kk

Mitotic Spindle

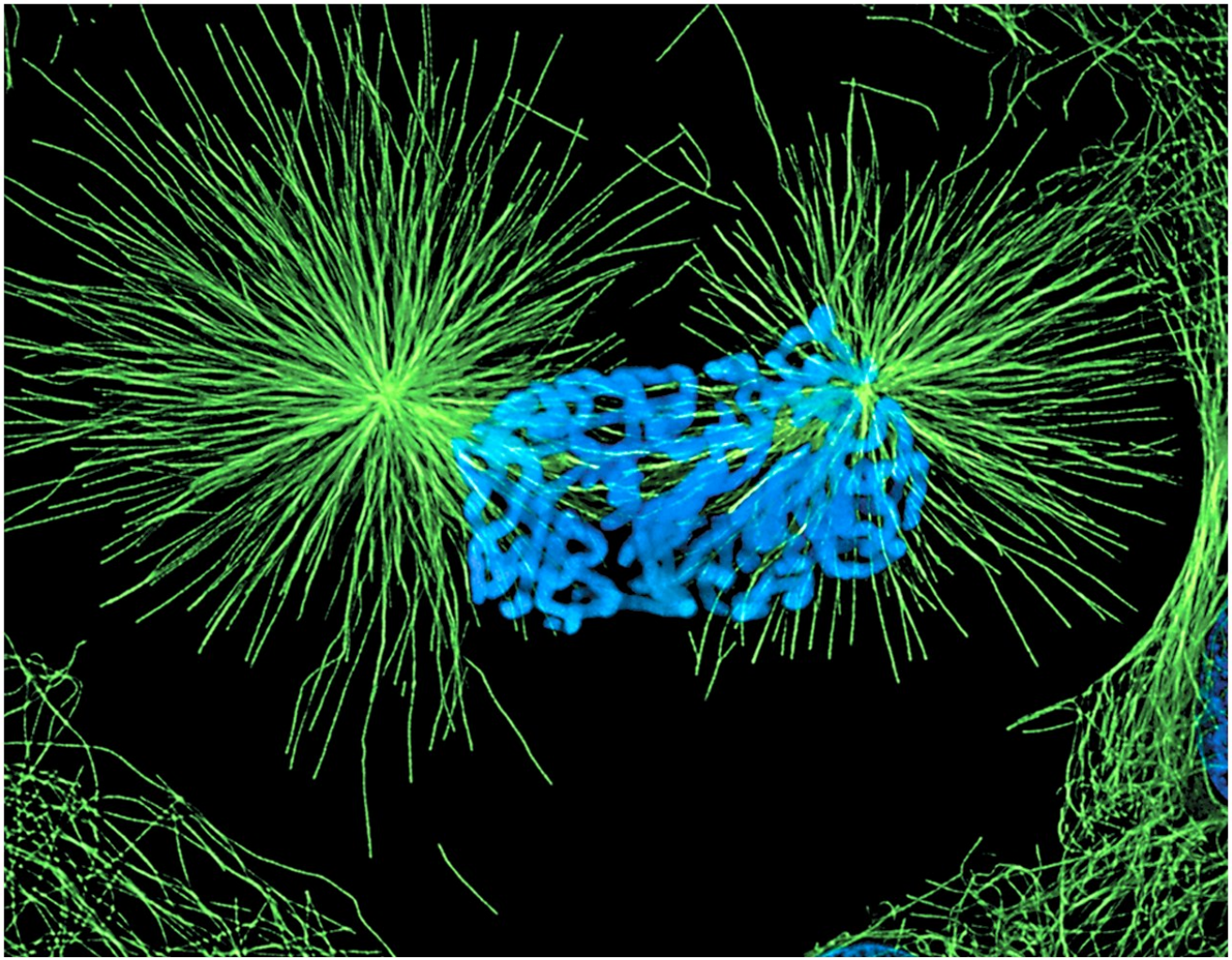


Figure 14-20 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Cell from Newt lung in prometaphase

Flourescent image of 2 micortubules growing…
Astral microtubules. Changes just before metaphase…
As they create the mitotic spindle, it becomes a "net".

Some Major Events of Mitosis

- Centrosome duplication (G_2).
- Mitotic spindle formation (*prophase*).
- Chromosome condensation (*prophase*).
- Breakdown of nuclear envelope (*prometaphase*).
- Chromosome attachment to MTs begins (*prometaphase*).
- The metaphase plate.
- Anaphase.

Chromosome pair that will separate into two sister chromatids.
Just watch a video and read this slide. Spindle handles the compartmentalization
Prophase mitotic spindle begins to “organize” chromosomes?
-Anaphase, sister chromatids move to different folds.

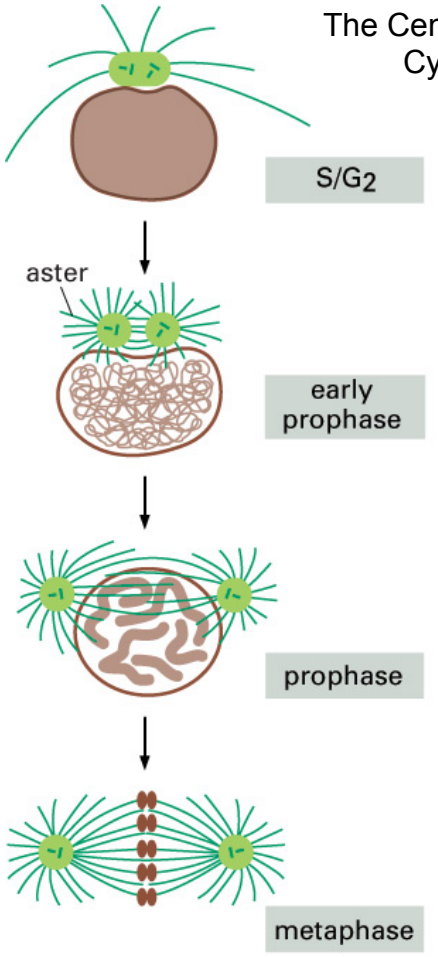


Figure 18–7. Molecular Biology of the Cell, 4th Edition.

Increased Dynamic Changes

(by phosphorylation)

M-Cdk ⟶ { ↓MAP activity ; ↑Kinesin activity } ⟶ ↑Dynamic changes of MTs

Recall from previous lecture:

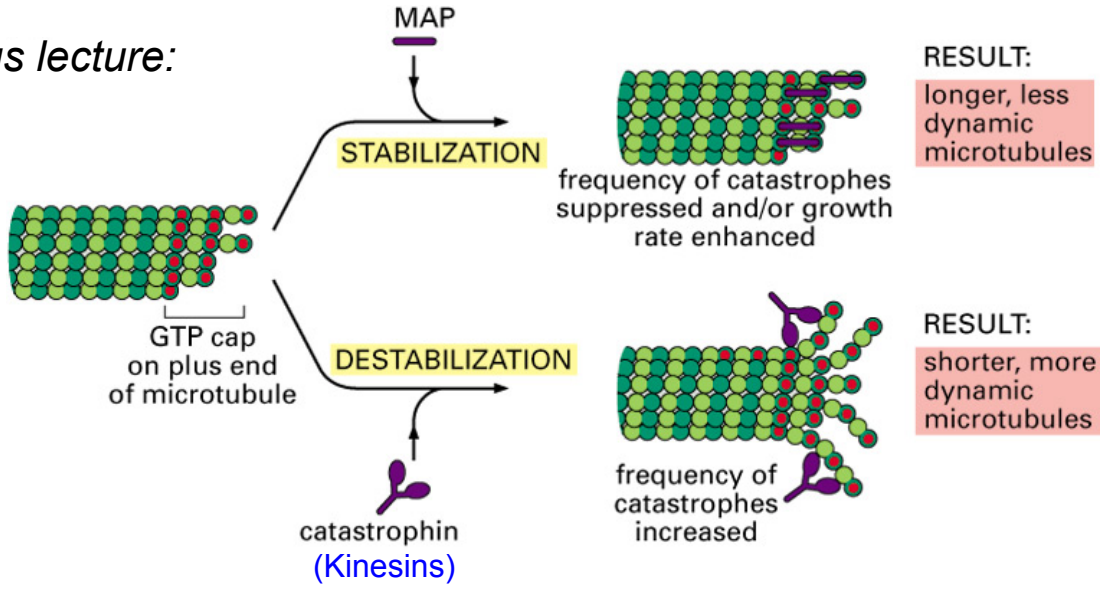


Figure 16–36 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

MAP = microtubule associated proteins.
catastrophin = kinesins. Accesory protein that can modify the structure of microtubules. This happens as mCDK is activated.

Components of the Mitotic Spindle

1. Microtubules
- Astral
- Kinetochore
- Overlap

2. Motor proteins
- Kinesin-related (+)
- Dynein (-)

3. Chromosomes (chromatids)

4. Centrosome
- Centrioles
- PCM

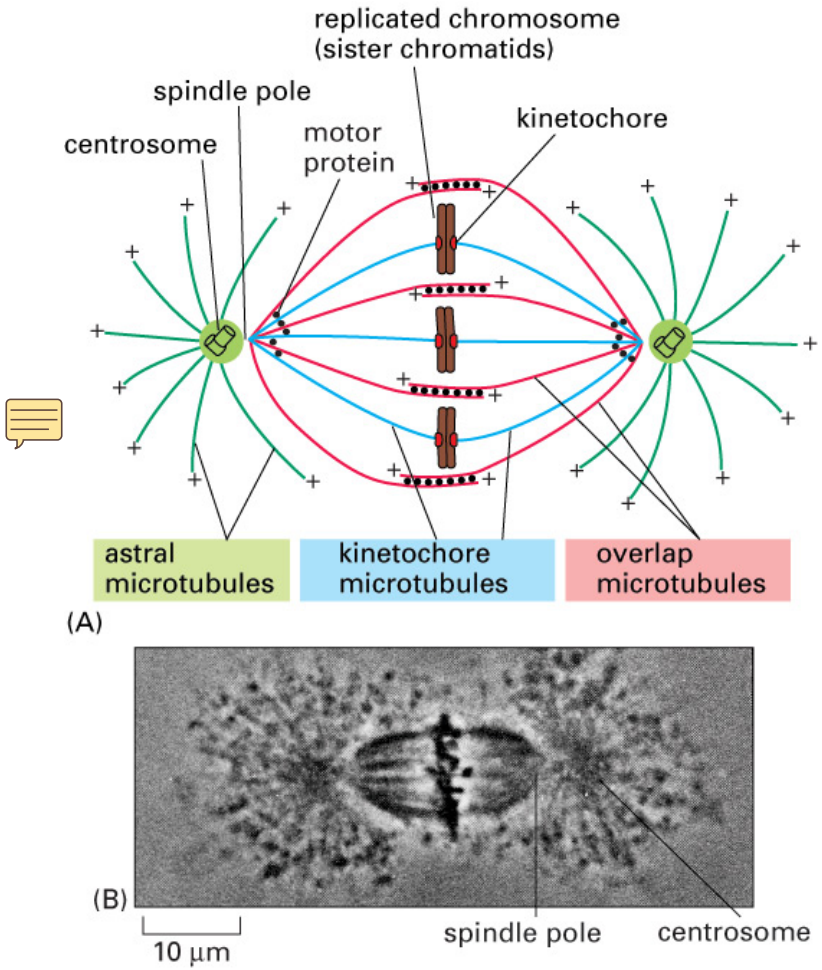


Figure 18–10. Molecular Biology of the Cell, 4th Edition.

Motor Proteins Contribute to Spindle Assembly

- Multimeric motor proteins "*cross-link*" antiparallel MTs.
- (+) end-directed kinesin-related proteins (KRPs, e.g. BimC or kinesin-5).
- KRPs self-associate with each other at their tail domains.
- Slide overlap MTs past each other.
- Promotes elongation of mitotic spindle.

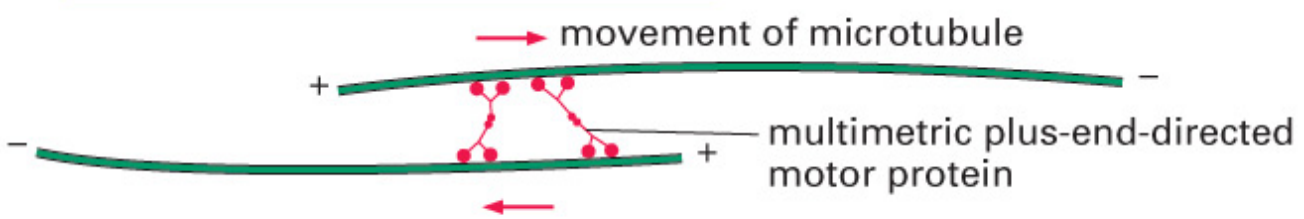
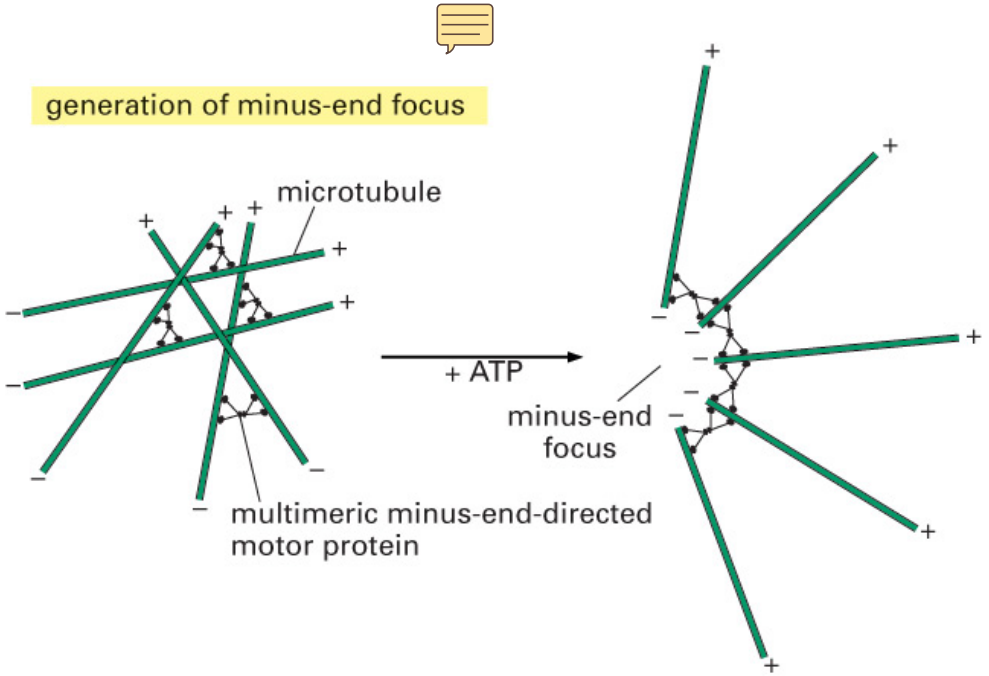


Figure 18–13. Molecular Biology of the Cell, 4th Edition.

