Engineering Molecular Cell Biology
Lecture 23, Fall 2010

Cell Cycle III:

S phase & DNA replication

G_2 phase & G_2-M transition

Mitosis and Cytokinesis

Chapters 42, 43, & 44

Final Exam: Group Assignment

Group ID	Student 1	Student 2	Student 3	Student 4
1	Mike McCann	Anupama Kuruvilla	Jacob Sheu	
2	Stephanie Chang	Emily Fredrich	Kush Mangal	
3	Will Kowalski	Chia-Yuan Chen	Stephen Kustra	Piyawat Chalermkanjana
4	John Goldman	Shravya Mukka	Aishwarya Sukumar	

Final Exam: Paper Assignment

Group Chia-Yuan Chen, Stephen Kustra, William Kowalski, Piyawat Chalermkanjana

Nov-29

Delanoue et al, Dynein anchors its mRNA cargo after apical transport in the Drosophila blastoderm embryo, *Cell*, 122:97, 2005.

Group Emily Fredrich, Stephanie Chang, Kush Mangal

Dec-01

D. Liu et al, Sensing chromosome bi-orientation by spatial separation of Aurora B kinase from kinetochore substrates, *Science*, 323:1350, 2009.

Group Shravya Mukka John Goldman, Aishwarya Sukuma

Dec-06 (may need to be adjusted)

D. Levy & R. Heald, Nuclear size is regulated by importin α and Ntf2 in Xenopus, *Cell*, 143:288, 2010.

Group Mike McCann, Anupama Kuruvilla, Jacob Sheu

Dec-08 (may need to be adjusted)

S. Ally et al, Opposite-polarity motors activate one another to trigger cargo transport in live cells, *Journal of Cell Biology*, 187:1071, 2009.

Final Exam: Reports

- For each group, the presentation PPT file will serve as the final report.

- Students not presenting should submit a one-page report that consists of two sections
  - →Section I: critical comments on the paper
  - →Section II: your questions

Outline

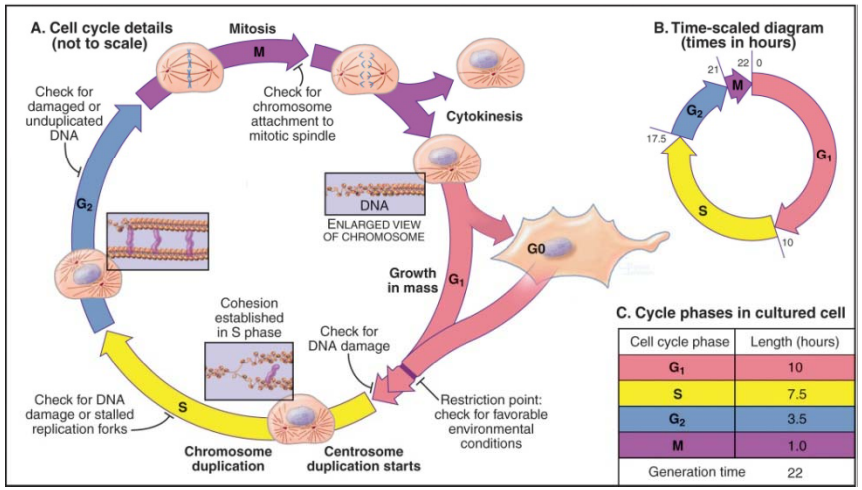
- DNA replication and its regulation in S phase
- S phase checkpoints and S-G_2 transition
- Overview of G_2 phase
- G_2 phase checkpoints and G_2-M transition
- Mitosis
- Cytokinesis

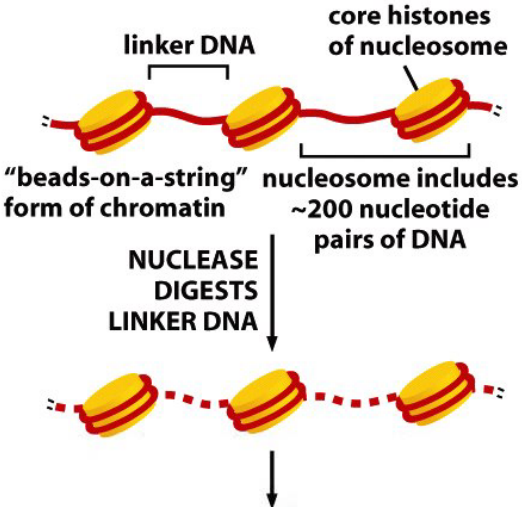
Overview of S Phase

- Main events:
  - Chromosome replication
    - → DNA replication
    - → Histone protein synthesis
  - Centrosome replication

- DNA replication is performed under precise regulation by a complex macromolecular machinery.

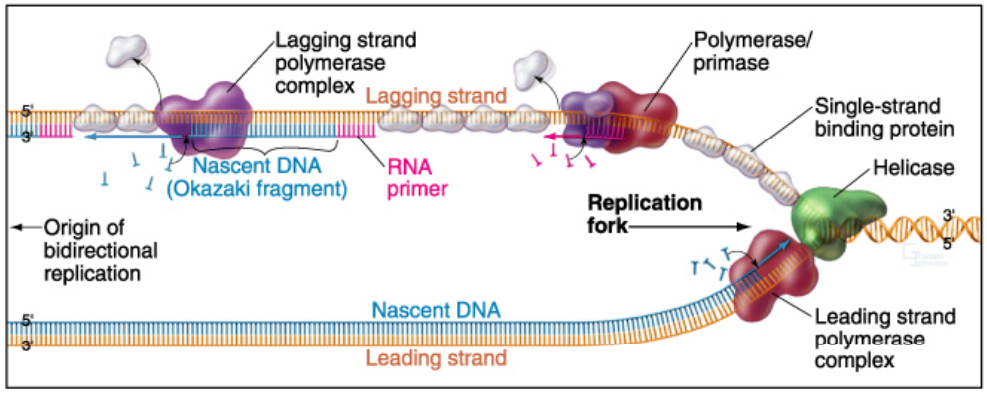
- Checkpoints:
  - DNA damages
  - Stalled replication forks
  - Completion of DNA replication

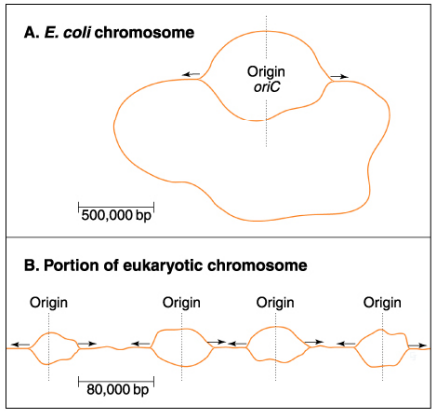


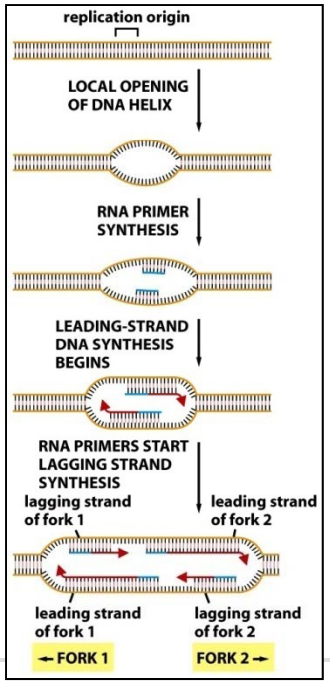


DNA Replication in S Phase (I)

- DNA synthesis proceeds in a 5' to 3' direction.
- DNA replication starts at origins of replication and proceeds bidirectionally.
- Eukaryotic cells use multiple origins to accelerate replication.
- A licensing mechanism ensures each origin is used once and only once.





