Mitosis: Riding the Protofilament Curl

More than 50 years ago, microtubule depolymerization was proposed as the force responsible for chromosome movement. New studies measure the force produced by depolymerization and show that protein ring complexes can couple depolymerization to movement. These results have implications for anaphase chromosome motility and mitotic evolution.

Lynne Cassimeris

Eukaryotic cell division typically couples anaphase A chromosome movement with depolymerization of spindle microtubules (Figure 1). Microtubule plus ends attach to kinetochores — protein complexes bound to centromeric DNA — and microtubule minus ends are at the spindle poles. In most cells, microtubule depolymerization occurs from the plus ends as chromosomes move toward the pole (Figure 1). Two general mechanisms operating at the kinetochore could power chromosome-to-pole movement: microtubule depolymerization could generate force and the kinetochore could hold on to this depolymerizing end, or minus-end-directed motor proteins could generate the force for chromosome movement as the microtubule depolymerizes.

The idea that chromosome movement could be powered by microtubule depolymerization originated from studies in which movement was induced experimentally by drug-mediated depolymerization [1]. More recent experiments, using *in vitro* mixtures of microtubules and isolated chromosomes or glass beads, showed that chromosomes or beads can move on depolymerizing microtubules in the absence of ATP [2–5]. Early models proposed mechanisms for microtubule force production [5,6], but questions remained regarding how much force could be produced by depolymerization and how something could remain tethered to the end of a microtubule that is falling apart. Several recent studies now address these questions [7–9].

Microtubules are polymers of α/β tubulin dimers arranged in 13 linear chains called protofilaments (Figure 1). Tubulins add to microtubule ends as GTP–tubulin dimers and hydrolyze GTP to GDP shortly after addition, resulting in a microtubule composed primarily of GDP–tubulin and a small cap of GTP–tubulin at the ends. Whether dimers are bound to GDP or GTP determines their preferred conformation. Protofilaments of GDP–tubulin bend away from the microtubule cylinder, while GTP–tubulin forms straight, or nearly straight, protofilaments [10]. The GDP–tubulin molecules in the microtubule are under strain, being held in a non-preferred straight conformation by the GTP–tubulin cap and lateral bonds with neighboring protofilaments. Loss of the GTP cap and lateral contacts allows protofilaments to relax to their preferred conformation and curl out from the microtubule wall [10] (Figure 1). Force generation by depolymerizing microtubules could be powered by GTP hydrolysis and the resulting structural change in the microtubule lattice [6,11,12].

Grishchuk and co-workers [7] have now measured the force produced by a depolymerizing microtubule. By attaching streptavidin-coated glass beads to biotinylated microtubules, the force generated on the bead as the microtubule depolymerizes was measured using optical tweezers and estimated at about 5 pN. Given the size and shape of the bead and the cylindrical shape of the microtubule, it is likely that only one or two protofilaments bind to the bead and exert force. The total force possible from all 13 protofilaments is about 30–65 pN.

How does the force measured for depolymerizing microtubules relate to the force generated during anaphase A? An applied force of about 700 pN is required to stall a chromosome in anaphase [13]. Assuming about 15 microtubules run from kinetochore to pole in the grasshopper spermatocytes used in these experiments, each microtubule contributes about 50 pN to poleward force [13], a force that could be produced by 13 peeling protofilaments [7].

Microtubule depolymerization may generate sufficient force to move a chromosome, but this mechanism requires a way to couple the chromosome to the plus ends of microtubules as these ends peel apart. Hypothetical models suggested that the coupler has the structure of a collar or ring [5,6,12]. Several recent studies now provide strong support for a ring-shaped coupler in budding yeast. The ten-protein Dam1 complex forms rings around microtubules *in vitro* [14,15]. This protein complex assembles into a ring that is about 54 nm wide with a central opening of about 32 nm [14,15]. Microtubules are 25 nm wide so the Dam1 rings do not bind directly to the walls of the microtubule. Instead, there is a gap of about 4 nm between the microtubule and the ring. Dam1 rings form preferentially around microtubules composed of GTP–tubulin, suggesting that rings may form preferentially at the ends of microtubules.

