Separating Duplicated Chromosomes in preparation for Cell Division

Part 3: Moving the chromosomes to the spindle poles: the mechanisms of Anaphase A

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How do chromosome approach the poles in anaphase A?

- The Motor Hypothesis: enzymes, like a kinesin or a dynein, drive chromosomes to the poles
- But MTs must shorten in Anaphase A, suggesting:
- The Depolymerization Hypothesis: MT shortening drives chromosome motion

Test the motor hypothesis by gene deletion

- Inactivating even a single motor often makes problems in mitosis, like an increase in rate of chromosome loss, but many parts of mitosis still go fine.
- Turn to yeasts for a "definitive" experiment. Delete the genes for all minus end-directed motors and ask what happens to the rate of chromosome-to-pole motion?

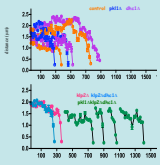
Fission yeasts lacking all minus end-directed motors still move their chromosomes poleward at a normal rate

pkl1D klp2D dhc1D

kinetochore retrieval and anaphase

Grishchuk and McIntosh EMBO J 25:4888(2006)

Deletion of all the minus-end directed motors in fission yeast does not slow pole-directed chromosome movement

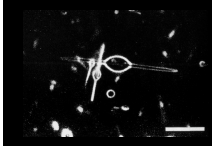


Budding yeast too can move its chromosomes to the poles without pole-directed motors

(Tanaka et al., J.Cell Biol. 178:268(2007)

- Such motion could be caused by a non-microtubule component of the spindle, or
- It could be that microtubule depolymerization itself is a motor
- How to find out? Try experiments in vitro.

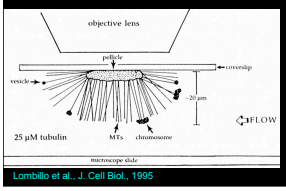
Microtubule polymerization can do mechanical work, e.g.,



Actin polymerization too can do work. See the iBio Seminar by Julie Theriot

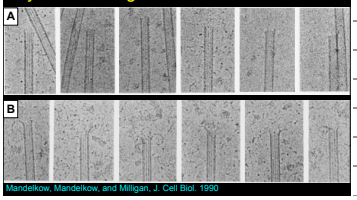
Miyamoto and Hotani, Proc. Tanaguichi Symp 14:220(1988)

Experimental system to see if microtubule **DE**polymerization



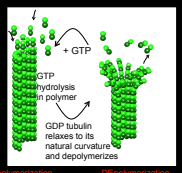
Lombillo et al., J. Cell Biol., 1995

How could depolymerization pull on anything? Structure of the ends of frozen-hydrated MTs gives a clue: tubulin bends!



Mandelkow, Mandelkow, and Milligan, J. Cell Biol. 1990

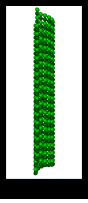
Where does this bend come from? The mechanochemical cycle of microtubule dynamics



Molecular-mechanical model of microtubule; Molodtsov et al., *Biophys. J.* 2005

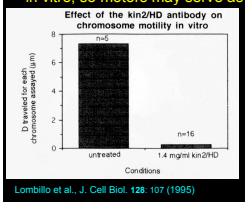
Are remarkable polymers, they are hollow tubes 25 nm in diameter formed by tubulins. They grow many microns by tubulin addition at the tip PROTEINs. Grow and shrink – length must change for chromosome motion

Calculated simulation of MT depolymerization



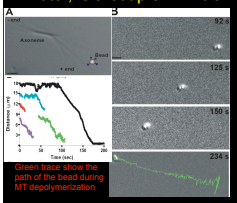
How can the cell take advantage of this mechanism to do work?
It needs a "Coupler" that will attach a load to the shortening microtubule end.

Antibodies to a kinesin strongly inhibit MT depolymerization-driven, chromosome motion in vitro, so motors may serve as couplers.



Lombillo et al., J. Cell Biol. **128**: 107 (1995)

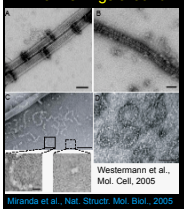
Kinesin 8, a plus end-directed motor, is a coupler in vitro



Green trace show the path of the bead during MT depolymerization

Grissom et al., Mol Biol. 2008

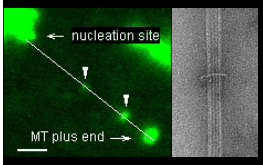
There are also couplers that are not motors: The Dam1 complex forms rings around MTs



Westermann et al., Mol. Cell, 2005

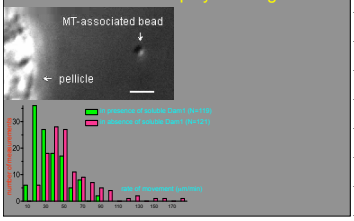
Miranda et al., Nat. Structr. Mol. Biol., 2005

DAM/DASH-GFP on MTs with rhodamine-tubulin caps; Collaboration with Barnes, Westermann, and Drubin, Berkeley, CA