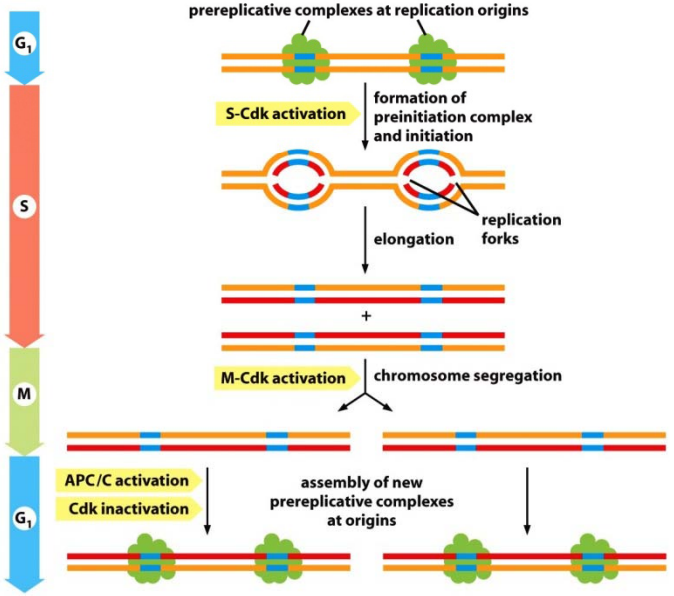


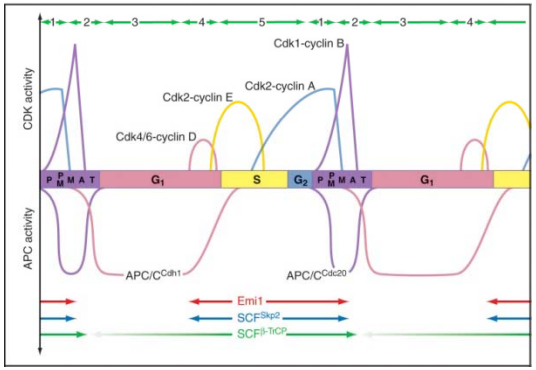
DNA Replication in S Phase (II)

- Origins of replication
  - *E. coli*: oriC
  - Budding yeast: ARS (autonomously replicating sequences)
  - Mammalian cells: several known origins; overall much less is known.

- Assembly of prereplication complex before the restriction point provides "licenses" to replication origins.

- An inducer, a combination of kinases (e.g. Cdk2-Cyclin E, Skp2 & Cdc7P), triggers the G_1-S transition and formation of the preinitiation complex.





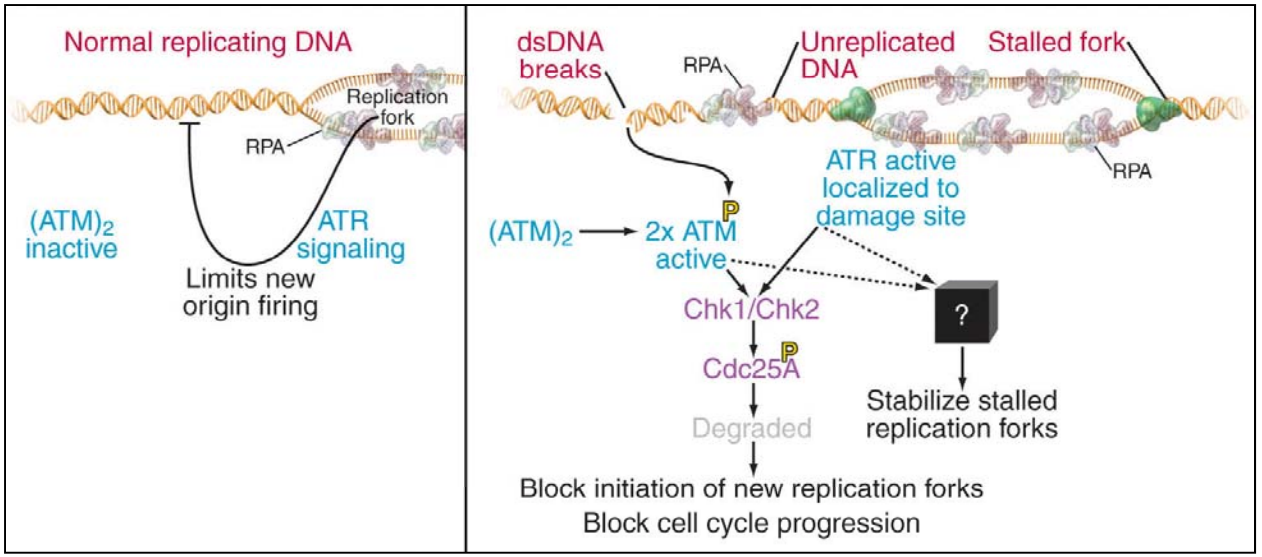
DNA Replication in S Phase (III)

- Binding of a hexameric Mcm (minichromosome maintenance) to the prereplication complex is critical to the licensing process.
- DNA replication is triggered by an inducer, a combination of protein kinases, especially Cdk2-cyclin E.
- DNA replication are divided into three phases: initiation, elongation, and termination.
- DNA replication is organized based on clusters of replication origins (replication foci; each with ~5-6 replication origins).
- Different domains of the genome are replicated at different time points under precise regulation.

Checkpoints in S Phase

- A group of three check points control the progression of the S phase.

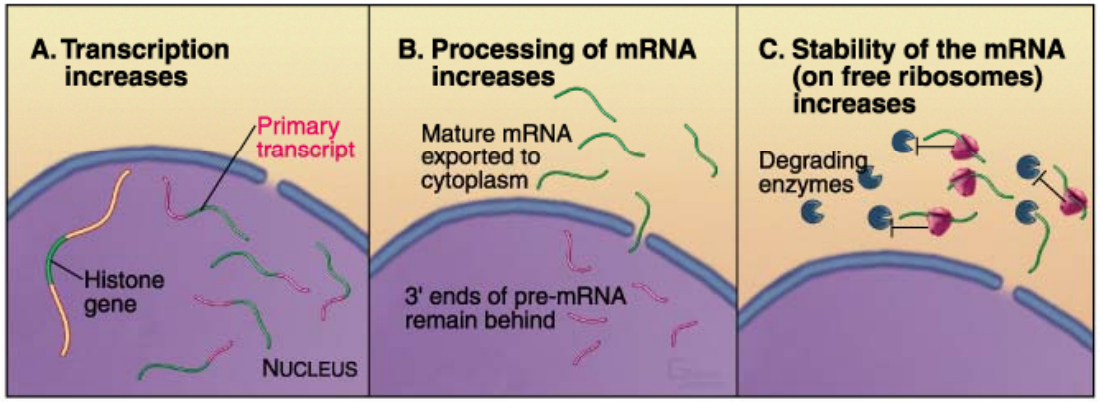
  - Check for DNA breakages
  - Check for stalled DNA replication forks
  - Delay cell-cycle until DNA replication is complete



Other Events of S Phase

- Histone protein synthesis
  - Human genome: 3.2×10^9bp, 200 bp/nucleosome
    $\rightarrow$ 3.2×10^9 nucleosomes

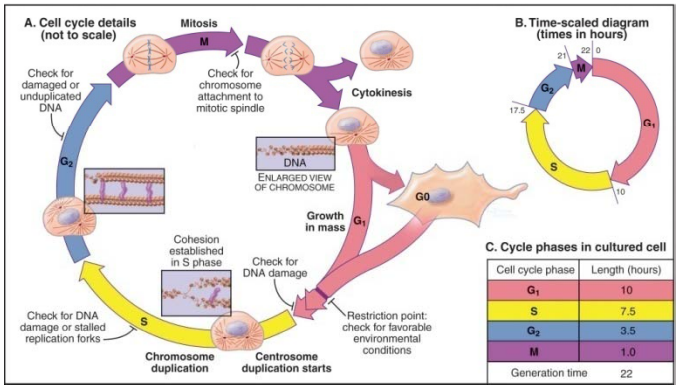
  - Synthesis of histone is substantially enhanced in S phase