Motor Proteins Contribute to Spindle Assembly

- Motor proteins cross-link adjacent MTs.
- Multimeric (-) end-directed dyneins and kinesin-14 arrange minus ends.
- Creation of foci at spindle poles.



Spindle Pole Separation

- Prophase
- KRPs push overlap MTs.
- Dyneins pull astral MTs.

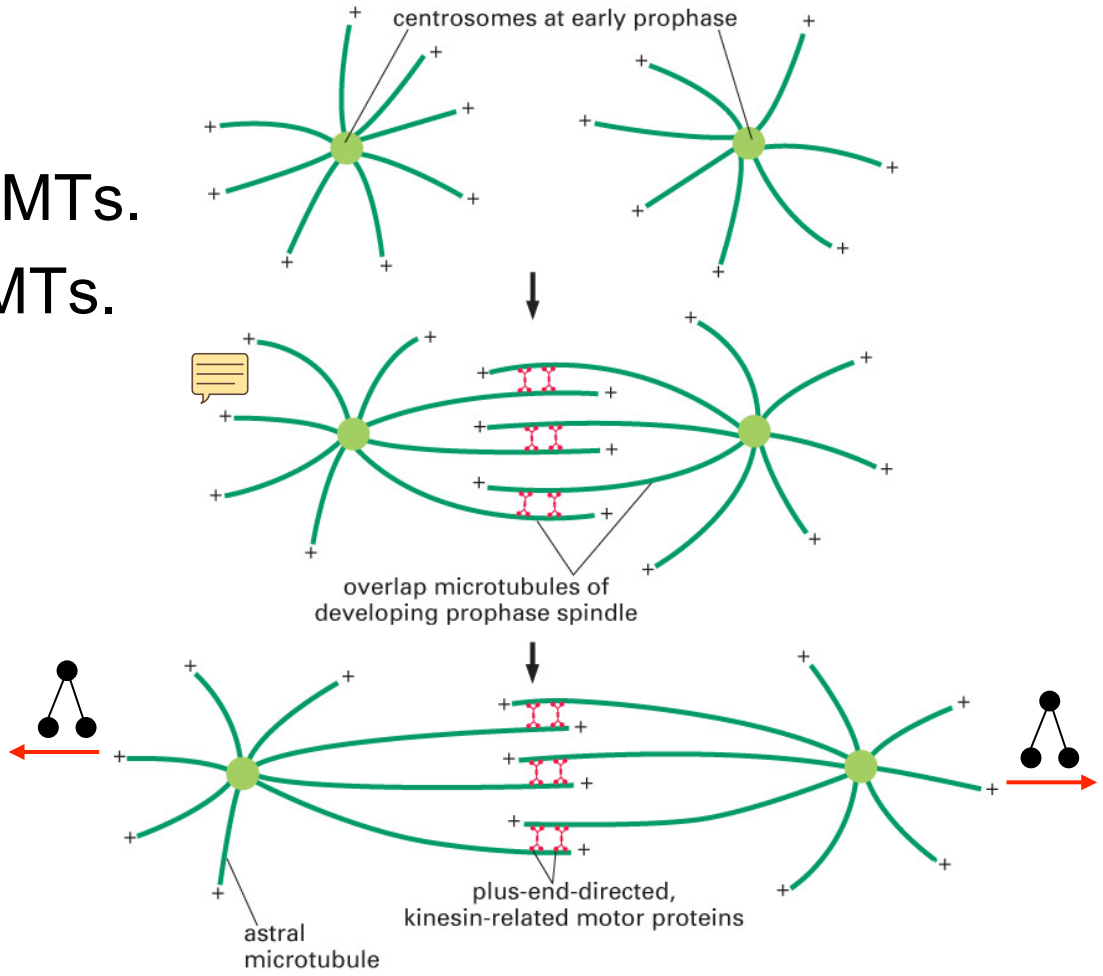


Figure 18–14 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Chromosome Condensation

- Occurs at prophase.
- Cohesin and condensin have similar ATP and DNA-binding domains.
- Cross-linking.
- Most cohesin is replaced by condensin during prophase.
- Condensation dependent on ATP hydrolysis and phosphorylation of condensin by M-Cdk.

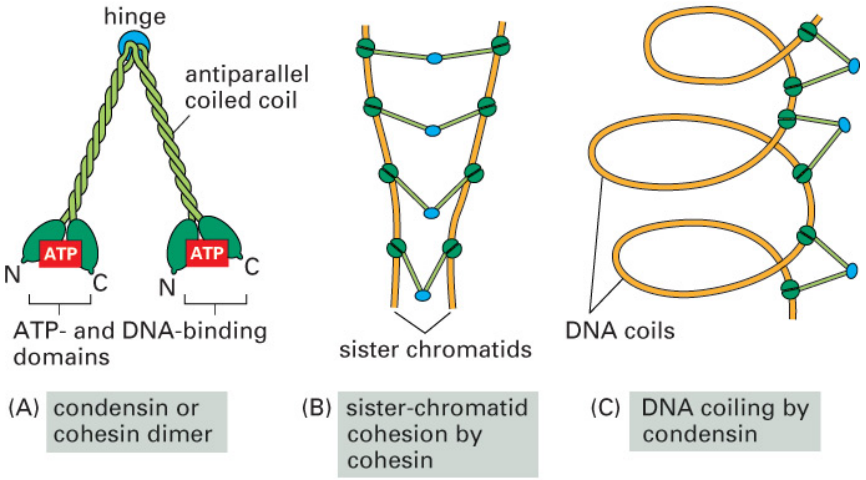
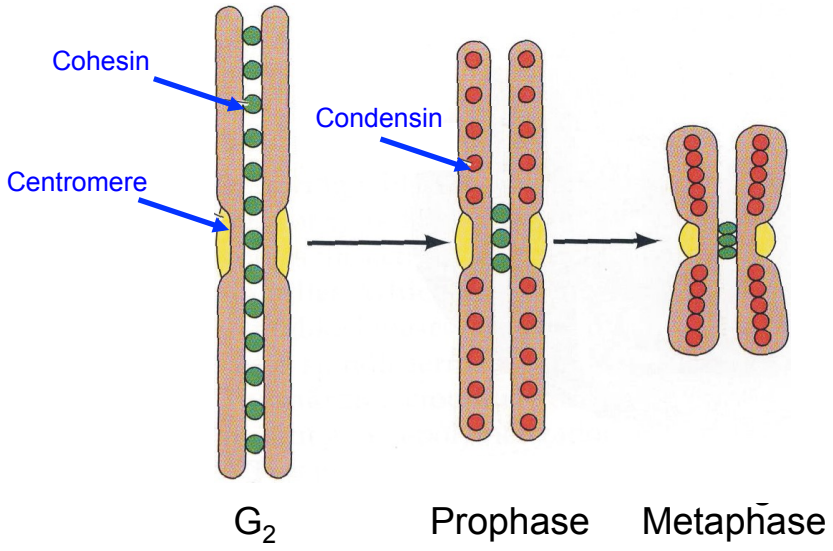


Figure 18–3. Molecular Biology of the Cell, 4th Edition.



