Lecture 8
Integrating spindle dynamics and chromosomes

Outline:

- Maintenance of the metaphase spindle
- Anaphase initiation and progression
- Cytokinesis

Paper: Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1
Establishment of the bipolar spindle

Microtubules become highly dynamic at the onset of mitosis. Localized activity of MAPs and catastrophe promoting factors.

Both kinetochores and chromosome arms contribute to spindle stabilization and congression.

Some spindles form without centrosomes (e.g., meiosis).
Bipolar spindles without centrosomes?
Xenopus egg extract
and DNA-coated beads (○)

MICROTUBULES ARE NUCLEATED IN REGION AROUND BEADS

PLUS-END-DIRECTED MOTORS (→)
BUNDLE AND SORT MICROTUBULES

PLUS-END-DIRECTED MOTORS PUSH MINUS ENDS OF MICROTUBULES AWAY FROM BEADS AND MOVE ANTIPARALLEL MICROTUBULES APART

MINUS-END-DIRECTED MOTORS (●) FOCUS SPINDLE POLES

(A) 10 μm
How is bipolar spindle maintained in metaphase?

Three Spindle Motors

**Kinesin 5**
- cross-links MTs
- pushes poles apart
- promotes bipolarity

**Kinesin 14**
- provides opposing force
- pushes poles together

**Dynein**
- at cortex - pulls poles apart
- at pole - focuses minus ends
Motors create balance of forces independent of kinetochore

Kinesin 5
Kinesin 14
Dynein

KIN N bipolar tetrameric plus end-directed
KIN C dimeric minus end-directed
Dynein/dynactin minus end-directed

push poles apart
push poles together
pull poles to cortex
focus -ends at poles
Dynein pulls poles to cortex

Dynein/dynactin minus end-directed
Useful system to explore balance of forces: Drosophila syncytial embryo

many nuclei in common cytoplasm synchronous divisions (8-10 min!!)

inject labeled tubulin
disrupt motor function:
mutants
microinjection of antibodies

control

injected with antibody to bipolar Kinesin 5

spindle collapse
Poleward Flux

another kind of movement in the spindle

MTs turnover

requires ATP hydrolysis

Fluorescence Speckle Microscopy

spike with fluorescent tubulin (low concentration)
Photobleaching of ‘Caged’ Fluorescent Tubulin

release fluorescence only in photobleached area follow over time

movement toward poles
but kinetochores remain at plate
how do kinetochores remain at the plate?

add rhodamine-labeled tubulin to identify newly polymerization

classify with rhodamine-labeled tubulin to identify newly polymerization

newly polymerized tubulin at (+) ends

depolymerize at poles

and kinetochores remain attached (Dam, linkers)
Implications of poleward flux

MT ends are free to exchange tubulin subunits in the spindle, dynamic attachments contribute to force balance.

MT movement could be harnessed to move chromosomes poleward in anaphase.
Stability of metaphase spindle: chromosomes still oscillate

flux
kinetochore: motors and MT polymerization
global changes in microtubule dynamics: less catastrophe

local regulation of microtubule dynamics
poleward flux of MTs

balance of dynamic forces generates metaphase state

organization by microtubule-based motor proteins
at kinetochores
on chromosome arms
on spindle and astral microtubules

disrupt balance to enter anaphase
The Onset of Anaphase

Balance of forces- MT dynamics, motors and chromosomes

- Asters pushing on cortex (dynein)
- Polar MTs and motors (kinesins) pushing poles together
- Antipoleward forces on arms (MTs and chromokinesins)
- Poleward flux of MTs (assembly at + end disassembly at - end)

MT assembly and motors (kinesins) at kinetochores causes oscillations
Sister separation resisted by cohesion and bipolar orientation of sister kinetochores
What’s needed to initiate Anaphase?

Tension as a regulator:
- signals anaphase onset
- influences microtubule-kinetochore attachments and movement

Evidence:
- laser ablation of unattached kinetochore induces anaphase

Checkpoint:
- unattached kinetochore sends WAIT! signal
- signal depends on having all kinetochores attached to the spindle (Spindle Assembly Checkpoint (SAC) or mitotic checkpoint)
  - responds to tension or attachment, or both
Nicklas lab - insect spermatocyte micromanipulation

1) only bipolar attachments are stable

Grasshopper meiosis I spindle

Micromanipulation

Spontaneous kinetochore detachment

Artificial opposing force stabilizes attachment

1969!
2) tension regulates anaphase onset

sex chromosome segregation:
2 X chromosomes pair with Y, unstable
What’s needed to initiate Anaphase?