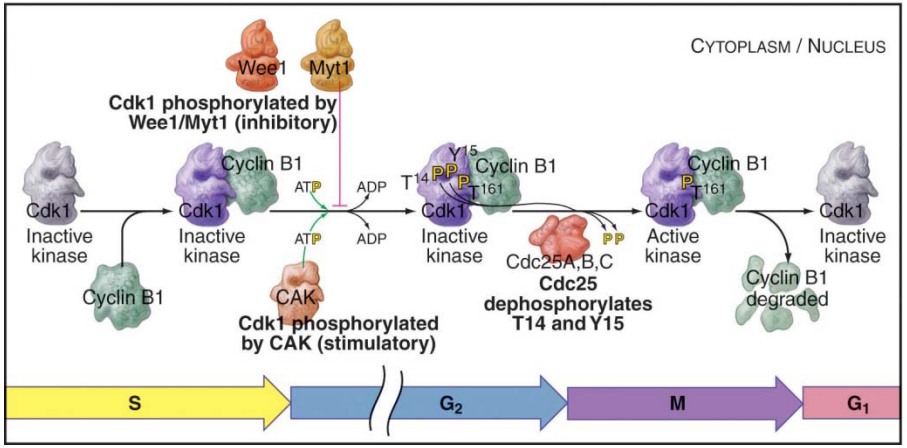


- Centrosome replication

Overview of G_2 Phase

- G_2 phase is relatively short. G_2-M transition is rapid.
- G_2-M transition is regulated primarily through Cdk1-cyclin B1.
- Cdk1-cyclin B1 burst is controlled by one stimulatory kinase (CAK) and two inhibitory kinases (Wee1 & Myt1).
- G_2-M transition is triggered by three Cdc25 phosphatases.



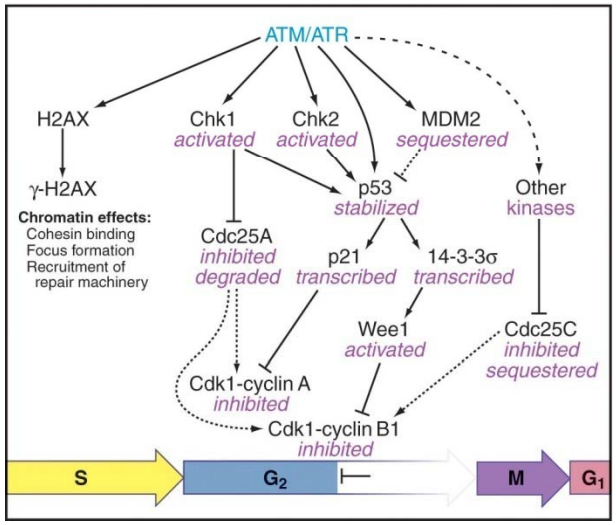


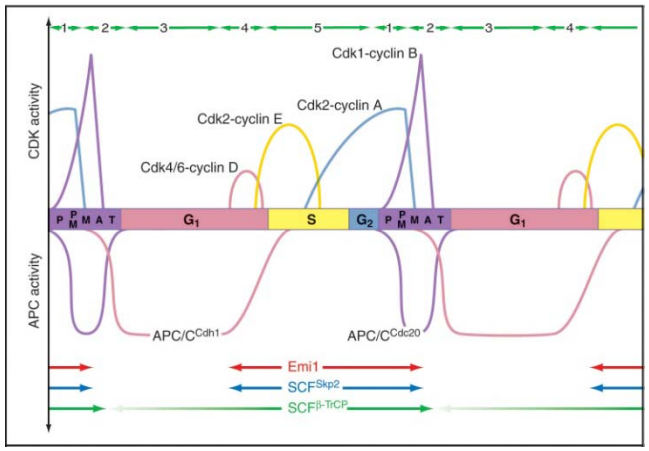
G_2 Checkpoints & G_2-M Transition

- G_2 checkpoints monitor
  - DNA damages
  - Completion of DNA replication

- G_2 checkpoints are mediated by ATM/ATR and their downstream molecules.

  → Critical final check before cell division



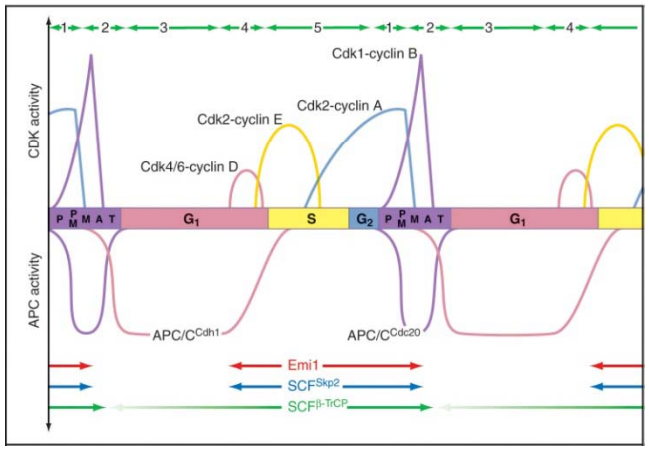


Major Events in G_2-M Transition

- Cdc25 triggers the activation of Cdk2-cyclin A and Cdk1-cyclinB.

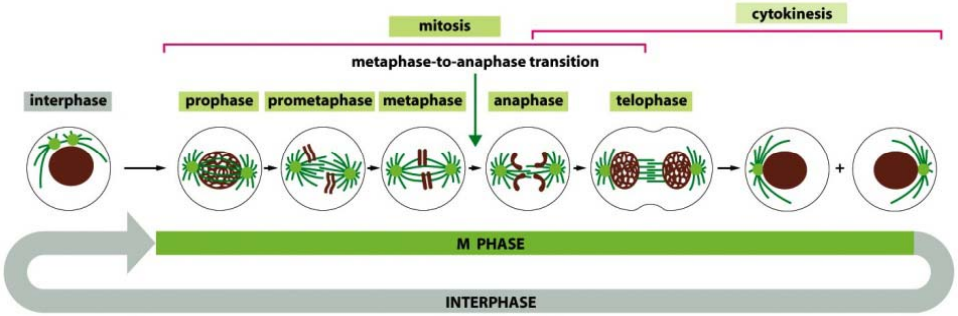
- Events:
  - Higher microtubules dynamics
  - Chromosome condensation.
  - Centrosome migration.

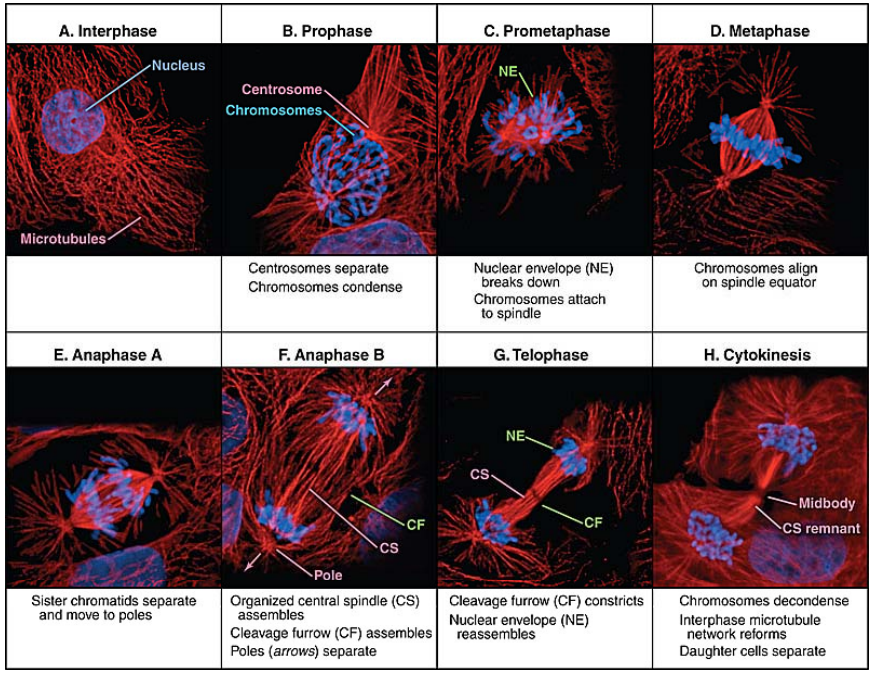
- The complex stimulatory and inhibitory signaling mechanism ensures rapid G2-M transition while offers regulatory options.