Cooper and Hausman, 2008

Cohesin and Condensin are similar.
-They have DNA binding domains.
sister chromatid. bridged by coh con.
-Condensin is dependent on ATP hydrolysis.
hydrolysis and phosphorylation. You need to hydrolize for condensin.
-cells are coordinating prep. for nuclear division.

MT – Kinetochore Attachment

- Prometaphase
- Astral MTs “capture” kinetochores.
- Increases MT stability.
- Astral MTs become kinetochore MTs.
- Bipolar attachment.

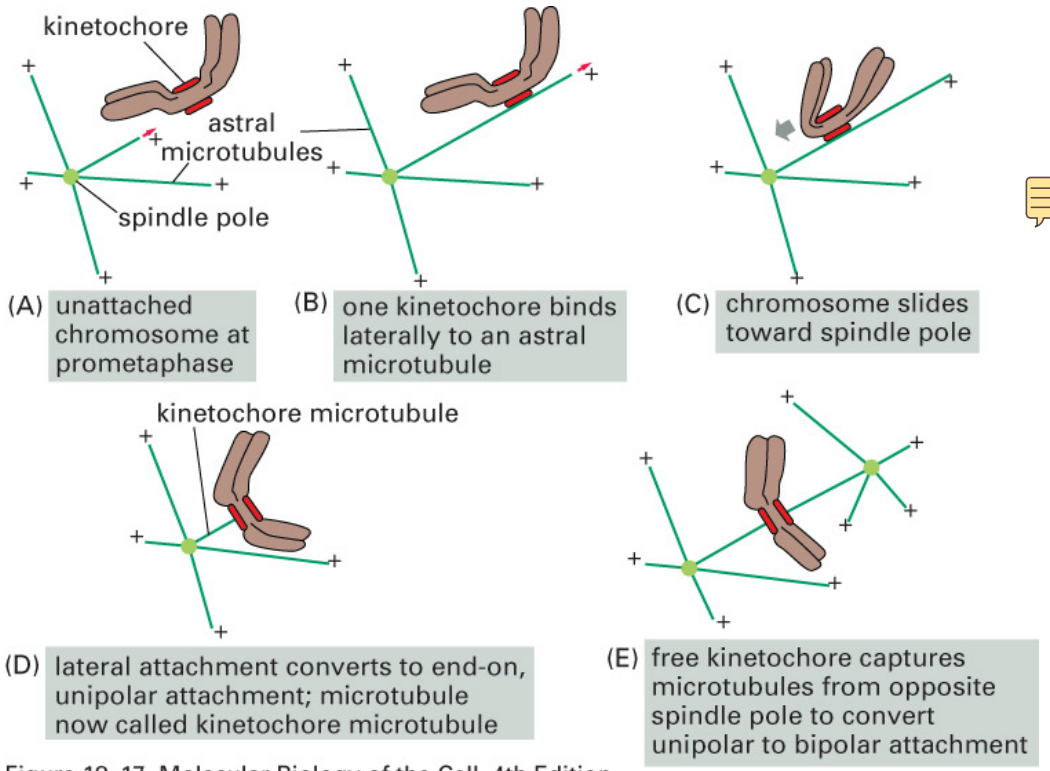


Figure 18–17. Molecular Biology of the Cell, 4th Edition.

MT – Kinetochore Attachment

- (+) end attached “end-on” to kinetochore.
- Metaphase
- Formation of *metaphase plate*.
- (+) and (-) end-directed motor proteins provide stability of chrosomose along equator of spindle.

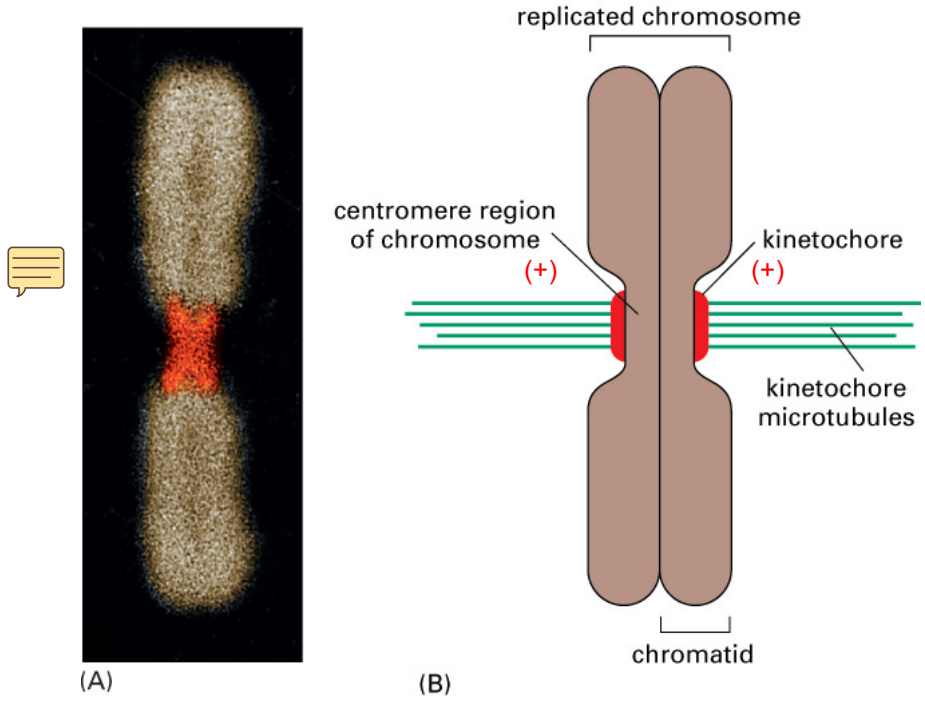


Figure 18–16. Molecular Biology of the Cell, 4th Edition.

MT – Kinetochore Attachment

Motor proteins: balance at the MT-kinetochore interface.