- disrupt balance of forces
- remove sister cohesion
  - allows separated sister kinetochores to move poleward
- maintain polar MTs pushing poles toward the poles
- maintain poleward flux of MTs, BUT depolymerize MTs at kinetochore
- maintain antipoleward forces on arms
Anaphase initiated by release of cohesion

triggered by activation of the APC (Anaphase Promoting Complex)
cleaves Mitotic Cyclin
cleaves inhibitor (securin) of cohesion protease (separase)
Anaphase is really two stages

Anaphase A
initial poleward chromosome movement after cohesin is degraded
motor activity (dynein) and shortening of K-MTs at kinetochores,
plus some depolymerization at poles (flux)

Anaphase B
poles separate, after sisters separate, moves sisters further apart
motor activity (dynein) pulls poles to cortex
motor activity (kinesins) and MT assembly at overlap between polar MTs
Anaphase A (chromosome-to-pole movement)

Anaphase B (pole-pole separation)
Anaphase A

kinetochore MTs shorten as chromosomes are transported poleward

2 models (not mutually exclusive)

1) pacman:
   kinetochore MTs disassemble at (+) ends

2) traction fiber:
   kinetochore MTs disassemble at (-) ends
   = poleward flux

Experiments:
photoactivation, in vitro reconstitution
predicted fate of photoactivated tubulin subunits

depolymerization at (+) end

depolymerization at (-) end

Anaphase A:
"Pacman" Kinetochore

Anaphase A:
Poleward Microtubule Flux
flux

CENP-E

dynein

pacman

MCAK/XKCM1
Evidence for Pacman model

tissue culture cells:
- chromosome movement 2 µm/min
- flux 0.5 µm/min
- kinetochores move past photoactivated region

in vitro:
- chromosomes can maintain attachment to shrinking MTs
Tetrahymena pellicle experiments

grow MTs from pellicle in flow chamber

flow in chromosomes

depolymerize MTs by diluting tubulin

chromosomes stay attached and move toward pellicle
ATP not required

blocked by antibodies to CENP-E

beads coated with (+) end-directed kinesin also work, but not dynein
Dam1 complex provides another linking mechanism

Ring complex moves processively for several micrometres at the ends of depolymerizing microtubules without detaching from the lattice

Xenopus in vitro spindles:

- flux and anaphase same rate, ~2 µm/min
- same pharmacology
- requires ATP, taxol insensitive
Drosophila embryo experiments: antibody injection and movies
2 KinI kinesins required for chromosome alignment and segregation

KLP59C: at kinetochore
  inhibition blocks pac-man
KLP10A: at spindle poles
  inhibition blocks flux

Anaphase B

poles separate

2 mechanisms:

sliding of antiparallel microtubules

pulling of astral microtubules by cortex
Isolated Diatom spindle in vitro
Cande Lab
Diatom spindle

central region of microtubule overlap

reduced overlap of polar MTs in Anaphase B
sliding of antiparallel microtubules

motor candidate: kinesin-like-protein MKLP1/CHO1
pulling of astral microtubules by cortex

motor candidate: cytoplasmic dynein
Cytokinesis

coordination between **microtubules** and **actin**

1) cleavage plane specification
2) cleavage furrow assembly
3) contraction of cleavage furrow
4) cleavage furrow seals

**membrane insertion**
Cytokinesis occurs at Midbody region of overlap between polar MTs at end of mitosis associated with contractile ring.
Evidence that sites of cytokinesis are determined by poles

Cell with two spindles: Savoian et al. MBC 10, 297 (1999)
1) Astral relaxation model:
signals from asters cause relaxation of the cortex
cleavage occurs where signal is weakest = spindle midzone

CONCEPTS: Astral MTs important
Contractile activity motile

2) Astral stimulation model:
signal from where MTs from opposite poles interact at cortex
push astral MTs away from cortex at midzone
no furrowing on that side

CONCEPT: MTs required for furrowing
3) Equatorial stimulation from spindle mid-zone

Evidence:

- physical barrier between spindle midzone and cortex blocks cytokinesis

- if midzone forms, then cytokinesis proceeds

- midzone proteins are required
Central spindle/midzone proteins required for cytokinesis

proteins:

motor proteins
- chromokinesin KLP3A
- CHO1/MKLP1

chromosome passengers
- INCENP
- BIR-1

kinases
- polo
- aurora-type

GTPases
- rho

roles:

- stabilize MT bundles
- transport furrow components?
- relocate from chromosomes to midzone at anaphase
targets kinases
- localization of motors
- MT and actin regulation
- And many more…
midbody forms between the two spindles:
MKLP1/CHO1 and INCENP recruited
If ectopic midbody doesn’t form - no furrowing
Actin and Myosin: formation of the contractile ring
Local rise in calcium concentration associated with contraction of an amphibian egg injected with calcium indicator dye.

What is the impact of increasing calcium concentration?
Evidence for membrane insertion step to fuse membranes at end of cytokinesis

syntaxins required
target vesicles for fusion

Brefeldin A inhibits closure of intercellular bridge
inhibits GTPase required for membrane targeting

Golgi membrane delivery is involved
major source of membrane

model:
terminal step is analogous to secretion, requires vesicle trafficking