Separating Duplicated Chromosomes in preparation for Cell Division

Part 2: Understanding mitosis through experimentation

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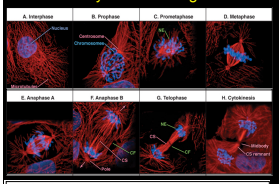
The jobs a cell must do to achieve accurate chromosome segregation

- Make the mitotic spindle
- Attach the chromosomes to the spindle and organize them
- Segregate the chromosomes and elongate the spindle

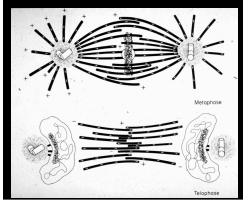
Our approaches to understanding the cell's solutions to these issues

- For the pathways of spindle formation, go look at the third iBio Seminar by Ron Vale
- We will focus on the mechanical properties of the spindle and see how they affect both chromosome attachment and chromosome segregation
- Start with spindle properties, then go to spindle elongation, because it is simpler than the processes of chromosome attachment and motion to the spindle poles

Regardless of the pathway for its formation, the spindle has fundamentally the same organization

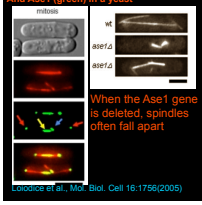


How are the two halves of the spindle connected to form a bipolar structure?



A MT-cross-linking protein, Ase1, is important for keeping the two half-spindles linked

Localization of tubulin (red)
And Ase1 (green) in a yeast



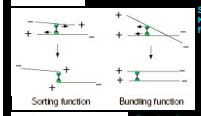
When the Ase1 gene is deleted, spindles often fall apart

Loïodice et al., Mol. Biol. Cell 16:1756(2005)

Motor enzymes too are required to establish spindle bipolarity

Schematic of Kinesin-5 organization

A homo-tetrameric motor enzyme, Kinesin-5, is required in most cells to get the half-spindles to interact

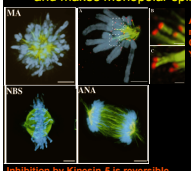


Schematic of Kinesin-5 function

Results of Kinesin-5 action

Walczak et al., Current Biology 8:903 (1998)

The small molecule, "Monastrol" inhibits the action of mammalian Kinesin 5 and makes monopolar spindles



A monastrol-generated, monopolar spindle includes Ca++ stable kinetochore MTs; Yellow=MT; red=kinetochore

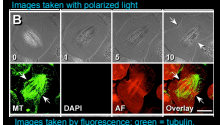
Inhibition by Kinesin-5 is reversible

Kapoor et al., J. Cell Biol. 150:957(2000)

Surprisingly, chromosomes are not required to have a bipolar spindle

A microneedle has been used to pull the chromosomes Away from a forming spindle, yet biopolarity is maintained And the spindle induces cell cleavage in a normal way

Images taken with polarized light

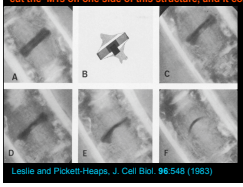


Images taken by fluorescence; green = tubulin, red = actin

Alsop, Zhang J. Cell Biol. 162:383 (2003)

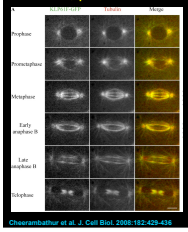
When chromosomes are present, there is an INWARD force acting on the spindle poles.

In diatoms, the inter-polar spindle is ordered at metaphase and easy to see with polarized light. A UV micro-beam can cut the MTs on one side of this structure, and it collapses.



Leslie and Pickett-Heaps, J. Cell Biol. 96:548 (1983)

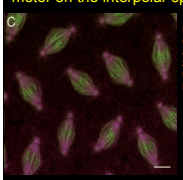
Why doesn't the spindle normally collapse? Kinesin-5, pushes outward from the middle



Chesarambathur et al. J. Cell Biol. 2008:182:429-436

Localization and function of KLP61F-GFP in D. melanogaster embryo mitosis. (A) Micrographs from a time-lapse video of a representative spindle showing KLP61F-GFP (left), rhodamine-tubulin (center), and double-label fluorescence (right) at various stages of mitosis. The plots (far right) are line scans extending pole to pole along an ipMT (10 pixels wide; ~0.129 μm/pixel) for KLP61F (green) and tubulin (red). The y axis shows normalized fluorescence intensity. Bar, 5 μm. (B) Spindle pole dynamics in wild-type embryos, GFP-KLP61F rescued mutant embryos, and anti-KLP61F microinjected wild-type embryos showing how bipolar spindles collapse into monoasters after the loss of KLP61F function. Pole–pole separation dynamics are very similar in wild-type and rescued mutant embryos.

