tournebize, et al. (1996). "Self-organization of microtubules into bipolar spindles around artificial chromosomes in Xenopus egg extracts." Nature 382: 420-425. Hyman, A. A. and E. Karsenti (1996). "Morphogenetic properties of microtubules and mitotic spindle assembly." Cell 84(3): 401-10. Caudron, et al. (2005). "Spatial coordination of spindle assembly by chromosome-mediated signaling gradients." Science 309(5739): 1373-6. Yokoyama, et al. (2008). "Cdk11 is a RanGTP-dependent microtubule stabilization factor that regulates spindle assembly rate." J Cell Biol 180(5): 867-75. Khodjakov, A. et al. (2003). "Minus-end capture of preformed kinetochore fibers contributes to spindle morphogenesis." J Cell Biol 160(5): 671-83.

4. The continued flux of spindle MTs towards the two poles suggests that each spindle MT is elongating by the addition of tubulin subunits at its plus (pole-distal) end, sliding under the action of motors, and depolymerizing at the pole. The site of tubulin addition could be identified by injecting a small amount of brightly labeled tubulin into a mitotic cell and seeing where it added to the spindle. It could also be found by photobleaching various part of the spindle and asking where the unbleached fluorescence returned into the now-dim region of the spindle. Finding sites of depolymerization is more difficult. It could be done by using a labeled tubulin

whose fluorescence can be activated by irradiation with near UV light. (Thereafter, its fluorescence is followed with blue light.) Such reagents are available either as alleles of the green fluorescent protein or as synthesized chemical reagents.

5. Flux almost certainly requires motors to push the sliding of MTs away from the spindle midplane, factors (probably motors) that induce MT depolymerization at spindle poles, factors that keep these moving MTs associated with one another where they interdigitate near the midplane and associated with the poles, where they are depolymerizing. Tests of these ideas would involve identifying the relevant motors and cross-bridging protein and modifying their function by genetic, immunological, or pharmacological methods.

6. This condition does arise in nature, and it is one of the sources of errors in chromosome segregation. It is called a “merotelic attachment”, and it is particularly common in cells whose normal mitosis has been bothered by genetic or other experimental treatment. Cimini, D., B. Howell, et al. (2001). "Merotelic kinetochore orientation is a major mechanism of aneuploidy in mitotic mammalian tissue cells." J Cell Biol **153**(3): 517-27. Cells can deal with it by having a mechanism for loosening the strength of the attachment between a kinetochore and the spindle MTs. Such a mechanism is found in the action of the protein kinase called Aurora-B, which is localized to the kinetochores. Cimini, D., X. Wan, et al. (2006). "Aurora kinase promotes turnover of kinetochore microtubules to reduce chromosome segregation errors." Curr Biol 16(17): 1711-8. Just how it accomplishes this loosening job is not yet known. Interesting field for future work.

7. The diagram is straightforward. The point to recognize is that the MTs associated with one kinetochore must elongate while the ones associated with the other kinetochore must shorten for the chromosome to move. The forces that move the chromosome could be the pushing forces from the poles, identified in the Rieder experiment described in the lecture. They could also come from a chromosome knowing by some mechanism that it is not at the spindle midplane and manipulating its kinetochores to get it to the right place. This hypothesis has been called the “Smart kinetochore” model.

8. The force necessary to move a chromosome against viscous drag can be estimated very roughly with the Stokes equation, F = 6p?rv, in which F is the viscous drag force measured in Newtons, ? is the viscosity of the medium (the solution all around the chromosome) measured in Pascal-seconds, r is the radius of the sphere we will

use to represent the chromosome measured in meters, and v is the speed at which the chromosome is moving, measured in meters/sec. The viscosity of water is about $1x10^{-3}$ Pascal-sec, and the medium of the spindle is unlikely to be more than 100 times this viscous. r is about $0.5x10^{-6}$ M, i.e., half a micrometer, and a high value for v is $1x10^{-7}$ M/sec. This works out to about 10^{-13} N, about one 50th of the force developed by a single motor enzyme. Thus, the amount of force necessary to move chromosomes against the cell's viscous drag is VERY small.