- KRP (i.e. depolymerase)
- CENP-E (centromere-associated protein E)
- Dynein

Note: Ndc80 not shown

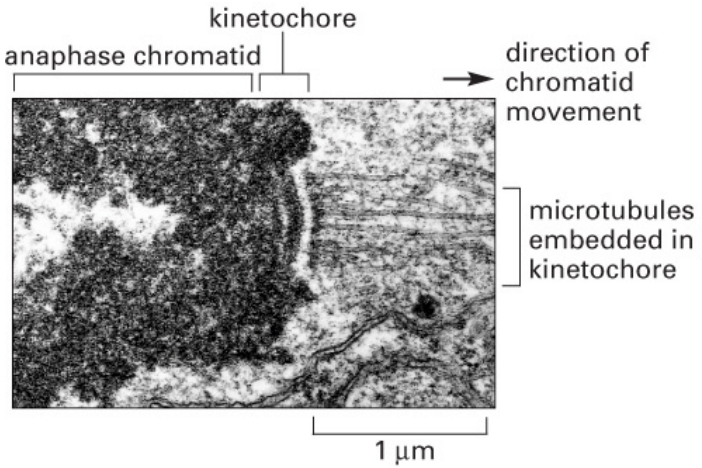
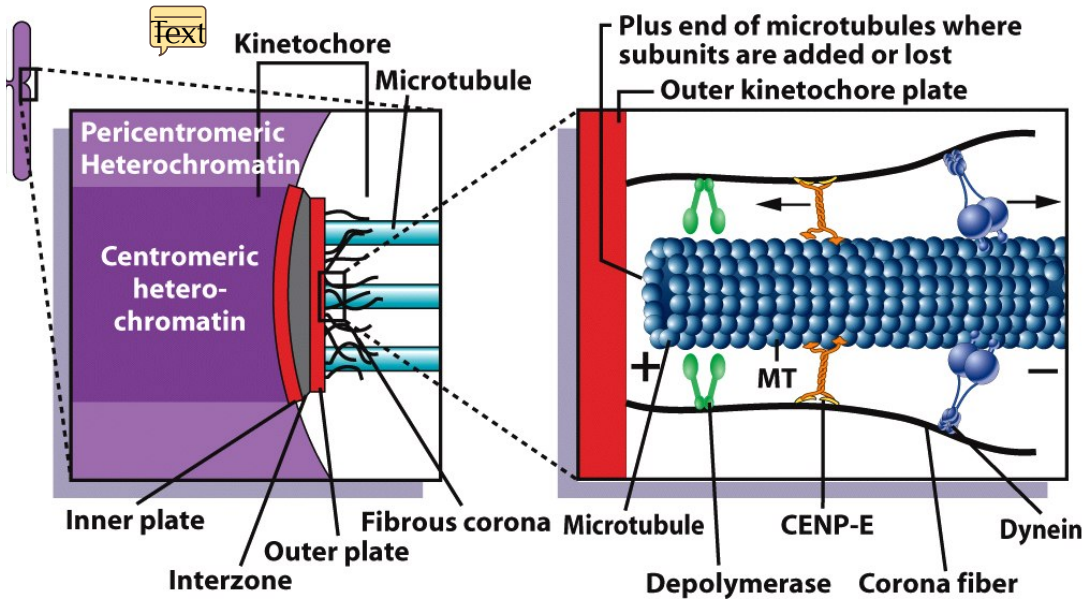


Figure 18–19. Molecular Biology of the Cell, 4th Edition.



(KRP/catastrophin)

(Karp 2008)

MT – Kinetochore Attachment

Motor proteins: balance by astral ejection force.

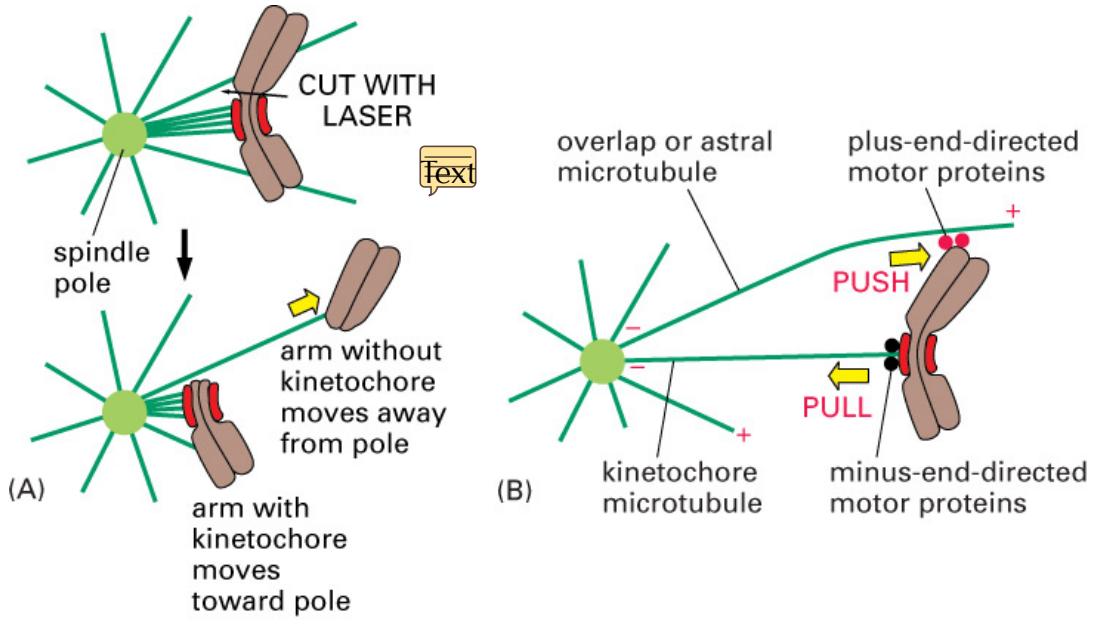


Figure 18–22. Molecular Biology of the Cell, 4th Edition.

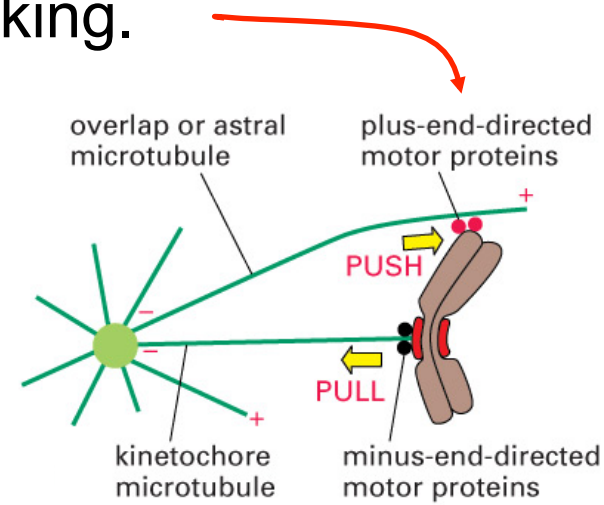
Consequence of Missing a Motor Protein

Upper

- Normal plate formation.

Lower

- Chromosomes trapped near (-) end of MT (poles).
- Kinesin protein (Kid) is lacking.