But Kinesin-5 is not the only motor on the interpolar spindle

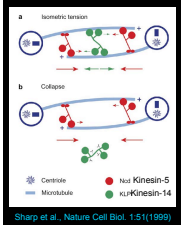


Kinesin-14s, which act in the opposite direction from Kinesin-5, are localized near the spindle mid-zone (green), while tubulin (red) is stronger near the poles, making purple.

Kinesin-5 and Kinesin-14 make a balance of push and pull

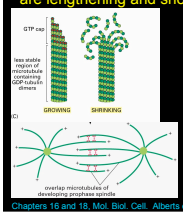
Endow and Komma, J. Cell Sci. 109, 2429 (1996)

A model for how these motors might create a balance of forces



Sharp et al., Nature Cell Biol. 1:51(1999)

But the spindle is also dynamic in the sense that its component MTs are lengthening and shortening



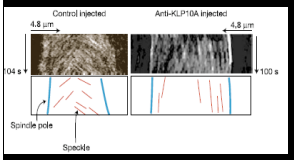
Chapters 16 and 18, Mol. Biol. Cell. Alberts et al.

Even when spindle length is constant, MTs are polymerizing and depolymerizing; they "treadmill."

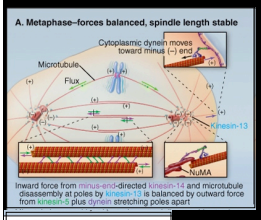
Fluorescent Speckle Imaging: Zoomed, cropped Xenopus extract spindles.

Desai, Waterman and Salmon from http://www.bio.unc.edu/Faculty/Salmon/lab

A depolymerization motor, Kinesin-13, is important for MT flux: an antibody to this kinesin slows MT flux (speckled MTs)



Rogers et al., Nature 427:364(2004)

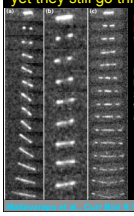


© Elsevier. Pollard et al: Cell Biology 2e.

How does all this relate to chromosome segregation?

- If the MTs of a metaphase spindle are in flux, anaphase A could happen simply as a result of separating the sister chromatids and letting each chromosome copy join the flow
- Anaphase B could happen by stopping the disassembly of spindle MTs at the poles, so the poles are forced apart by the growing and sliding MTs
- Is this how mitosis really works? It is probably a good part of the story, BUT

As usual, biology is not that simple. Not all spindles show flux, yet they still go through anaphase



Progress of spindle elongation in fission yeast, seen with fluorescent tubulin in vivo

Each spindle is bleached by a microbeam at line 2. Successive times show the behavior of the bleach as time goes by.

Mitchison and Salmon, Nature Cell Biol. 3:E17(2001)

In many fungi and some other cells spindles elongate a lot; this depends on more than pushing from the spindle midzone

Target	Schematic	Rate of separation* μm/min ± 1 SD
Unirradiated		7.6 ± 1.2
Spindle		22.4 ± 12.9
Nucleoplasm	N	8.6 ± 1.7
Cytoplasm	N	6.1 ± 1.5

Aist and Berns, J Cell Biol. 91:445(1981)

Fungi and some other cells have a motor that pulls on the poles. Genetics suggests that the pull comes from dynein in the cortex interacting with astral MTs

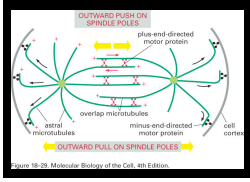
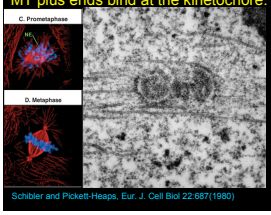
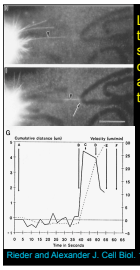


Figure 18-29, Molecular Biology of the Cell, 4th Edition.

Now we can ask, how do chromosomes attach to the spindle? MT plus ends bind at the kinetochore.