Overview of M Phase (I)

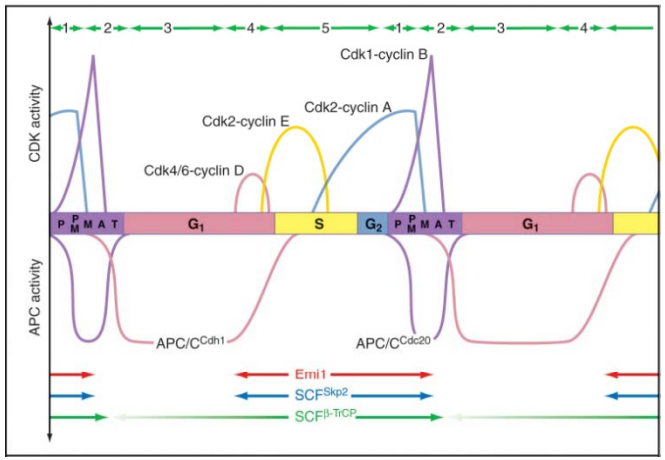
- In metaphase cells undergo dramatic and complex changes in its structure and organization. Only apoptosis is comparable.
- Mitosis is the most complex process in the cell cycle.





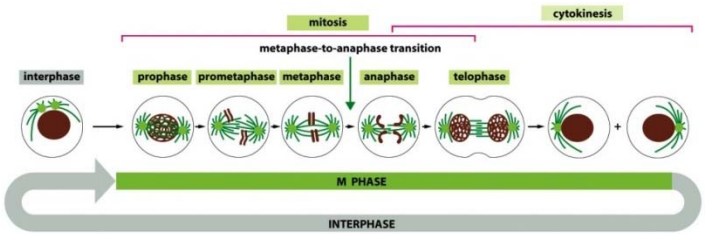
Overview of M Phase (II)

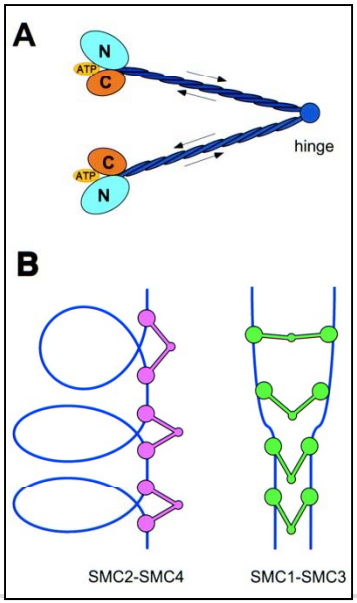
- Cell division is usually symmetric.
- An important exception is the asymmetric division of stem cells.
- Key regulators of metaphase include Cdk2-cyclin A, Cdk1-cyclin B and APC/C^{Cdc20}.



Prophase (I)

- Prophase is the transition phase of G_2 into mitosis.
- Chromosome condensation
  - → H1 and H3 are phosphorylated by Cdk1 and Aurora-B, respectively.
  - → Condensin enters nucleus.
  - → These activities are not essential for chromosome condensation.
- Disassembly of nucleolus

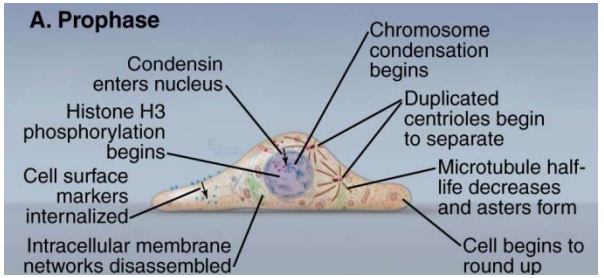


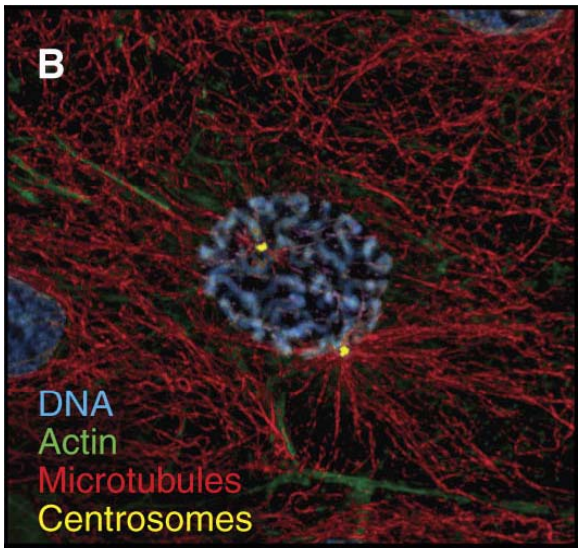


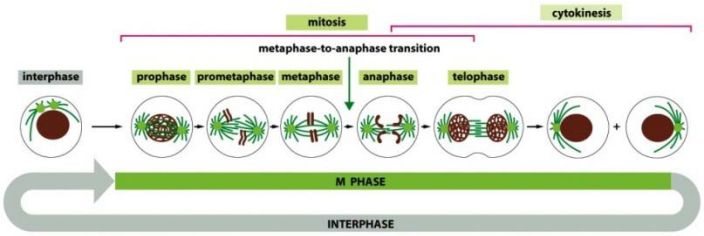
Hirano T, *Genes Dev.* 1999;13:11-19

Prophase (II)

- Microtubules
  - → Microtubules become more dynamic and much shorter.
  - → Increased nucleation at centrosomes.
  - → Microtubules become organized into two radial arrays.
- Intermediate filaments and actin disassemble.
- Transcription stops.
- Intracellular organelles
  - → Golgi and ER fragment.
  - → Membrane-mediated events greatly decrease.
- Cell surface
  - → Endocytosis and exocytosis are suppressed.
  - → Surface receptors are internalized.
- Cell shape becomes rounded.







Overview of Prometaphase

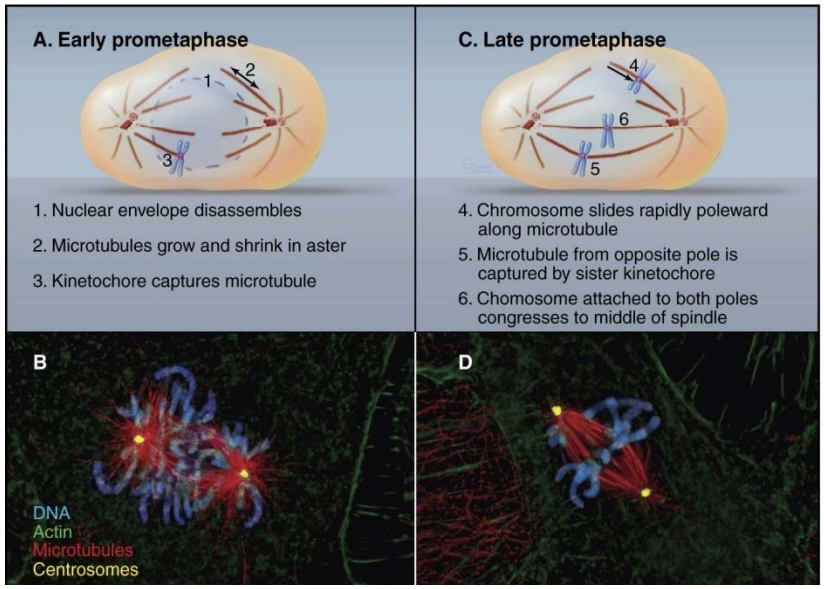
- Major events

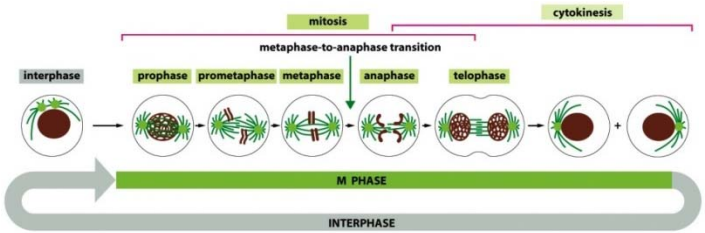
  - Nuclear envelope breaks down.

