Mini-Review

A New Role For Motor Proteins as Couplers to Depolymerizing Microtubules

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THE segregation of chromosomes during mitosis involves dynamic interactions between the kinetochores of mitotic chromosomes and spindle microtubules. At the metaphase–anaphase transition, sister chromatids separate and move toward opposite poles of the spindle. During anaphase A, the chromosome to pole distance shortens, and subsequently during anaphase B, the spindle elongates. Anaphase A–like movements also occur during metaphase as chromosomes oscillate toward and away from poles (18). Spindle-marking experiments performed during anaphase A using photobleaching and photoactivation of labeled tubulin have shown that the major site of depolymerization of kinetochore microtubules is at the kinetochore, at least in vertebrate mitosis (1, 3). These studies imply that the force for poleward chromosome movement is predominantly generated at kinetochores. Current attention is focused on two potential mechanisms for force generation: activity of minus-end–directed ATPase motor proteins on kinetochores and/or harnessing of energy released by microtubule depolymerization.

The idea of harnessing depolymerization to move chromosomes has a distinguished history dating back to Inoue (6, 7). However, the idea that kinetochores move using ATPase motor activity has gained force from recent discoveries of dynein and kinesin-like motors at kinetochores (16, 22, 23). Two recent papers from Lombillo, McIntosh, and co-workers (10, 11) go some way towards resolving this question by showing that kinetochores may use motor proteins as the molecular interface that couples microtubule depolymerization to chromosome movement.

Purified Chromosomes and Microtubules: A Brief History

The studies by McIntosh and co-workers represent a culmination of the use of purified mitotic chromosomes as tools to investigate the interactions of chromosomes with microtubules in vitro. The first in vitro studies on mitotic chromosomes showed that kinetochores could nucleate microtubules by incubating chromosomes with tubulin, fixing, and processing for electron microscopy (20) or immunofluorescence (reviewed in reference 13). This fixed time point approach was extended to show that kinetochores could capture preformed microtubules, and these microtubules could slide over kinetochores in the presence of ATP (5). Motor protein activity was more convincingly demonstrated by real time analysis of ATP-dependent microtubule sliding, which revealed the presence of two motors of opposite polarities on kinetochores whose activity could be differentially regulated by thiophosphorylation (5).

ATP-independent movement of kinetochores, driven by microtubule depolymerization, was observed by two different approaches. First, anaphase A in lysed cells could be driven by calcium-induced microtubule depolymerization in the absence of ATP (19). Second, using isolated chromosomes, Koshland and co-workers showed that kinetochores could reel in depolymerizing microtubules in the absence of ATP (8). However, the fixed time point assays used by the latter relied on statistical analysis of microtubule lengths to demonstrate movement. Skeptics argued that depolymerization could have occurred while microtubule ends were transiently detached from kinetochores. Furthermore, these experiments did not demonstrate that significant amounts of force could be generated by depolymerization. These shortcomings, and perhaps also the rather ambitious title of the Koshland et al. paper, may have provided part of the inspiration for McIntosh and co-workers to develop a real time assay for depolymerization-driven chromosome movement (9). Their elegant innovation was to use *Tetrahymena* pellicles (detergent-extracted cortices of deciliated *Tetrahymena* cells) as a microtubule-nucleating template. The pellicles were attached to the coverslip of a perfusion cell, and microtubules were elongated from the exposed basal bodies, providing a dense, plus end out array of microtubules. Isolated chromosomes in buffer with tubulin were perfused into the cell, where they bound to the nucleated microtubules. Microtubule depolymerization was then initiated by diluting out the tubulin, and chromosome movement was observed in real time by DIC optics. This assay demonstrated unambiguously that chromosomes could be pulled toward microtubule minus ends by depolymerization in the absence of ATP, and furthermore, that this mechanism could generate appreciable force against a counter-flow of buffer (1).