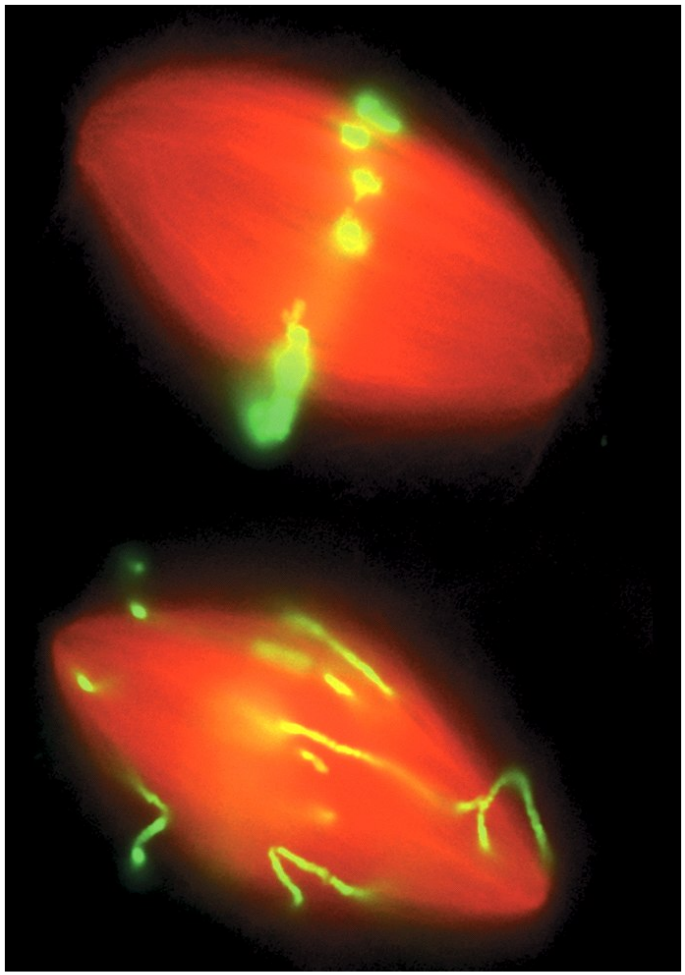


Figure 14-21 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Poleward flux

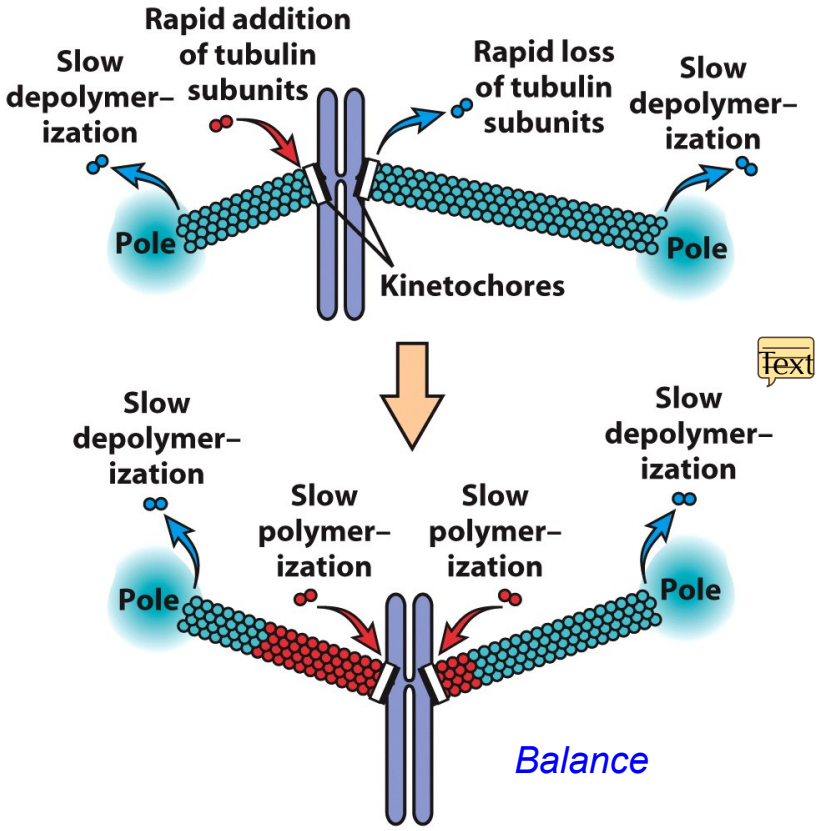


Figure 14-22 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

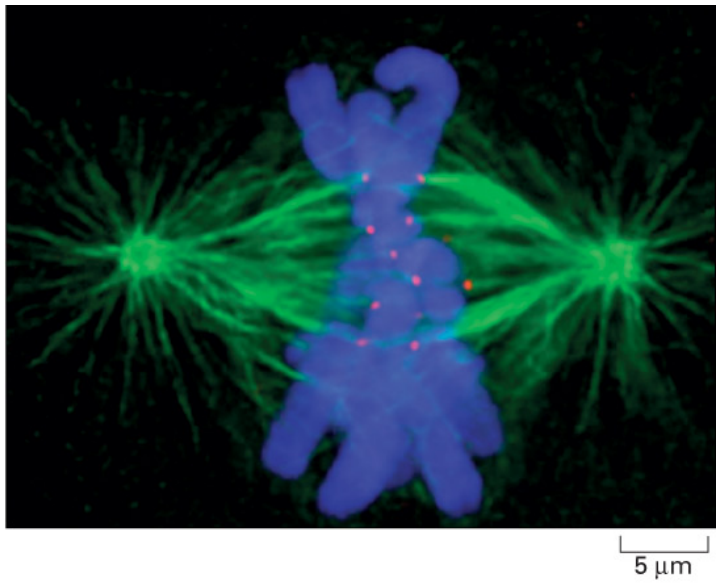


Figure 18–18. Molecular Biology of the Cell, 4th Edition.

Summary: balance at metaphase plate

1. Motor proteins
2. Poleward flux

Anaphase A Separation

- Poleward (-) movement of chromatids by shortening of kinetochore MTs.

1. MT depolymerization at (+) ends by KRPs.
2. Continual loss of tubulin at (-) ends (i.e. as in poleward flux) without addition at (+) ends.

