BIOS 95: Cell Division and Chromosome Dynamics
Part II

October 27, 2008
Tubulin heterodimers associate head-to-tail (like actin) but aggregate as 13 protofilaments into a hollow cylinder!
Addition promotes hydrolysis of underlying tubulin
At steady state – EACH microtubule randomly switches between growth and shortening

-> Dynamic Instability
Microtubules are nucleated AND organized by the centrosome

- Nucleating sites (γ-tubulin ring complexes)
- Pair of centrioles
- Microtubules growing from γ-tubulin ring complexes of the centrosome

Notice polarized array
Microtubules generate railway system

Railway is polarized . . .
Polarized railway . . . You go to the plus-end, and I’ll go to the minus-end:

Opposing motors

Dynein moves Toward Minus-end

Kinesin moves Toward Plus-end
Phases in the life of a cell – and in making another cell

Setting up for mitosis requires 2 key duplications in S-phase
1. Chromosomes replicate AND Sister chromatids pair

DNA Replication

Cohesins

Cohesin degradation

Chromosome segregation

G1

S

G2

M
2. Centrosomes duplicate during S-phase,

Two sets of anti-parallel polarized Microtubules arrays
Early mitosis:

Kinesins help Drive poles apart

1. polarized Microtubules arrays separate via kinesin complexes
Early mitosis

2. Duplicated chromosomes condense

Centrosomes (spindle poles) separate

plate of spaghetti model
Prophase -> Nuclear Envelope Breakdown -> Prometaphase

Cytoplasmic microtubules can now be captured by chromosomes
Microtubules captured by discrete protein complexes on each Sister chromatids

Skibbens et al
Centrosomes

Microtubules

Pulling Forces

**Kinetochore** - protein complex assembled onto centromere DNA

Microtubule capturing complex
Kinetochore: structure relates to function

CENP A - centromere specific histone variant

Figure 18–19. Molecular Biology of the Cell, 4th Edition.
Kinetochore: structure relates to function

CENP E – Kinesin-like microtubule motor
Review

Structural features
1. Centrosomes
2. Microtubule array
   -> bipolar spindle
3. Kinetochores
   -> each chromatid
Dynamic view

www.scripps.edu/cb/sullivan/movies
Kinetochores allow for dynamic instability at captured plus-end.

Microtubule flux in the spindle

Pac-man model of chromosome movement

Loss?
Kinetochore: structure relates to function
CENP E - plus-end directed motor (outer plate)

Figure 18–19. Molecular Biology of the Cell, 4th Edition.

Modified from
Maiato et al., 2004
DASH collar-like structure maintains Kinetochore-Microtubule attachment even to depolymerizing microtubule!
The bulk of chromosome movement in our cells is generated by Kinetochore/Microtubule Plus-end interaction.
Cohesin degradation allows sister chromatids to segregate.
Figure 18-26 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

ANAPHASE A

- Shortening of kinetochore microtubules, movement of daughter chromosomes to poles; forces generated mainly at kinetochores

ANAPHASE B

- (1) A sliding force is generated between overlap microtubules from opposite poles to push the poles apart;
- (2) A pulling force acts directly on the poles to move them apart

Microtubule growth at plus end of polar microtubules

Figure 18-26 part 2 of 2. Molecular Biology of the Cell, 4th Edition.
Evidence of tension:

Laser ablation and anaphase onset

Changes in Kinetochore phosphorylation
Kinetochore poleward forces are resisted by sister chromatid pairing forces.

There is a net tension between sisters!
Static view

Figure 18–18. Molecular Biology of the Cell, 4th Edition.
Source of tension

Flux

Fluorescent Speckle Imaging: Zoomed, cropped Xenopus extract spindles.

www.bio.unc.edu/faculty/salmon/lab
Flux – microtubule minus-end depolymerization maintains tension within spindle

Why is tension important?
Microtubule capture by Kinetochore is random . . .

Lack of tension allows for microtubule release and new kinetochore capture events that might be correct.

From Maiato et al., 2004

1. Tension functions in Error Correction
Aurora kinase phosphorylates Ndc80

Phosphorylated Ndc80 lets go of microtubule

Under tension - Ncd80 pulled away from Aurora kinase -> Kt-Mt linkage stabilized
2. Tension is monitored by the cell to regulate anaphase onset

Flux and K-Mts

From Maiato et al., 2004
1. Unattached kinetochore emits ‘wait’ signal

2. Tension is monitored by the cell to regulate anaphase onset

Flux and K-Mts

From Maiato et al., 2004
MADs

Mitotic Arrest Deficient

BUBs

Budding uninhibited by Benomyl

Cells mutated or deficient for MAD/BUB activity are unable to Arrest the cell cycle (M-phase progression) in response to Spindle damage or mono-oriented chromosomes

Checkpoint proteins (Surveillance proteins) act as Brakes
Checkpoint proteins block cohesin degradation —> keep sisters paired

Unattached kinetochore

Anaphase Promoting Complex

Cohesins hold sister chromatids together
Checkpoint proteins reside at unattached kinetochores to monitor tension and regulate anaphase onset.
Sources of aneuploidy

Mutations in checkpoint proteins
- anaphase onset before all sisters are bi-oriented

Centrosome over-duplication
- loss of geometry

Mutations in cohesin proteins
- sisters never bi-orient
  - Gene mis-expression
Fighting Cancer

Paclitaxel (taxol) – originally from Pacific Yew tree
Taxol blocks protofilament curvature - >

Microtubules never disassemble

Side effects
pretty dramatic
Other issues in Cancer

Aneuploidy

Metastasis – inappropriate movement of cells