Pure Motors on Beads Can Couple Bead Movement to Depolymerizing Microtubules

A key question in any experimental situation where microtubule depolymerization drives motility (1, 2) is the nature of the molecular interface coupling subunit loss to physical movement. Theoretical studies suggested a requirement for

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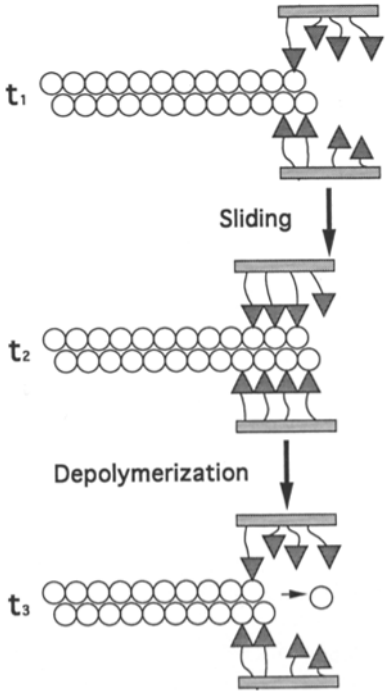

Figure 1. A model for kinetochore movement driven by microtubule depolymerization. A kinetochore is cartooned as comprising multiple coupling proteins (*triangles*) attached to a structural matrix. A microtubule (*circles*) penetrates it, making contact with several coupling proteins. The microtubule is drawn as imposing order on randomly arranged couplers. The motile mechanism is broken down into two basic processes, diffusional sliding of the kinetochores over the microtubule lattice, and depolymerization of the microtubule. Comparing t_1 and t_2, the free energy of the system has decreased by formation of new coupler-microtubule interactions. Comparing t_2 and t_3, these favorable interactions have been lost, but this is offset by decreased free energy caused by microtubule depolymerization. Comparing t_1 and t_3, the only energy change is the net decrease in free energy resulting from depolymerization. The mechanism is a form of Brownian rachet. In this scheme, the rate of movement is governed by the rate of subunit loss, the rate of coupler detachment, and the Brownian diffusion of the kinetochore. The force generated is dependent only on the energy difference between t_1 and t_3 and the distance moved. The energy term depends on how much of the free energy of the GTP hydrolysis that accompanied microtubule polymerization is stored and released during depolymerization. For more quantitative derivations, see reference 4. (Adapted from reference 4.)

multiple tubulin-binding sites on the moving structure that are capable of sliding readily between adjacent tubulin molecules in the microtubule lattice while maintaining a significant favorable interaction energy, as shown in Fig. 1 (4). Motor proteins have similar requirements during their mechanochemical cycles, making them candidates for constituting the coupling interface (Fig. 1, *triangles*). Certain motor proteins have been shown to allow one-dimensional diffusional sliding of bound microtubules under conditions where their ATPase activity is prevented (21), consistent with a role in coupling depolymerization to movement. Lombillo, McIntosh, and co-workers have addressed this potential role for motors in two ways. In one paper, they tested the properties of beads coated with pure motor proteins in their depolymerization assay (11). In the paper in this issue, they use antibodies to probe the role of specific motors in depolymerization-driven chromosome movement in vitro (10).

In the experiments with pure motors on beads, depolymerization was found to drive ATP-independent, minus-end–directed motility of three different proteins. These included the plus-end–directed motor kinesin, a chimeric kinesin-like protein (KLP)[1], and flagellar dynein in the presence of ATP-vanadate. The last two proteins are incapable of ATP-driven motility, but they support one-dimensional diffusion of microtubules. Although these experiments do not mimic precisely any specific in vivo motility process, they clearly demonstrate that motor proteins can couple depolymerization to movement in the absence of ATP, increasing our confidence that polymer disassembly is a potentially important cellular force generating mechanism.