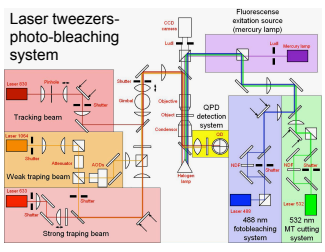


Grishchuk et al., Proc. Natl. Acad. Sci. 2008

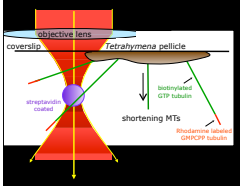
The Dam1 Complex can Couple a microbead to a depolymerizing MT



Laser tweezers-photo-bleaching system

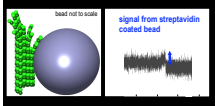


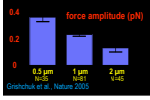
Detecting the bending of tubulin protofilaments



Grishchuk et al., Nature 2005

Depolymerizing MTs can exert enough force on a bead to move a mitotic chromosome

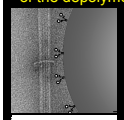




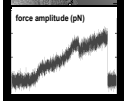
Grishchuk et al., Nature 2005

Noise from brownian movement, 5pN is similar to kinesin, introduce coupler

Dam1/DASH ring can capture much of the depolymerization force



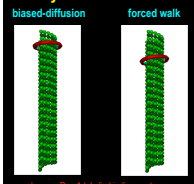
MT and Dam1 ring from an electron micrograph. Bead drawn to scale with cartoon antibodies on it.



Force developed on a Bead as the MT shortens; Measurement made with Laser tweezers.

Grishchuk et al., PNAS 2008

Possible modes of ring motility on the MT surface

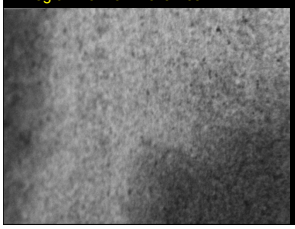


Efremov et al., PNAS 2007

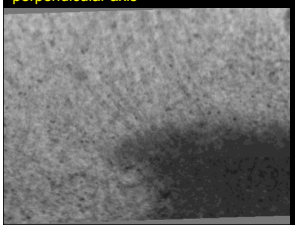
Is the DAM/DASH complex the kinetochore-MT coupler?

- In budding yeast, DAM is in the spindle; it binds to chromatin, and it is essential for proper chromosome attachment and segregation
- In wild type fission yeast DAM is NOT essential, although it localizes to the spindle
- Outside of the fungi, the proteins of this complex have not been found
- Go looking at the K-MT connection with a powerful structural tool : EM tomography

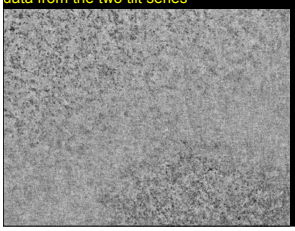
Tilted views of the kinetochore regionin a mammalian cell



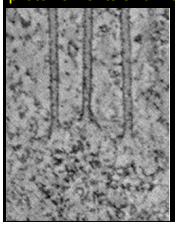
A second set of tilted views about a perpendicular axis



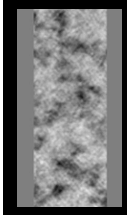
A 3-D reconstruction, made by combining data from the two tilt series



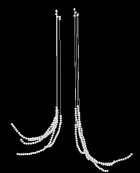
Mammalian KMTs in prometaphase; bent protofilaments and many fibrils, but no rings



Multiple slices that contain an MT axis

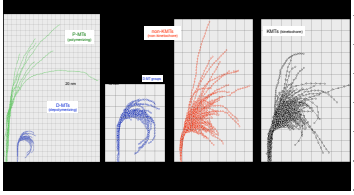


A Model of many PFs from one MT

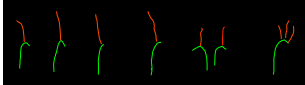


McIntosh et al., Cell, 2008

Comparison of polymerizing and depolymerizing PFs in vitro with kinetochore and non-kinetochore PFS in vivo

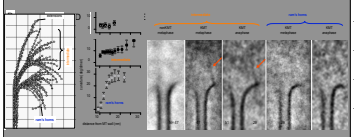


Bending protofilaments are connected to chromatin by slender filaments

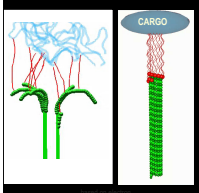


McIntosh et al., Cell, 2008

Protofilaments of intermediate shape (between polymerizing and depolymerizing) are bound to chromatin by straight fibrils



Fibrillar couplers can in principle support processive motion



McIntosh et al., Cell, 135:322 (2008)

Similar fibrils connect KMTs and kinetochores of mammals, yeasts and nematode. So they are common, but what are these fibrils made from?

- The kinetochore-localized motors, Kinesins 7 and 8 look like fibrous proteins. Perhaps they contribute?
- NDC80 is a fibrous, kinetochore-localized, MT-binding protein (not a motor) that is essential for MT-chromosome connections wherever it has been found
- Other fibrous proteins are common in kinetochores; some might serve as connectors

The molecular nature of the coupling between kinetochores and spindle microtubules is not yet known

Evidence from localization of proteins, from genetic disruption of particular components, and from biochemistry suggests that the coupling involves several different proteins, and it is probably somewhat different in different organisms, depending on their mitotic physiology.

A great problem for future work!