  - Capture of chromosomes by MTs.

  - Chromosomes establish bipolar attachment at kinetochores.

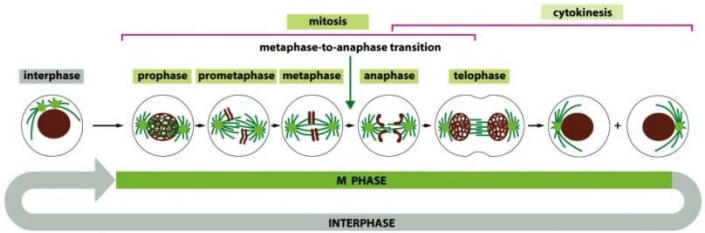
  - Correction of attachment errors under the control of spindle checkpoint molecules.

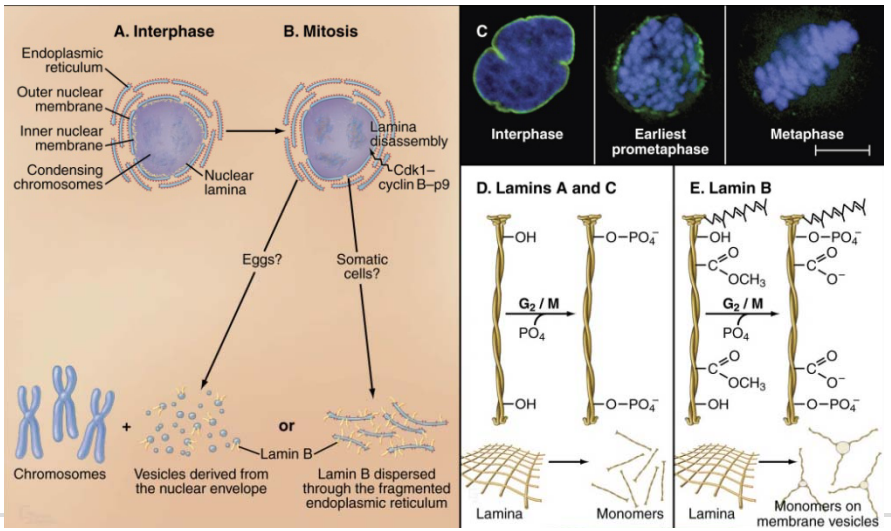




Prometaphase: Nuclear Envelope Breakdown

- Nuclear membrane bilayers are removed.
- Nuclear pores disassemble.
- Nuclear lamina meshwork disassemble.
- Broken nuclear envelope components are organized differently in different cells.

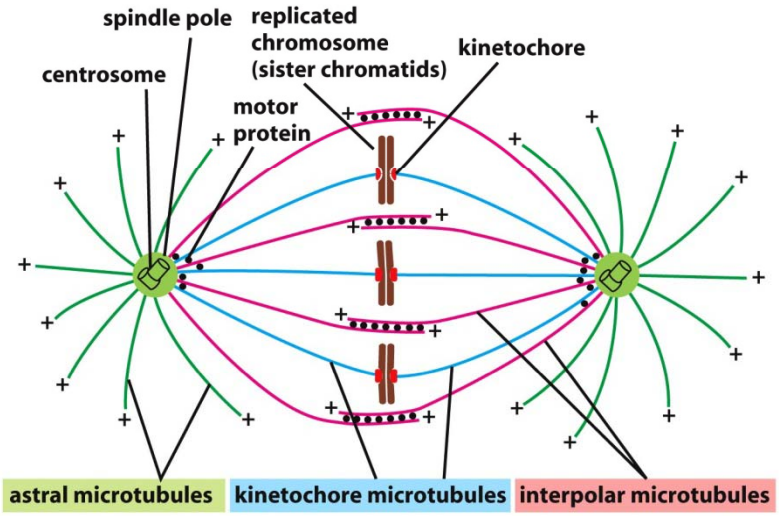


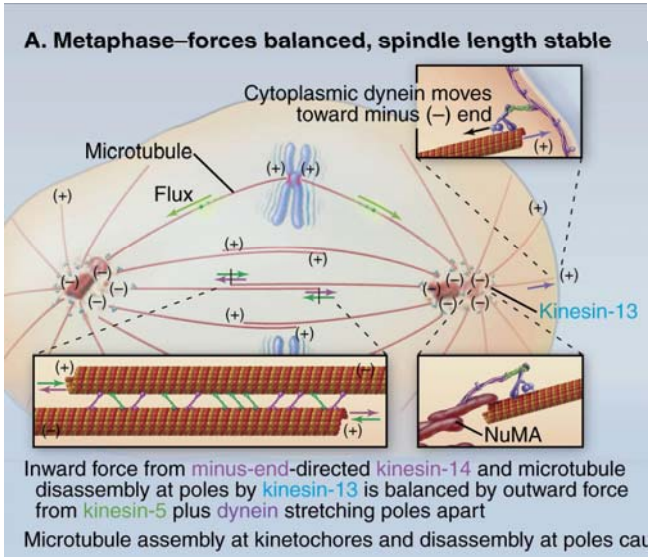


Prometaphase: Mitotic Spindle Organization (I)

- Three groups of microtubules
  - Kinetochore MTs
  - Interpolar MTs
  - Astral MTs

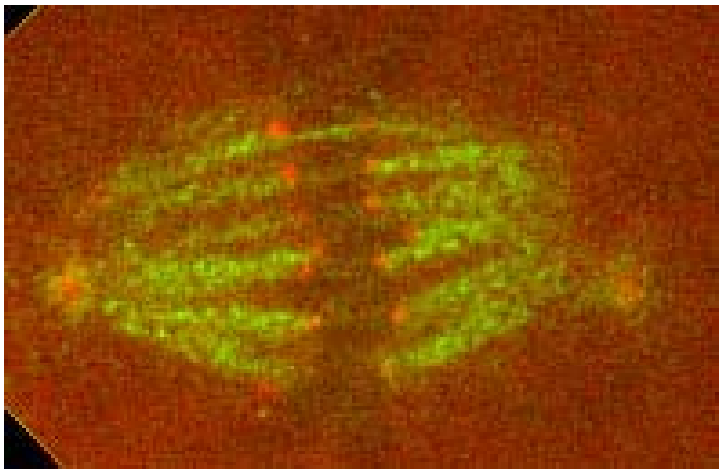
- Molecular motors play critical roles in maintaining the dynamic architecture of the mitotic spindle.



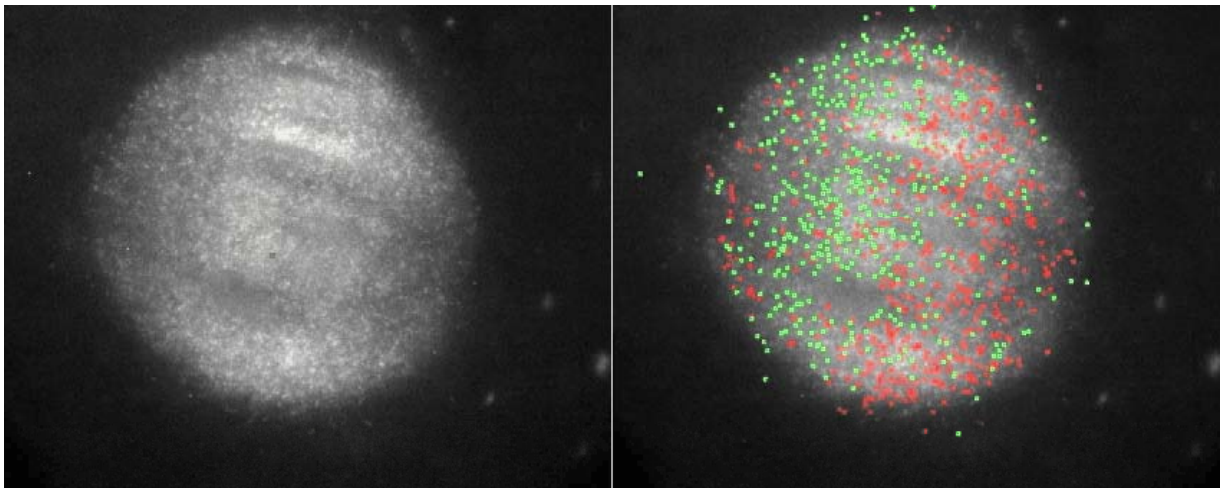


Prometaphase: Mitotic Spindle Organization (II)

- Constant addition of tubulin at MT plus ends is balanced by depolymerization at MT minus ends. This generates microtubule flux.