Another intriguing observation made by the authors using the motor-coated beads was the ability of beads coated with the chimeric KLP to dramatically stimulate the depolymerization rate of the microtubules to which they were coupled. The coupling mechanism requires a favorable free energy of interaction between the bead-bound motors and tubulin. Since both tubulin–tubulin and tubulin–motor bonds need to be broken to release a subunit from the depolymerizing end of a microtubule with a bead attached, one would predict that microtubules with beads attached would depolymerize more slowly than those with free ends. Slowing of depolymerization was indeed observed with kinesin-coated beads. Unexpectedly, the chimeric KLP has the opposite effect, speeding up depolymerization. The authors do not mention whether the density of the chimeric KLP on beads is different than that for kinesin, and although the mechanism of this effect is unclear, resolving it might tell us a lot about the nature of the depolymerizing end and perhaps indirectly about the mechanism of dynamic instability. Regulation of microtubule polymerization dynamics is a novel role for motor proteins, adding to the biological possibilities of these versatile molecules.

Chromosome Movement In Vitro

The results of the pure motor bead experiments lead to the hypothesis that motor proteins at the kinetochore are responsible for coupling chromosome movement to depolymerizing microtubules. The paper in this issue attempts to identify the specific motor protein(s) at the kinetochore that acts as the coupling factor diagrammed in Fig. 1. To date, two KLPs and cytoplasmic dynein have been localized to mammalian kinetochores. Neither function-blocking antidynein antibodies nor UV–vanadate cleavage of cytoplasmic dynein affected chromosome movement in the assay. In contrast, the inhibitory effects of an antibody that recognizes multiple KLP motor domains prompted further investigation of kinetochore KLPs. Antibodies to two specific KLPs were tested for inhibitory effects in the assay: centromeric protein-E (CENP-E), a 312-kD KLP with an NH_2-terminal motor domain present in kinetochores and the midzone of anaphase spindles (23) and mitotic centromere-associated kinesin (MCAK), a 90-kD KLP with a central motor domain present in centromeres and spindle poles (22). Three out of four

1. *Abbreviations used in this paper*: CENP-E, centromeric protein-E; KLP, kinesin-like protein.

polyclonal antibodies to different regions of CENP-E, when preincubated with chromosomes, inhibit the depolymerization-driven movement of chromosomes, with one of them blocking it completely, while antibodies to MCAK had no effect.

These results clearly implicate CENP-E in depolymerization-driven chromosome movement, but with some caveats. It is always a concern that antibody inhibition may be steric rather than direct with a nearby protein being the real coupling factor. On a technical note, the anti–CENP-E antibodies were not affinity purified, and they were used at rather high concentrations to see a functional effect, far higher than the concentrations at which their specificity was tested by immunoblot and immunofluorescence methods. The latter concern is largely alleviated by the effects of three independent antibodies. More seriously, the morphological data in the paper do not prove that the microtubule–chromosome connection is mediated by kinetochores in the depolymerization assay. However, kinetochores are known to be strongly preferred binding sites for microtubules on chromosomes in vitro (14). Overall, the balance of evidence is strongly in favor of the authors' conclusion that CENP-E is important in coupling chromosome movement to microtubule depolymerization.

An important point in the paper that makes CENP-E an attractive candidate for mediating anaphase A movement in vivo is the observation by immunofluorescence that the protein is still attached to kinetochores at this stage of mitosis. Previously, it was thought that CENP-E was at kinetochores only until metaphase, and that it began to relocate to the spindle midzone at the metaphase–anaphase transition (23). By reexamining the localization in a different cell line with more prominent anaphase A, the authors show that this relocation is incomplete, and that CENP-E remains attached to kinetochores during anaphase A. In combination with the results of the in vitro analysis, this revised localization strongly suggests that CENP-E may play an important role in chromosome movement during anaphase A.