A new computational model suggests that the Dam1 ring complex has the right shape to harness the force produced by a depolymerizing microtubule [8]. The powerstroke occurs as each protofilament peels away from microtubule wall. Curling protofilaments push on the ring's edge, moving the ring toward the opposite microtubule end [8]. Force production requires space between the ring and the microtubule so that the protofilaments can break lateral bonds with neighboring protofilaments and curl away from the microtubule. Maximum force production is predicted for a ring that is spaced 5–7 nm from the

microtubule wall [8], nearly identical to that of the Dam1 ring [14,15]. If the ring fits more snugly against the microtubule it will inhibit protofilament peeling. If the ring fits more loosely it will be too far from the site of force production — the point where lateral bonds are broken and the protofilaments begin to peel away [8].

Modeling and force measurements suggest that the Dam1 rings should slide with a microtubule tip as it depolymerizes, but do they? Westermann *et al.* [9] now report direct observation of Dam1 ring movement on shortening microtubules *in vitro*. In their assay, Dam1 rings and microtubules were labelled with separate fluorescent tags to allow real-time visualization of both components. For microtubules decorated with Dam1 rings spaced along their length, depolymerization collected the rings at the depolymerizing end (Figure 1). Rings moved with the depolymerizing end for 2 μm or more, indicating that individual rings could move processively for distances longer than the length of the budding yeast spindle. Beads attached to Dam1 rings also moved along with depolymerizing microtubules, showing that Dam1 rings can function as a coupler and link movement of cargo to depolymerization.

The picture emerging from *in vitro* measurements of the force produced by depolymerizing microtubules and the ability of Dam1 rings to couple depolymerization to movement suggests that anaphase A could be powered solely by microtubule depolymerization. Showing that depolymerization is sufficient to move chromosomes in a cell is not so easy. In budding yeast, the Dam1 complex is part of the outer kinetochore plate and functions in microtubule attachment [16], but it is not known whether the Dam1 complex forms rings *in vivo* [17]. Whether kinetochore rings exist in other organisms is also unknown because homologs of the Dam1 complex have not been identified in organisms other than yeasts [17]. In all eukaryotic cells examined, motor proteins play prominent roles in spindle assembly and chromosome movements in prometaphase, metaphase and anaphase (for example, see [18–20]), making it difficult to study possible depolymerization-driven anaphase movement *in vivo*.

Perhaps the movement of Dam1p rings by depolymerizing microtubules is showing us a glimpse of the earliest eukaryotic mitotic mechanism. Movement of chromosome(s) to opposite ends of the cell could be powered simply by linking DNA to a depolymerizing microtubule. Tubulins self-assemble into dynamic microtubules and require no other components to convert energy from GTP hydrolysis into a force sufficient to move chromosomes. Strong selective pressure would favor the addition of components to increase the accuracy of chromosome segregation to daughter cells. The result of this selection may be the many motor and non-motor proteins that contribute to chromosome segregation in today's cells.

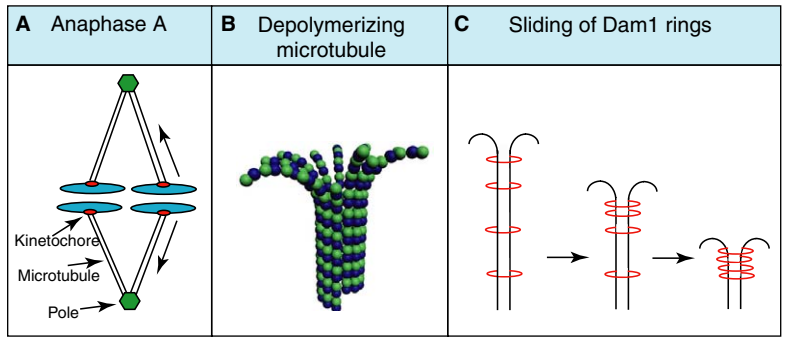

Figure 1. Motility coupled to microtubule depolymerization.

(A) Schematic diagram of spindle organization and anaphase A chromosome motility. Chromosomes (blue) attach to spindle microtubules at kinetochores (red) and move toward the spindle poles (green) as microtubules depolymerize (arrows). (B) Microtubules are composed of alpha and beta tubulins (blue, green) arranged in 13 linear protofilaments to form a microtubule cylinder. When microtubules depolymerize the protofilaments curl out from the wall of cylinder, breaking lateral contacts between protofilaments [10]. Protofilament curling generates force [8]. (C) Rings (red) composed of the ten-protein Dam1 complex from budding yeast kinetochores assemble around microtubules (black lines) *in vitro* [14,15] and are pushed by the curling protofilaments as the microtubule shortens [9].

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Department of Biological Sciences, 111 Research Drive, Lehigh University, Bethlehem, Pennsylvania 18015, USA.
E-mail: lc07@lehigh.edu

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