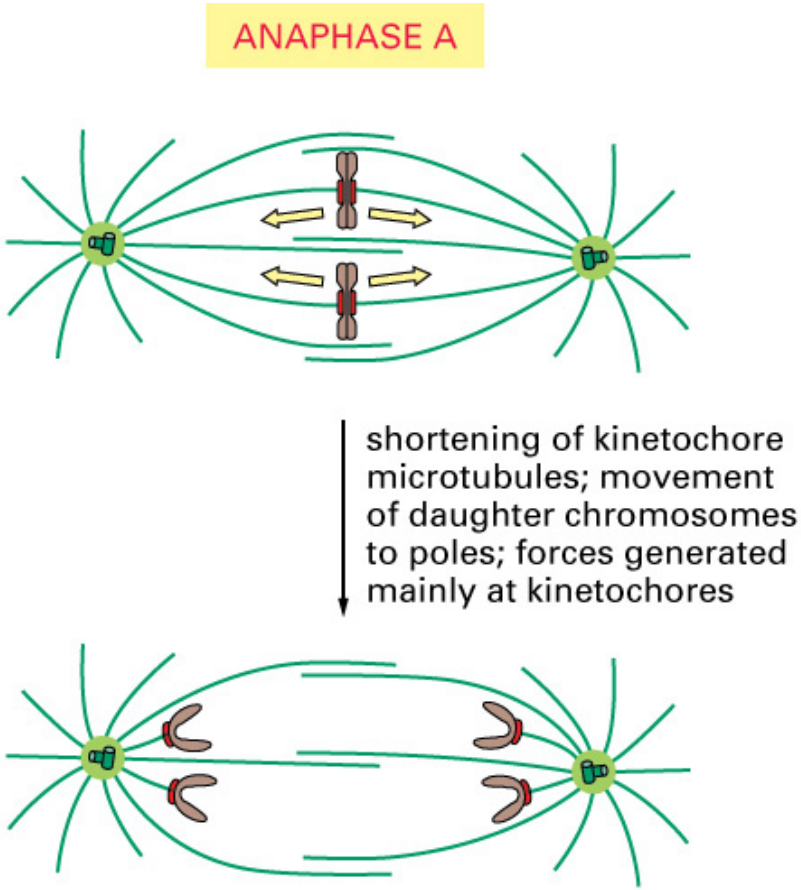
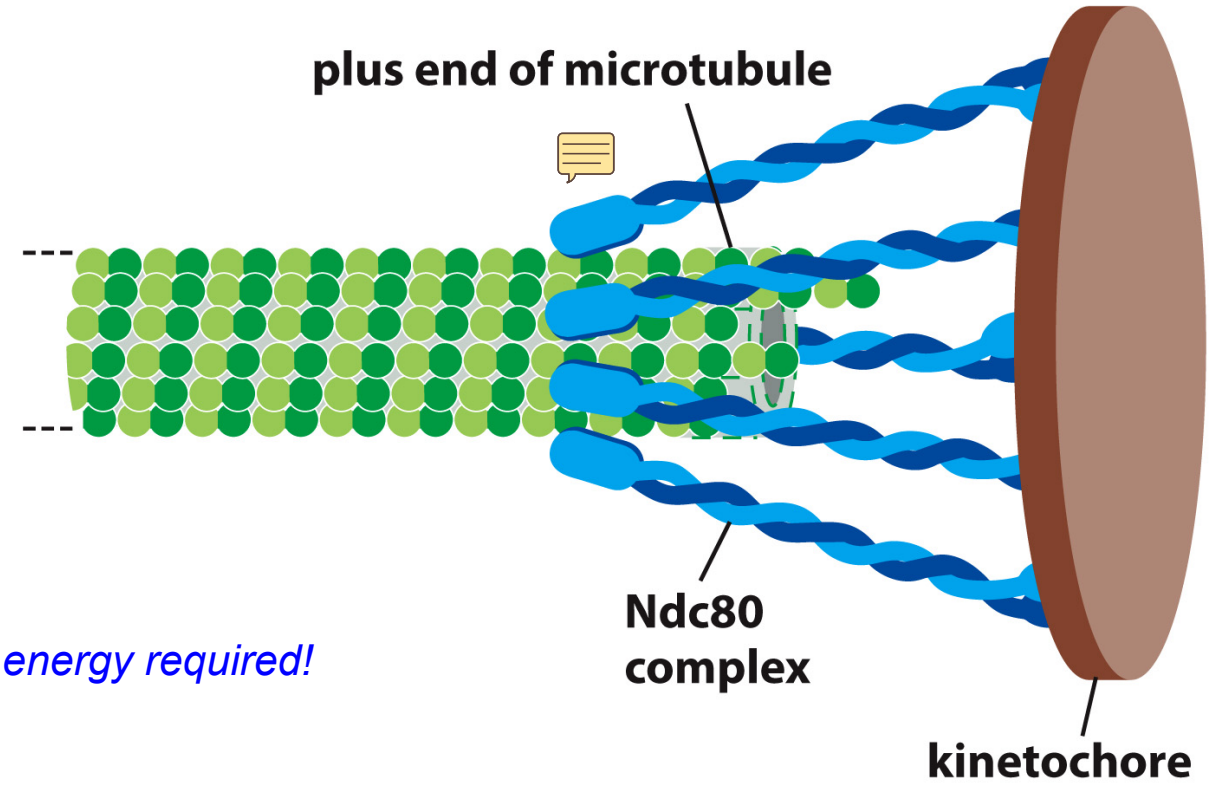


Figure 18–26 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

2 Separation Forces

1. MT disassembly drives chromatid movement (below).
2. Poleward (or microtubule) flux at onset of anaphase.



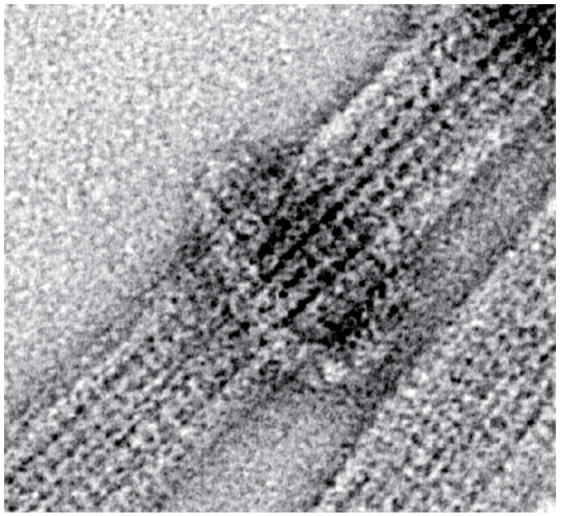
No additional energy required!

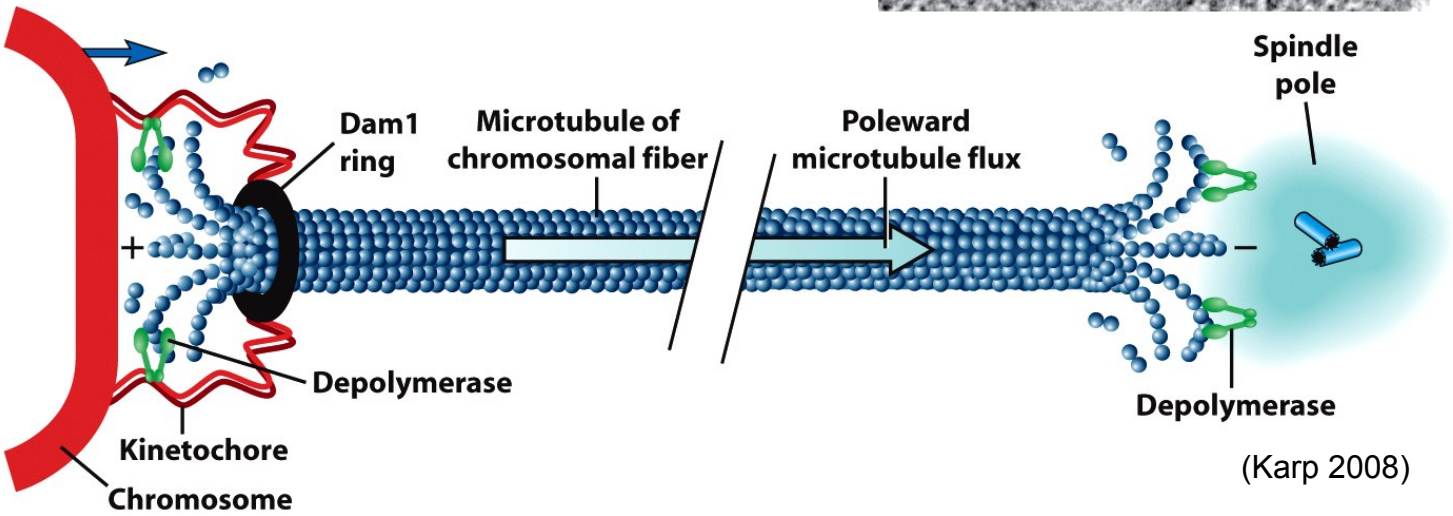
Figure 17-31c Molecular Biology of the Cell 6e (© Garland Science 2015)

Combined Model for Anaphase A

- Kinesin-13 depolymerizes at both ends.
- Dam1 ring maintains attachment between MT and chromatid in yeast.
- Depolymerization powers movement.