One surprise in the paper is the observation that preincubation of chromosomes with antibodies that inhibit their depolymerization-driven movement does not inhibit microtubule capture by kinetochores. The pellicle assay is not ideal for quantitating capture, so the significance of this observation is unclear. However, it may suggest that distinct molecules mediate capture and movement. In favor of this idea, recent in vitro work has identified distinct proteins (a motor protein Kar3 [12] and unidentified microtubule-binding proteins [17]) that interact with the centromere-binding CBF3 complex in *Saccharomyces cerevisiae.* Alternatively, CENP-E may mediate both capture and movement in mammalian chromosomes, with the antibodies blocking only the latter activity. The most potent CENP-E–specific antibody interacts with the neck of CENP-E, where it might block movement of the head on the stalk without blocking microtubule binding. Neck flexibility is probaby required in the ATPase mechanochemical cycle, but its importance during motor-mediated diffusional sliding is less clear. Addressing the effects of the various antibodies on the interaction of pure CENP-E with microtubules would help address this issue. Another useful extension of the current work would be to look at the behavior of pure CENP-E on beads in the depolymerization assay. It will be interesting to see if CENP-E enhances or decreases microtubule depolymerization in the pellicle assay.

Anaphase A In Vivo

The use of microtubule depolymerization to power anaphase A movement represents an elegant way to use the energy stored in the labile lattice of the polymer to do work in the cell. The thermodynamic drive to depolymerization derives from the GTP hydrolysis that accompanies polymerization. It is somewhat ironic to see an ATPase motor protein coupling this energy to movement in the absence of ATP hydrolysis. Proving that microtubule depolymerization drives anaphase A in vivo and that CENP-E is the coupling protein will be challenging. Domain analysis of CENP-E and microinjection of inhibitory antibodies will be important future approaches, as will genetic tests of all putative anaphase mechanisms.

Having a motor as the candidate coupling factor adds an extra element of uncertainty in interpreting such experiments because the role of the motor as a force generating ATPase will have to be distinguished from its coupling role. The extent of this ambiguity might be addressed by extending the analysis of pure motors attached to beads to determine how motors with different intrinsic rates and polarities behave in the depolymerization assay in the presence of ATP. The authors' analysis of kinesin-coated beads in the presence of ATP uses extremely fast microtubule depolymerization rates, making it difficult to interpret their data. Performing their assay using motors of different polarities and rates at physiological depolymerization rates will be very informative. For CENP-E, distinguishing between its motor activity and its role as a coupling factor during anaphase A will depend in part on its polarity. If it is a plus-end–directed motor, then any role in anaphase A force production could only be as a coupling factor. The same would be true if it is not a motile ATPase despite its similarity to kinesin, though absence of motile function would be hard to prove. If, however, it turns out to be a minus-end–directed motor (as suggested by Thrower, D. A., M. A. Jordan, B. Schaar, T. Yen, and L. Wilson. 1994. *Mol. Biol. Cell.* 5[Suppl.]:40*a*), then the distinction will be more difficult. However, the two papers by Lombillo, McIntosh, and co-workers define a new function for motor proteins as molecules that couple their attached cargo to depolymerizing microtubules and their identification of CENP-E as being the kinetochore coupling factor in vitro will help elucidate the mechanism of anaphase A in vivo.

In view of space limitations, we apologize to those whose work was not directly cited. We would like to thank Claire Walczak for helpful comments about the manuscript. A. Desai is a Howard Hughes Medical Institute predoctoral fellow.

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References

1. Coue, M., V. A. Lombillo, and J. R. McIntosh. 1991. Microtubule depolymerization promotes particle and chromosome movement in vitro. *J. Cell Biol.* 112:1165–1175.
2. Gliksman, N. R., and E. D. Salmon. 1993. Microtubule-associated motility in cytoplasmic extracts of sea urchin eggs. *Cell Motil. Cytoskeleton.* 24:167–178.
3. Gorbsky, G. J., P. J. Sammak, and G. G. Borisy. 1987. Chromosomes move poleward in anaphase along stationary microtubules that coor-

