

**CONCEPTS AND MECHANISMS IN MITOSIS**  
**PBIO 558**  
Minicourse offered by Chip Asbury & Linda Wordeman

Spring Quarter, 2007

<b>MEETING TIMES AND LOCATION</b>	Mondays, 9:30 – 11:30 am, room T474A
<b>COURSE WEBSITE</b>	<a href="http://courses.washington.edu/pbio558">http://courses.washington.edu/pbio558</a>
<b>INSTRUCTOR CONTACT INFORMATION</b>	Linda, <a href="mailto:worde@u.washington.edu">worde@u.washington.edu</a> Chip, <a href="mailto:casbury@u.washington.edu">casbury@u.washington.edu</a>

**BRIEF COURSE DESCRIPTION** In this 10-week (2 credit) mini-course, we will examine how the mitotic spindle organizes and separates duplicated chromosomes during cell division. The course begins with a brief overview of spindle components and key mechanistic concepts. Students then choose specific areas for discussion, focusing on recent and/or classic papers from the primary literature.

**PRIMARY GOALS** The main purpose of the course is to review our current understanding of the mechanisms underlying chromosome organization and movement during cell division. The secondary goal is to provide experience for the students in reading, evaluating, and presenting published results from the literature. Most of the course will involve presentations and discussion by the students.

**ENROLLMENT** PBIO students can register freely, other graduate students may register by permission of the instructors

**SCHEDULE**

*WEEK 1: Introduction -- Chip*

1. What the spindle does and why it is important.
2. Description of major parts of the spindle
3. Stages of mitosis:  
spindle assembly, KT capture, biorientation, congression, metaphase, anaphase A/B
4. Likely sites of force production and signalling
5. What are the big questions still left unanswered?
6. Sign-up for presentations

*WEEK 2: Dynamic instability of microtubules & the GTP cap hypothesis*

1. Describe microtubule structure, and the classic two-state dynamic instability.
2. What is known about filament tip conformations during growth and shortening?
3. Describe the GTP cap hypothesis and evaluate some evidence in favor of it.
4. How might a microtubule-binding protein influence filament dynamics?

**WEEK 3: *Discovery of spindle motors***

1. Briefly explain what a molecular motor is / does.
2. Where have motors been found in the spindle? List some of them.
3. Choose one or two, and describe how altering their function(s) affect mitosis.
4. What does this suggest about the role these motors play in the process?

**WEEK 4: *Two differing views -- polymer driven vs. 'muscle-like' mitosis***

0. Describe the arrangement of microtubules in the spindle in animal cells and yeast.
1. Describe the essence of the polymer-driven hypothesis
2. What evidence supports this picture? Explain some of the problems with it too.
3. Describe the 'muscle-like' hypothesis for mitosis
4. What evidence supports this picture? Explain some of the problems with it too.

**WEEK 5: *'Flux' -- where are tubulin subunits being added and removed?***

1. Describe where tubulin subunits are added to and removed from the spindle.
2. Describe the experiments used to observe flux (photo-bleaching, -activation, speckles).
3. Does flux occur in monopolar spindles or does it require bipolarity? Teach the controversy!
4. What would either result imply about the mechanism driving flux?

**WEEK 6: *Assembly is eerily robust – more ways than one to build a spindle***

1. How can search and capture lead to biorientation?
2. What evidence supports this picture?
3. Describe two alternative methods for biorientation, and evaluate some evidence for these.
4. Explain how spindles form even without spindle poles and kinetochores.

**WEEK 7: *Kinetochose movements – 'directional instability', tensiometer model***

1. Describe the movements of sister kinetochores after biorientation.
2. Describe how the tensiometer model can explain coordination of sister kinetochores.
3. What evidence supports the tensiometer model?
4. Can the tensiometer model explain congression? Why or why not?

**WEEK 8: *More micromanipulation – balance of forces in the spindle***

1. How much force does the spindle generate at kinetochores?
2. What are the 'polar winds'? How might they be generated?
3. Describe the experiments demonstrating 'polar winds' and measuring kinetochose forces.
4. How might chromosome position be dictated by the balance between these two?