Dam1 ring surrounding a MT

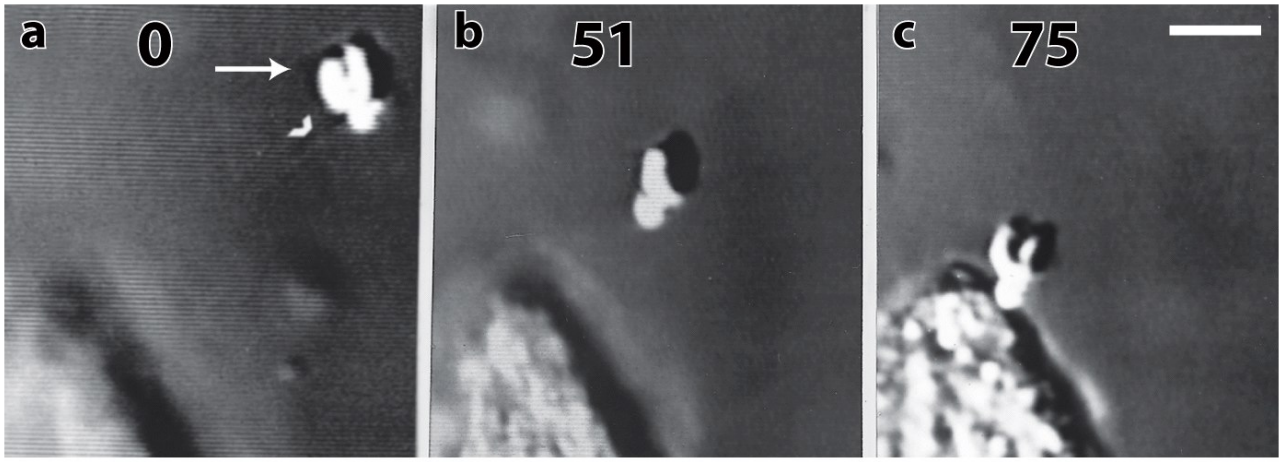




(Karp 2008)

Experimental Evidence

- MT depolymerization may drive chromatid movement.
- Nucleated MTs deprived of tubulin.
- Depolymerization of MTs pulls chromosome pair.
- Therefore, MT depolymerization provides sufficient energy.



(Karp 2008)

Anaphase B Separation

- Pulling by motor proteins at poles.
- Pushing by motor proteins at central spindle (i.e. at overlap MTs).
- Further elongation of spindle.

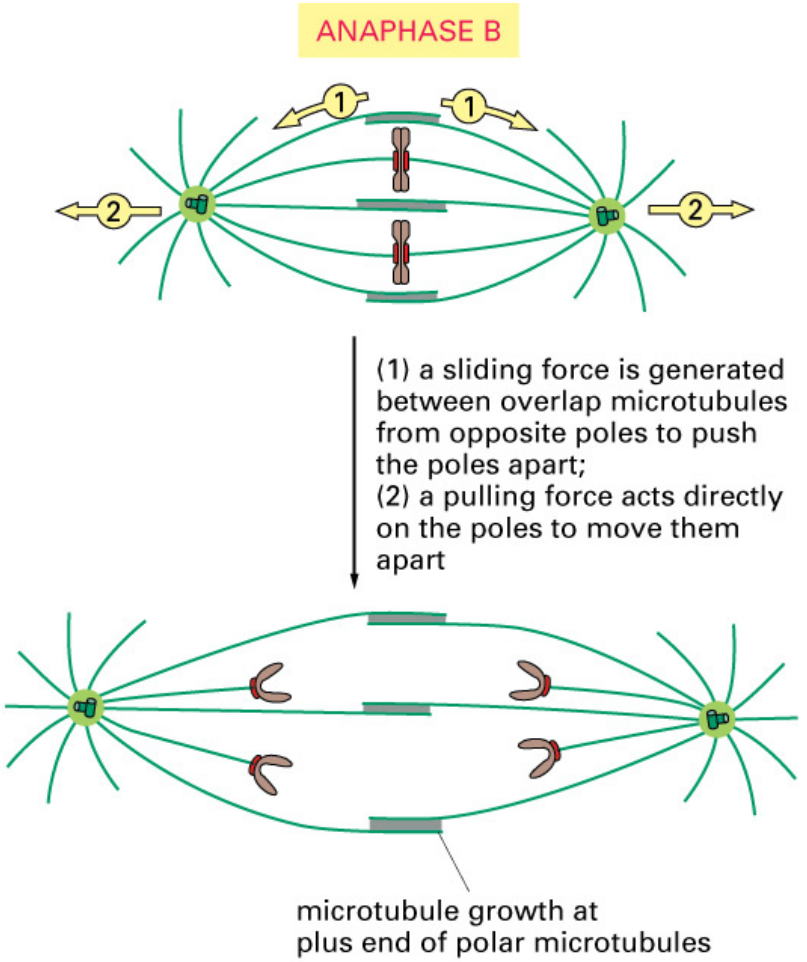


Figure 18–26 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Action of Motor Proteins in Anaphase B

- KRPs cross-link and push overlap MTs apart.
- Dyneins anchored to cell cortex pull themselves toward (-) end of astral MTs.

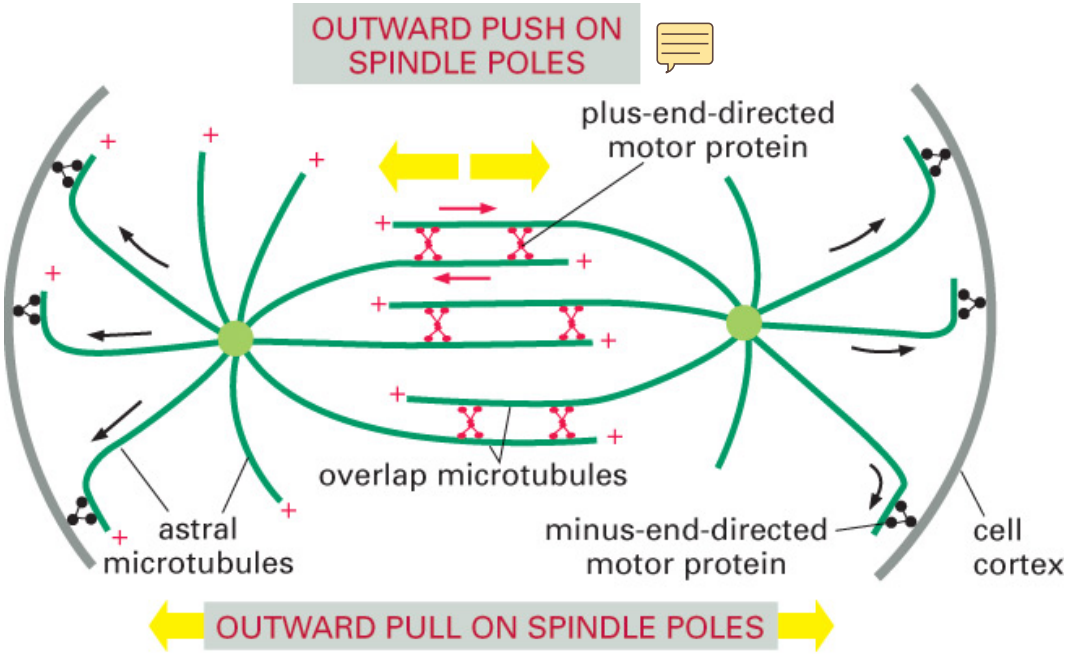


Figure 18–29. Molecular Biology of the Cell, 4th Edition.

Fly spindle

Things to Consider...

1. Are you familiar with the roles of each different type of MT during mitosis?

2. Motor proteins?

3. Think about how chromosomes are balanced at metaphase, and how chromatids separate during anaphase.