dinately disassemble from their kinetochore ends. *J. Cell Biol.* 104:9–18.
4. Hill, T. L. 1985. Theoretical problems related to the attachment of microtubules to kinetochores. *Proc. Natl. Acad. Sci. USA.* 82:4404–4408.
5. Hyman, A. A., and T. J. Mitchison. 1991. Two different microtubule-based motor activities with opposite polarities in kinetochores. *Nature (Lond.).* 351:206–211.
6. Inoue, S. 1952. The effect of colchicine on the microscopic and submicroscopic structure of the mitotic spindle. *Exp. Cell Res.* 2(Suppl.): 305–318.
7. Inoue, S., and H. Sato. 1967. Cell motility by labile association of molecules. The nature of mitotic spindle fibers and their role in chromosome movement. *J. Gen. Physiol.* 50(Suppl.):259–292.
8. Koshland, D. E., T. J. Mitchison, and M.W. Kirschner. 1988. Polewards chromosome movement driven by microtubule depolymerization in vitro. *Nature (Lond.).* 331:499–504.
9. Lombillo, V. A., M. Coue, and J. R. McIntosh. 1993. In vitro motility assays using microtubules tethered to *Tetrahymena* pellicles. *Methods Cell Biol.* 39:149–165.
10. Lombillo, V., C. Nislow, T. J. Yen, V. I. Gelfand, and J. R. McIntosh. 1995. Antibodies to the kinesin motor domain and CENP-E inhibit depolymerization-dependent motion of chromosomes in vitro. *J. Cell Biol.* 128:107–115.
11. Lombillo, V. A., R. J. Stewart, and J. R. McIntosh. Microtubule depolymerization can drive minus-end directed motion of kinesin-coated microspheres. *Nature (Lond.).* In press.
12. Middleton, K., and J. Carbon. 1994. KAR3-encoded kinesin is a minus-end directed motor that functions with centromere binding proteins (CBF3) on an in vitro yeast kinetochore. *Proc. Nat. Acad. Sci. USA.* 91:7212–7216.
13. Mitchison, T. J., and M. W. Kirschner. 1985. Properties of the kinetochore in vitro. I. Microtubule nucleation and tubulin binding. *J. Cell Biol.* 101:755–765.
14. Mitchison, T. J., and M. W. Kirschner. 1985. Properties of the kinetochore in vitro. II. Microtubule capture and ATP-dependent translocation. *J. Cell Biol.* 101:766–777.
15. Mitchison, T. J., and E. D. Salmon. 1992. Poleward kinetochore fiber movement occurs during both metaphase and anaphase-A in newt lung cell mitosis. *J. Cell Biol.* 119:569–582.
16. Pfarr, C. M., M. Coue, P. M. Grissom, T. S. Hays, M. E. Porter, and J. R. McIntosh. 1990. Cytoplasmic dynein is localized to kinetochores during mitosis. *Nature (Lond.).* 345:263–265.
17. Severin, F. F., P. K. Sorger, and A. A. Hyman. 1994. Microtubule binding activity of the *S. cerevisiae* kinetochore. Yeast chromosome structure, replication and segregation. FASEB Summer Conference, Santa Cruz.
18. Skibbens, R. V., V. P. Skeen, and E. D. Salmon. 1993. Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: a push-pull mechanism. *J. Cell Biol.* 122:859–875.
19. Spurck, T. P., and J. Pickett-Heaps. 1987. On the mechanism of anaphase A: evidence that ATP is needed for microtubule disassembly and not generation of polewards force. *J. Cell Biol.* 105:1691–1705.
20. Telzer, B. R., M. J. Moses, and J. L. Rosenbaum. 1975. Assembly of microtubules onto kinetochores of isolated mitotic chromosomes of HeLa cells. *Proc. Natl. Acad. Sci. USA.* 72:4023–4027.
21. Vale, R. D., D. R. Soll, and I. R. Gibbons. 1989. One-dimensional diffusion of microtubules bound to flagellar dynein. *Cell.* 59:915-925.
22. Wordeman, L., and T. J. Mitchison. 1995. Identification and partial characterization of MCAK, a kinesin-related protein that associates with centromeres during mitosis. *J. Cell Biol.* 128:95–105.
23. Yen, T. J., G. Li, B. T. Schaar, I. Szilak, and D. W. Cleveland. 1992. CENP-E is a putative kinetochore motor that accumulates just before mitosis. *Nature (Lond.).* 359:536–539.