**WEEK 9: *The spindle checkpoint -- attachment, tension, and error correction***

1. Describe the function of the spindle checkpoint.
2. Describe the protein signaling cascade that mediates the checkpoint delay.
3. How do we know the 'wait' signal is produced at the kinetochose?
4. Does the checkpoint sense only attachment, or attachment and tension?

**WEEK 10: *Ultrastructure of the kinetochose-microtubule interface***

1. Describe the 'tri-laminar' structure of the kinetochose, as seen by EM.
2. Where in this structure are motors located? Where are the checkpoint proteins?
3. How are the kinetochose microtubules arranged in mammalian cells, in budding yeast, in pombe?
4. Describe the arrangement and tip structures of kinetochose-attached microtubules.
5. How might the kinetochose stay attached to dynamic microtubules? How might it regulate their dynamics?

## READING LIST (PDF versions for most are posted on the course website)

### WEEK 1: *Classic views of mitosis, predating the discovery of microtubules*

1. Schrader, F. Hypotheses of mitosis. in *Mitosis: the movements of chromosomes in cell division*. Columbia University Press, New York (1953).
2. Ostergren, G., Mole-Bajer, J. & Bajer, A. An interpretation of transport phenomena at mitosis. *Annals NY Acad. Sci.* **90**:381-408 (1960).

### WEEK 2: *'Dynamic instability' of microtubules & the GTP cap hypothesis*

1. Mitchison, T. & Kirschner, M. Dynamic instability of microtubule growth. *Nature* **312**, 237-42 (1984).
2. Kristofferson, D., Mitchison, T. & Kirschner, M. Direct observation of steady-state microtubule dynamics. *J Cell Biol* **102**, 1007-19 (1986).
3. Walker, R.A. et al. Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies. *J Cell Biol* **107**, 1437-48 (1988).
4. Mandelkow, Mandelkow & Milligan Microtubule dynamics and microtubule caps: a time-resolved cryo-electron microscopy study. *J Cell Biol* **114**:977-991 (1991).
5. Chretien, D., Fuller, S.D. & Karsenti, E. Structure of growing microtubule ends: two-dimensional sheets close into tubes at variable rates. *J Cell Biol* **129**, 1311-28 (1995).
6. Caplow, M. & Shanks, J. Evidence that a single monolayer tubulin-GTP cap is both necessary and sufficient to stabilize microtubules. *Mol Biol Cell* **7**, 663-75 (1996).
7. Desai, A. & Mitchison, T. J. Microtubule polymerization dynamics. *Annu Rev Cell Dev Biol* **13**, 83-117 (1997).
8. Wilson, L. & Margolis, R. L. Microtubule treadmills and their possible cellular functions. *Cold Spring Harb Symp Quant Biol* **46 Pt 1**, 199-205 (1982).

### WEEK 3: *Discovery of spindle motors*

1. McDonald, H. B. & Goldstein, L. S. Identification and characterization of a gene encoding a kinesin-like protein in *Drosophila*. *Cell* **61**, 991-1000 (1990).
2. McDonald, H. B., Stewart, R. J. & Goldstein, L. S. The kinesin-like *ncd* protein of *Drosophila* is a minus end-directed microtubule motor. *Cell* **63**, 1159-65 (1990).
3. Steurer, E.R., L. Wordeman, T.A. Schroer and M.P. Sheetz. Localization of cytoplasmic dynein to mitotic spindles and kinetochores. *Nature* **345**, 266-268 (1990).
4. Wordeman, L. & Mitchison, T. J. Identification and partial characterization of mitotic centromere-associated kinesin, a kinesin-related protein that associates with centromeres during mitosis. *J Cell Biol* **128**, 95-104 (1995).
5. Yen, T. J., Li, G., Schaar, B. T., Szilak, I. & Cleveland, D. W. CENP-E is a putative kinetochore motor that accumulates just before mitosis. *Nature* **359**, 536-9 (1992).
6. Fuller, M. Riding the polar winds: chromosomes motor down east. *Cell* **81**:5-8 (1995).