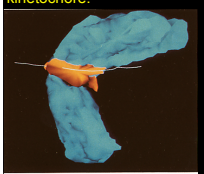
Schibler and Pickett-Heaps, Eur. J. Cell Biol 22:687(1980)



Live cells injected with fluorescent tubulin show MT growth from the spindle pole; soluble tubulin shows chromosome position. When a MT encounters a kinetochore, the chromosome moves poleward

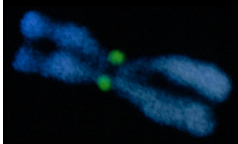
Rieder and Alexander J. Cell Biol. 110:81(1990)

3D electron microscopy shows the MT in grazing contact with the kinetochore.



Rieder and Alexander J. Cell Biol. 110:81(1990)

Cytoplasmic dynein is a component of prometaphase kinetochores. It may be responsible for these movements

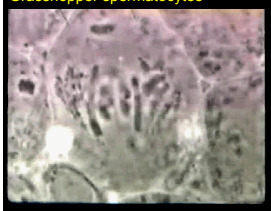


Pfarr et al., Nature 345:262(1990)

How do chromosomes make stable, bipolar attachments to the spindle?

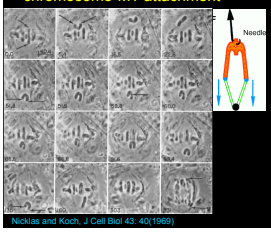
- **The central problem of mitosis is attaching sister kinetochores to sister spindle poles**
- If MTs are coming from both poles, how do the kinetochores know which ones they should bindto?
- Experiments suggest that they bind any MT plus end, but they form stable attachments only when the kinetochore-MT junction is under tension

Manipulation of chromosomes in living Grasshopper spermatocytes



Nicklas, R.B. Chromosoma 21:17(1967)

Tension promotes the stability of chromosome-MT attachment

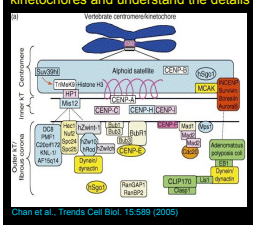


Nicklas and Koch, J Cell Biol 43: 40(1969)

What generates tension at kinetochores?

- Kinetochore dynein is one possibility
- But antibody injection experiments from our lab suggest that dynein doesn't matter for chromosome attachment, and most dynein leaves the kinetochore shortly after the chromosome attaches to MTs
- A direct test by mutation of dynein? Not currently possible because dynein is used by the cell for so many things, mitosis may not even start. Need a quick effect.
- Hard to get a good temperature-sensitive mutant for dynein, because the protein is so big. See iBio seminar by Ron Vale

Work is ongoing to learn the protein composition of kinetochores and understand the details of their function

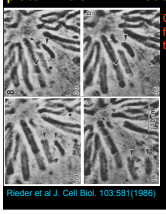


Chan et al., Trends Cell Biol. 15:589 (2005)

Understanding biochemical complexity in the kinetochore is important, but relating it to function is hard

- Commonly, one can mutate a gene and see the phenotype, but when many gene products are interacting, indirect effects are common
- The same applies for antibody and drug perturbations, even with specific reagents
- Many kinetochore proteins are modified post-translationally (e.g., by phosphorylation), so kinetochore state is subject to change
- Understanding the roles of each kinetochore protein will take data from many experimental approaches, as discussed in the next lecture

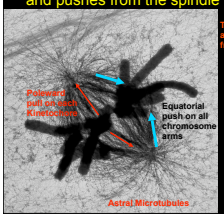
One final mitotic issue: how do the chromosomes get to the metaphase plate? Polar "Elimination Forces"



Cutting with a microbeam makes fragments without kinetochores that are pushed away from the pole.

Rieder et al J. Cell Biol. 103:581(1986)

Metaphase in some cells results from a balance of forces on kinetochores and pushes from the spindle poles



These pushes come from yet another kinesin and perhaps from MT polymerization

Mitosis still provides many puzzles for the interested biologist

- We must learn the biochemical basis of each spindle function:
  1) chromosome attachment,
  2) congression to the metaphase plate,
  3) regulation of anaphase onset, and
  4) the mechanisms of chromosome-to-pole motion
- The next lecture will present one approach to unraveling this kind of complexity: work in vitro on a specific subset of the problems