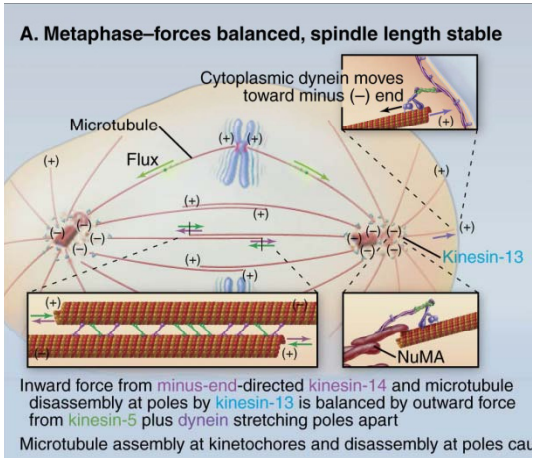
mitotic spindle



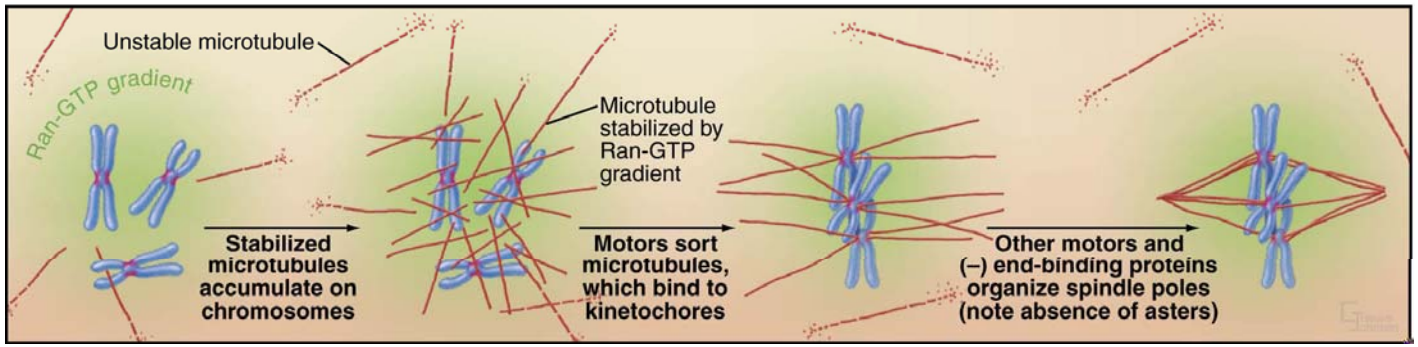
Yang et al., *J. Cell Biology,* 182:631-639, 2008

Spindle Assembly: MT Organization (I)

- Two pathways of microtubule assembly
  - Centrosome-mediated assembly
  - Centrosome-independent assembly

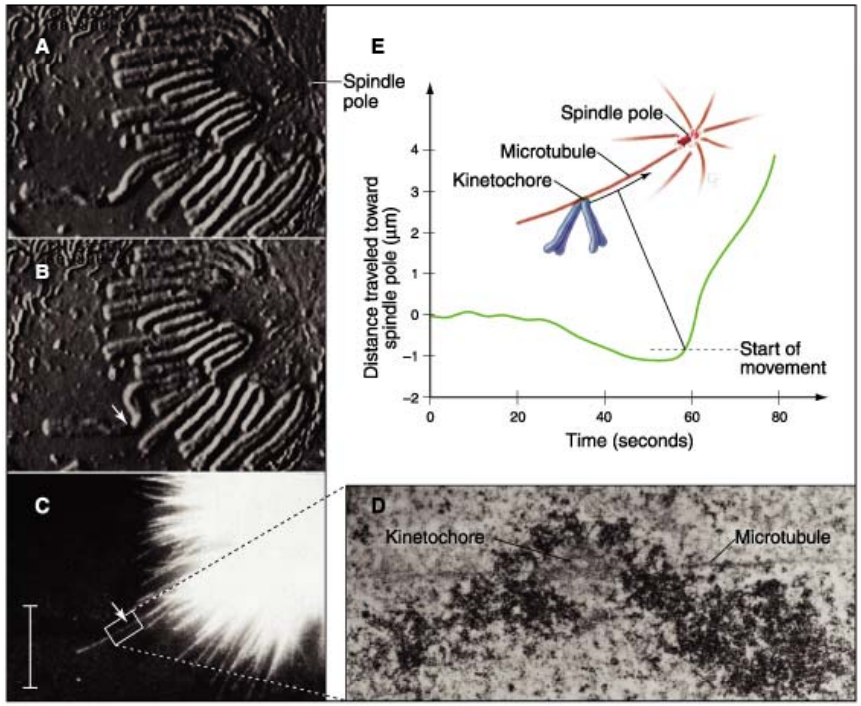


- Centrosome-independent spindle assembly depends on a Ran-GTP gradient.



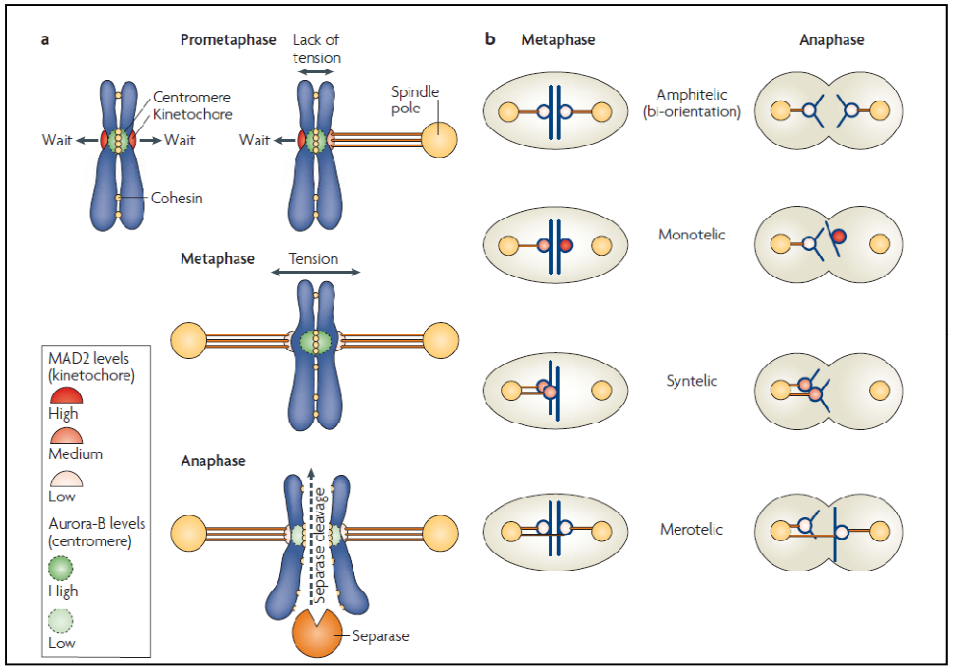
Spindle Assembly: Bipolar Attachment (II)

- Two mechanisms to establish bipolar attachment of microtubule and chromosomes
  - Search and capture
  - Chromosome mediated MT growth