### WEEK 4: *Two differing views – polymer-driven vs. 'muscle-like' mitosis*

1. Spurck, T.P. & Pickett-Heaps, J.D. On the mechanism of anaphase A: evidence that ATP is needed for microtubule disassembly and not generation of polewards force. *J Cell Bio* **105**:1691-1705 (1987).
2. Inoue, S. & Salmon, E. D. Force generation by microtubule assembly/disassembly in mitosis and related movements. *Mol Biol Cell* **6**, 1619-40 (1995).
3. Inoue, S. & Ritter H. Dynamics of mitotic spindle organization and function. in *Molecules and Cell Movement*, Inoue & Stephens, eds. Raven Press, New York (1975).
4. Coue, M., Lombillo, V. & McIntosh, J.R. Microtubule depolymerization promotes particle and chromosome movement in vitro. *J Cell Bio* **112**:1165-1175 (1991).
5. McIntosh, J.R., Cande, Z. & Snyder, J.A. Structure and physiology of the mammalian mitotic spindle. in *Molecules and Cell Movement*, Inoue & Stephens, eds. Raven Press, New York (1975).

6. McIntosh, J.R., Helper, P.K. & van Wie, D.G. Model for mitosis. *Nature* **224**:659-663 (1969).
7. Nicklas, R.B. Chromosome movement: current models and experiments on living cells. in *Molecules and Cell Movement*, Inoue & Stephens, eds. Raven Press, New York (1975).
8. Mitchison, T. & Salmon, E.D. Mitosis: a history of division. *Nat Cell Bio* **3**:E17-E21 (2001).

**WEEK 5: Flux -- where are tubulin subunits being added and removed?**

1. Mitchison, T. J. Polewards microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence. *J Cell Biol* **109**, 637-52 (1989).
2. Zhai, Y., Kronebusch, P.J. & Borisy, G. Kinetochore microtubule dynamics and the metaphase-anaphase transition. *J Cell Bio* **131**:721-734 (1995).
3. Maddox, P., Straight, A., Coughlin, P., Mitchison, T. J. & Salmon, E. D. Direct observation of microtubule dynamics at kinetochores in *Xenopus* extract spindles: implications for spindle mechanics. *J Cell Biol* **162**, 377-82 (2003).
4. Sawin, K.E. & Mitchison, T.J. Microtubule flux in mitosis is independent of chromosomes, centrosomes, and antiparallel microtubules. *Mol Bio Cell* **5**:217-226 (1994).
5. Mitchison, T.J., et al. Bipolarization and poleward flux correlate during *Xenopus* extract spindle assembly. *Mol Bio Cell* **15**:5603-5615 (2004).
6. Cameron, L. et al. Kinesin 5-independent poleward flux of kinetochore microtubules in PtK1 cells. *J Cell Bio* **173**:173-179 (2006).

**WEEK 6: Assembly is eerily robust – more ways than one to build a spindle**

1. Hayden, J. H., Bowser, S. S. & Rieder, C. L. Kinetochore capture astral microtubules during chromosome attachment to the mitotic spindle: direct visualization in live newt lung cells. *J Cell Biol* **111**, 1039-45 (1990).
2. Heald, R. et al. Self-organization of microtubules into bipolar spindles around artificial chromosomes in *Xenopus* egg extracts. *Nature* **382**, 420-5 (1996).
3. Maiato, H., Rieder, C. L. & Khodjakov, A. Kinetochore-driven formation of kinetochore fibers contributes to spindle assembly during animal mitosis. *J Cell Biol* **167**, 831-40 (2004).
4. Khodjakov, A., et al. Minus-end capture of preformed kinetochore fibers contributes to spindle morphogenesis. *J Cell Bio* **160**:671-683 (2003).
5. Caraxo-Salas, R.E., Gruss, O.J., Mattaj, I.W. & Karsenti, E. Ran-GTP coordinates regulation of microtubule nucleation and dynamics during mitotic-spindle assembly. *Nat Cell Bio* **3**:228-234 (2001).
6. Kapoor, T. M. et al. Chromosomes can congress to the metaphase plate before biorientation. *Science* **311**, 388-91 (2006).