This result poses several questions to which the answers now available are only speculation, but they are intriguing: why does a cell move its chromosomes so slowly? How is chromosome speed regulated so the motors that are present do not cause the chromosomes to speed up? Are the forces generated by the spindle being used for a more subtle purpose, such as tearing the spindle apart as the chromosomes move?

8. Explain or Teach These Concepts to a Friend

1. Explain why the existence of forces pulling the metaphase chromosomes toward both poles implies that there are also forces pulling the poles toward the spindle midplane.
2. Explain how two motors with opposite directions of action can work against each other as they cross-bridge the same microtubules.
3. Describe what is going on when spindle microtubules are fluxing towards the poles.
4. Explain how the idea of stabilizing the attachment of chromosomes to the spindle by tension allows the cell to select against inappropriate attachments, like the attachment of both kinetochores on one chromosome to a single pole.
5. Explain how pushing forces from the pole can help to get a prometaphase chromosome to the metaphase plate.

9. Research the Literature on Your Own

1. If two microtubules are cross-bridged by Ase1, how can they be made to slide over one another by the action of kinesin-5? (Hints: When a protein is bound to one site on a microtubule wall, how hard is it to move that protein to an identical site just one or two tubulin subunits away? This kind of question can be answered by measuring the rate at which a microtubule-bound protein will diffuse on the microtubule surface. There may not yet be data for this question on Ase1, but other microtubule-binding proteins have been studied.)

2. What really happens when two oppositely directed motors are trying to push the same object in opposite directions? (Hint: look for relevant data on the motion of vesicles or microbeads coated with motor proteins).
3. What are the molecular mechanisms for the forces that push chromosome arms away from the poles of at least some vertebrate spindles? (Hint: check out both forces developed by MT polymerization and by the special class of kinesin-like proteins called “chromo-kinesins”.) What factors are likely to make one of these mechanisms more important than the other?
4. The older literature on mitosis includes descriptions of movements of many kinds of structures that associate with spindles: not just chromosome arms but also granules of various kinds. Find data on such movements from a variety of organisms, including higher plants, and see if you can formulate a single hypothesis about spindle action that accounts for all these motions. (Hint: the best data for answering this question come from studies on comparatively big cells, because their large spindles allowed accurate observations on such movements.)
5. Several lines of experimentation have led to our current view that spindle MTs are dynamic (polymerize and depolymerize rapidly), but not all the data say the same thing. Which spindle MTs are the most dynamic, the least dynamic, and which are in between? How where these MT identities and their rates of turnover established? How reliable are the data from each method? Working with the data you find the most convincing, do the relative labilities of different MT groups (kinetochore-associated vs not, interdigitating MTs, astral MTs) make sense in terms of building a successful mitotic apparatus?

10. Papers for Journal Club

Mitchison, T., L. Evans, E. Schulze, M. Kirschner, (1986). "Sites of microtubule assembly and disassembly in the mitotic spindle." Cell 45(4): 515-27. *This classic paper provided the first solid evidence for the localization of sites to which tubulin added spindle microtubules.*

Pfarr, C. M., M. Coue, P.M. Grissom, T.S. Hays, M.E. Porter, J. R. McIntosh, (1990). "Cytoplasmic dynein is localized to kinetochores during mitosis [see comments]." Nature 345(6272): 263-5. *The first evidence for localization of dynein to kinetochores and related spindle structures.*

Echeverri, C.J., B.M. Paschal, K.T. Vaughan, R.B. Vallee, (1996). "Molecular characterization of the 50-kD subunit of dynactin reveals function for the complex in chromosome alignment and spindle organization during mitosis." J.Cell Biol. 132: 617-633. *Discovery that a protein subunit of the dynein-associated protein complex, dynactin, can interfere with dynein action in fascinating ways.*

Scholey, J. M., I. Brust-Mascher, A. Mogilner, (2003). "Cell division." Nature 422(6933): 746-52. *A thoughtful review of the importance of motor action in both mitosis and cytokinesis.*

Goshima, G. and R. D. Vale (2003). "The roles of microtubule-based motor proteins in mitosis: comprehensive RNAi analysis in the Drosophila S2 cell line." J Cell Biol 162(6): 1003-16. *A comprehensive analysis, using modern molecular technology, of the full complexity of motor involvement with spindle formation and function.*