Error Correction & Spindle Checkpoint

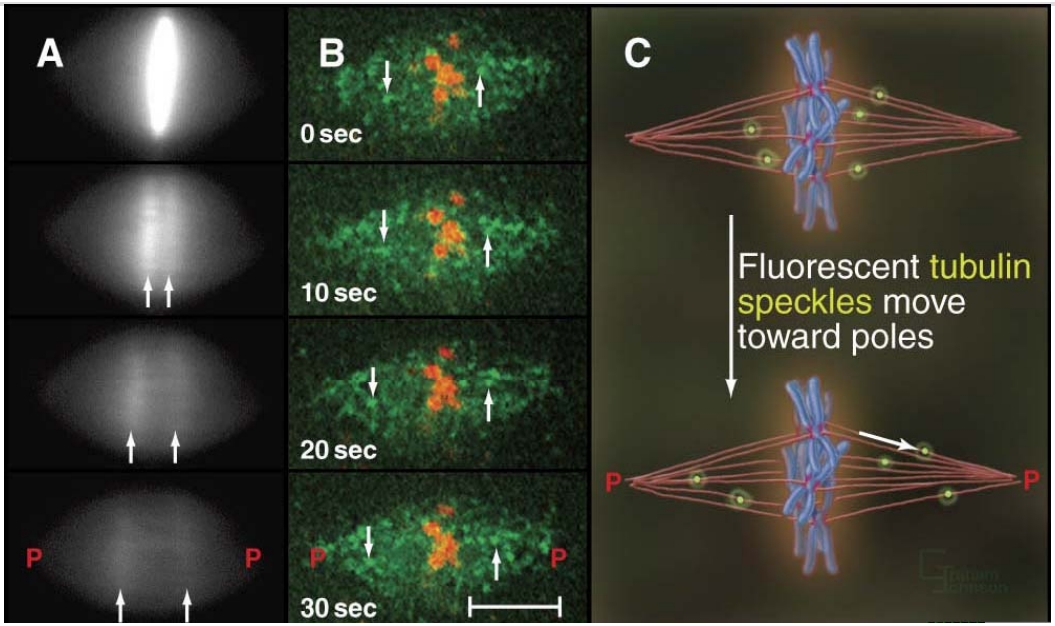
- Tension between sister chromatids is essential to error detection and correction.
- MAD1/2 & Aurora-B kinase play critical roles in spindle checkpoint.

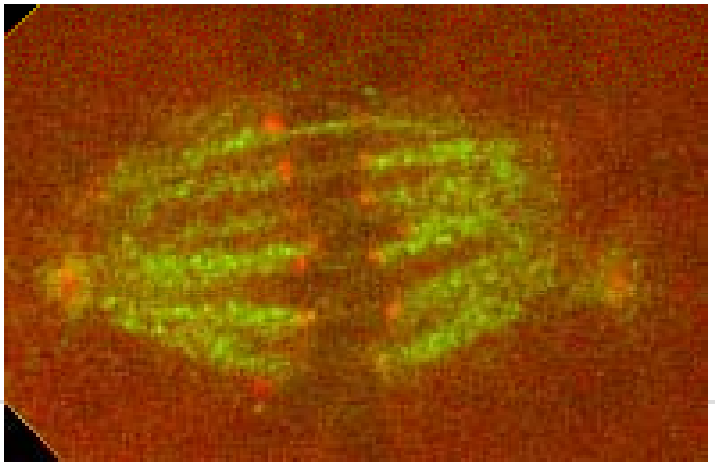


Musacchio & Salmon, *Nat. Rev. Mol. Cell Biol.* 8:319, 2007.

Metaphase

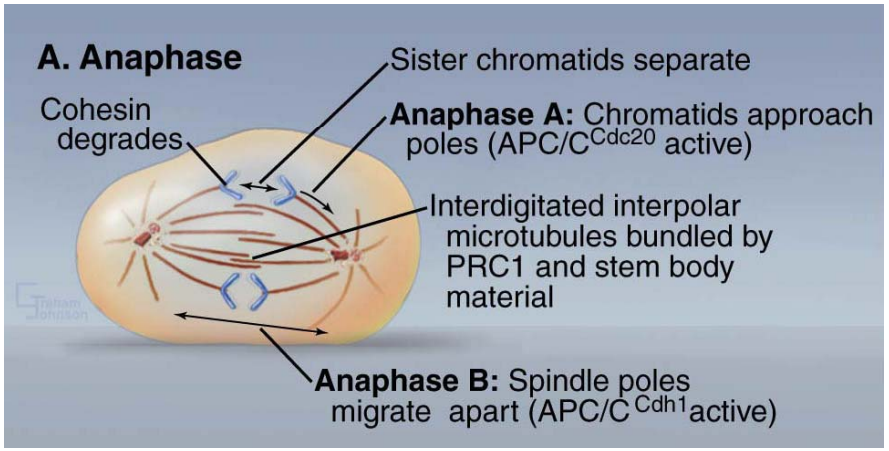
- Chromosomes become aligned at the metaphase plate.
- Microtubules undergo constant poleward flux.
- Chromosomes oscillation during metaphase.
- APC/C promotes the degradation of securin.

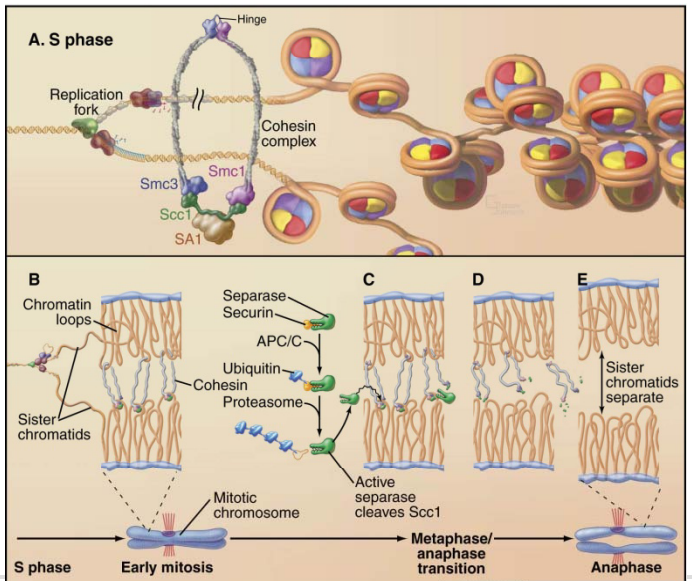




Anaphase A

- Movement of sister chromatids to the poles requires shortening of kinetochore MTs.
- Anaphase A follows activation of APC/C^{Cdc20}.
- After spindle checkpoint is turned off, APC/C^{Cdc20} triggers the degradation of securin.
- Reduced securin level allows separase to cleave cohesin.



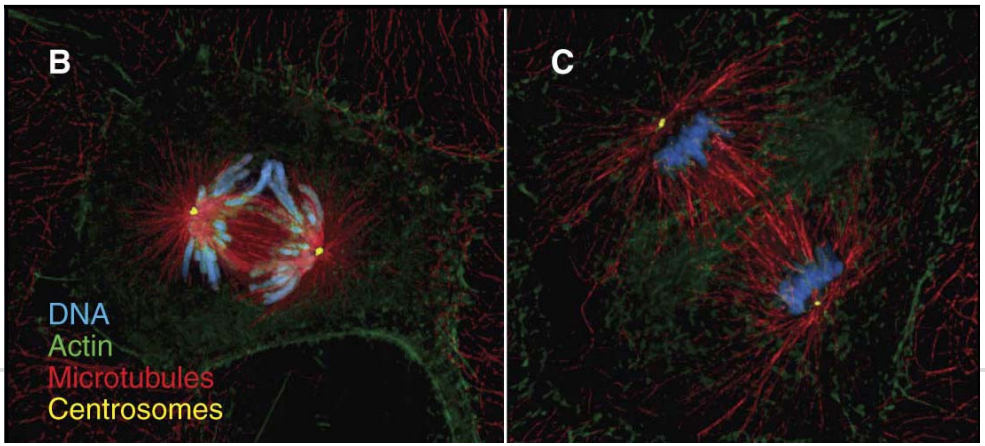


Anaphase B

- Spindle elongation pushes spindle poles apart in Anaphase B.