**WEEK 7: Kinetochore movements – ‘directional instability’, tensiometer model**

1. Skibbens, R. V., Skeen, V. P. & Salmon, E. D. Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: a push-pull mechanism. *J Cell Biol* **122**, 859-75 (1993).
2. Skibbens, R. V., Rieder, C. L. & Salmon, E. D. Kinetochore motility after severing between sister centromeres using laser microsurgery: evidence that kinetochore directional instability and position is regulated by tension. *J Cell Sci* **108 ( Pt 7)**, 2537-48 (1995).
3. Waters, J.C., Skibbens, R.V. & Salmon, E.D. Oscillating mitotic newt lung cell kinetochores are, on average, under tension and rarely push. *J Cell Sci* **109**:2823-2831 (1996).
4. Skibbens, R.V. & Salmon, E.D. Micromanipulation of chromosomes in mitotic vertebrate tissue cells: tension controls the state of kinetochore movement. *Exp Cell Res* **235**:314-324 (1997).
5. Fuller, M. Riding the polar winds: chromosomes motor down east. *Cell* **81**:5-8 (1995).

**WEEK 8: More micromanipulation – balance of forces in the spindle**

1. Rieder, C. L., Davison, E. A., Jensen, L. C., Cassimeris, L. & Salmon, E. D. Oscillatory movements of monooriented chromosomes and their position relative to the spindle pole result from the ejection properties of the aster and half-spindle. *J Cell Biol* **103**, 581-91 (1986).

2. Nicklas, R.B. The forces that move chromosomes in mitosis. *Ann Rev Biophys Biophys Chem* **17**:431-449 (1988).
3. Nicklas, R.B. How cells get the right chromosomes. *Science* **275**:632-636 (1997).
4. Rieder, C.L. & Salmon, E.D. Motile kinetochores and polar ejection forces dictate chromosome position on the vertebrate mitotic spindle. *J Cell Bio* **124**:22-233 (1994).

**WEEK 9: *The spindle checkpoint -- attachment, tension, and error correction***

1. Rieder, C. L., Cole, R. W., Khodjakov, A. & Sluder, G. The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores. *J Cell Biol* **130**, 941-8 (1995).
2. Li, X. & Nicklas, R. B. Mitotic forces control a cell-cycle checkpoint. *Nature* **373**, 630-2 (1995).
3. Biggins, S. & Murray, A.W. The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. *Genes & Dev* **15**:3118-3129 (2001).
3. Pinsky, B.A. & Biggins, S. The spindle checkpoint: tension versus attachment. *Trends Cell Bio* **15**:? (2005).
4. Dewar, H. et al. Tension between two kinetochores suffices for their biorientation on the mitotic spindle. *Nature* **428**:93-97 (2004).
5. Wong OK, Fang G. Plx1 is the 3F3/2 kinase responsible for targeting spindle checkpoint proteins to kinetochores. *J Cell Biol.* **170**, 709-19 (2005).

**WEEK 10: *Ultrastructure of the kinetochore-microtubule interface***

1. Jokelainen P.T. The ultrastructure and spatial organization of the metaphase kinetochore in mitotic rat cells. *J Ultrastruct Res.* **19**:19-44 (1967).
2. Euteneuer U, McIntosh JR. Structural polarity of kinetochore microtubules in PtK1 cells. *J Cell Biol.* **89**(2):338-45 (1981).
3. Mitchison T, Evans L, Schulze E, Kirschner M. Sites of microtubule assembly and disassembly in the mitotic spindle. *Cell.* **45**(4):515-27 (1986).
4. Rieder CL, Alexander SP. Kinetochores are transported poleward along a single astral microtubule during chromosome attachment to the spindle in newt lung cells. *J Cell Biol.* **110**(1):81-95 (1990).
5. McDonald KL, O'Toole ET, Mastronarde DN, McIntosh JR. Kinetochore microtubules in PTK cells. *J Cell Biol.* **118**(2):369-83 (1992).
6. Cooke CA, Schaar B, Yen TJ, Earnshaw WC. Localization of CENP-E in the fibrous corona and outer plate of mammalian kinetochores from prometaphase through anaphase. *Chromosoma.* **106**(7):446-55 (1997).
7. VandenBeldt, K.J. et al. Kinetochores use a novel mechanism for coordinating the dynamics of individual microtubules. *Curr Biol* **16**:1217-1223 (2006).
8. O'Toole, E.T., et al. Morphologically distinct microtubule ends in the mitotic centrosome of *Caenorhabditis elegans*. *J Cell Bio* **163**:451-456 (2003).