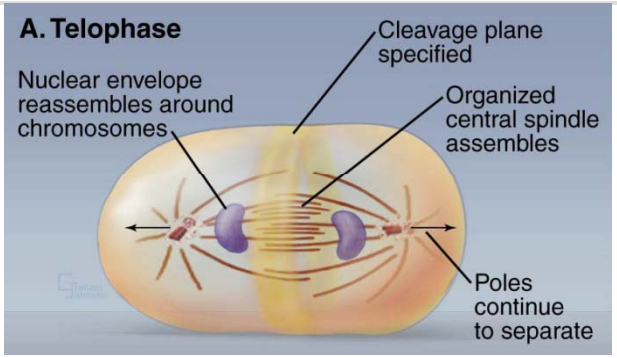
- Chromosome movement is driven by two factors
  - microtubule shortening and growth
  - microtubule flux

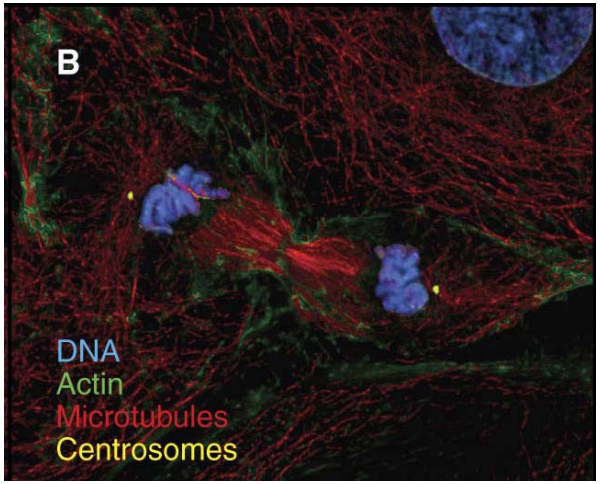
- Spindle elongation
  - Antiparallel sliding of microtubules
  - Microtubule growth
  - Spindle pole motility



Telophase

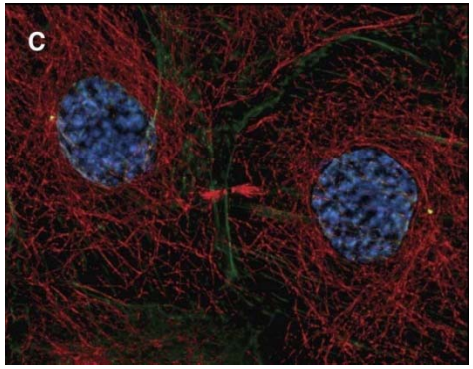
- Nuclear envelope starts to reassemble in late anaphase and is completed in telophase.
- Ran-GTP mediates nuclear envelope assembly.
- Nuclear lamina reassembles through recycling of disassembled lamin subunits.

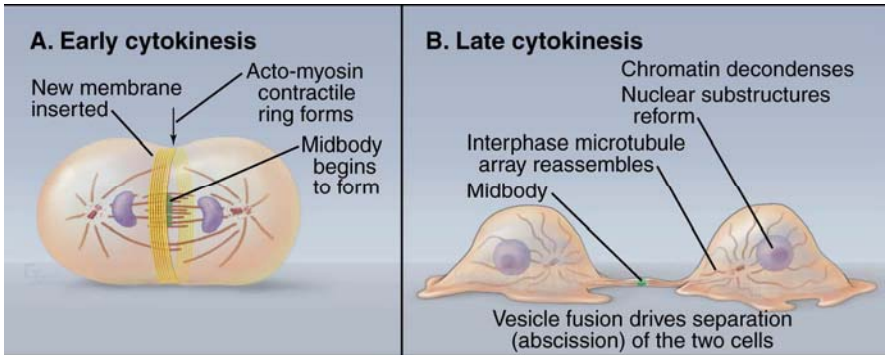




Cytokinesis (I)

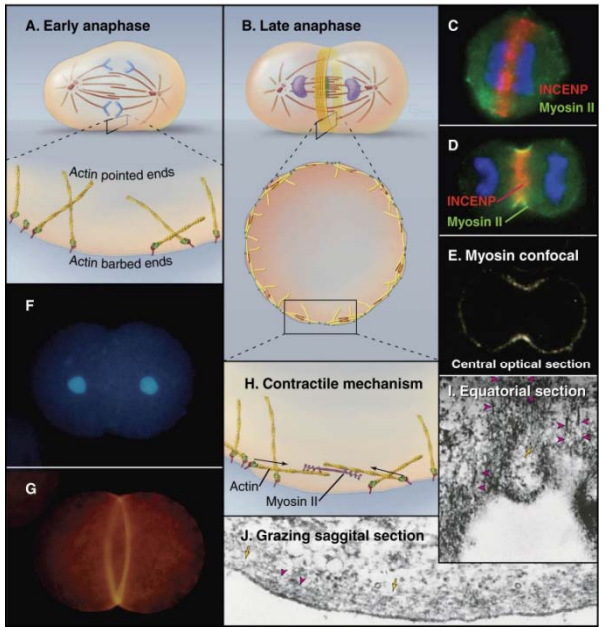
- Two daughter cells become separated through cytokinesis.
- Formation of the contractile ring requires actin and myosin-II.
- Separation of two daughter cells is accompanies by constriction and disassembly of the contractile ring.

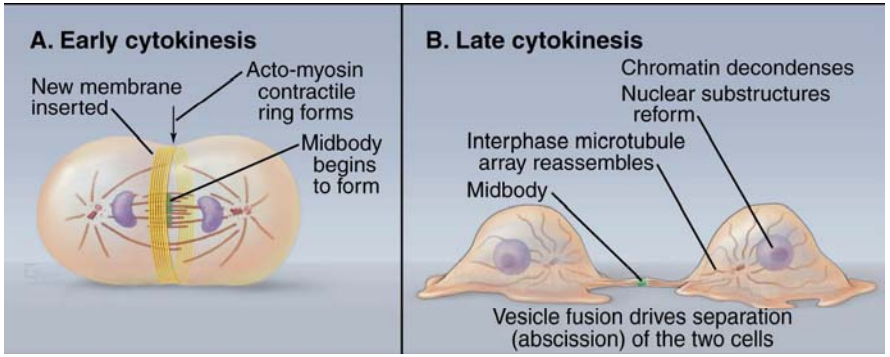




Cytokinesis (II)

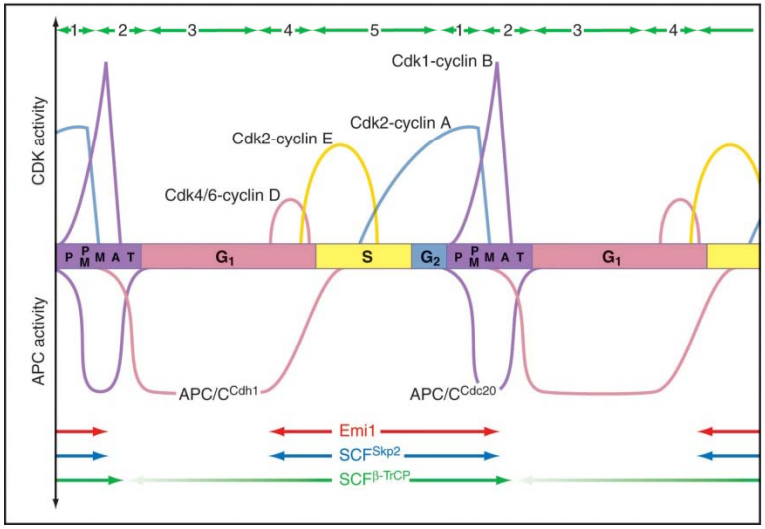
- Cytokinesis requires membrane addition and abscission.
- Secretory vesicles from the Golgi provides new membrane.
- Midbody contains many proteins involved in membrane trafficking.
- Intracelluar bridges may remain open to connect cells.





Exit From Mitosis

- Cdk1 must be inactivated for exit from mitosis.
- Much of what is known of exit from mitosis comes from budding yeast.
- Exit from mitosis in yeasts is mediated by the MEN GTPase.
- Lowered Cdk activities allow the release of MEN GTPases.
- Released MEN GTPases activate Cdc14p, which inhibits Cdks.



Questions?