BME 42-620 Engineering Molecular Cell Biology

Lecture 23:

Cell Cycle (II)

Chapter 17

Final Exam Schedule

Group ID	Students	Paper	Time & Location
1	Wing Chiu Tam, Zixuan He, Jian Zhang		
2	Kenneth Yan, April Watt, Daniel Engel	Shimamoto et al, *Cell*, 2011	Dec. 16, 10:30AM Mellon Institute 411
3	Rebecca Duffy, Kevin Kennedy, Stephanie Wong	Levy et al, *Cell*, 2010	Dec. 11, 9:00AM Mellon Institute 411
4	Priti Albal, Arun Sampath, Madhumitha Raghu, Sombeet Sahu		
5	Jackie Chen, Jaclyn Brackett, Pitirat Pholpabu		
6	Simone Costa, Christine Bronikowski, James Rockwell		

Final Exam Papers

1) R. Delanoue & I. Davis, Dynein anchors its mRNA cargo after apical transport in the Drosophila blastoderm embryo, *Cell*, 122:97-106, 2005.

2) D. Levy & R. Heald, Nuclear size is regulated by importin α and Ntf2 in Xenopus, *Cell*, 143:288-298, 2010.

3) S. Ally, A. G. Larson, et al, Opposite-polarity motors activate one another to trigger cargo transport in live cells, *Journal of Cell Biology*, 187:1071-1082, 2009.

4) Y. Shimamoto, Y. T. Maeda, et al, Insights into the micromechanical properties of the metaphase spindle, *Cell*, 145:1062-1074, 2011.

5) C. A. Wilson, M. A. Tsuchida, et al, Myosin II contributes to cell-scale actin network treadmilling through network disassembly, *Nature*, 465:373-377, 2010.

6) A. Levskaya, O. D. Weiner, W. A. Lim, C. A. Voigt, Spatiotemporal control of cell signaling using a light-switchable protein interaction, *Nature*, 461:997-1001, 2009.

Outline

- Overview of cell cycle
- Different phases of cell cycle
- Checkpoints; Cyclin and cyclin-dependent kinases
- A detailed look at M phase
- Quantitative system-level study of cell cycle
- Outlook

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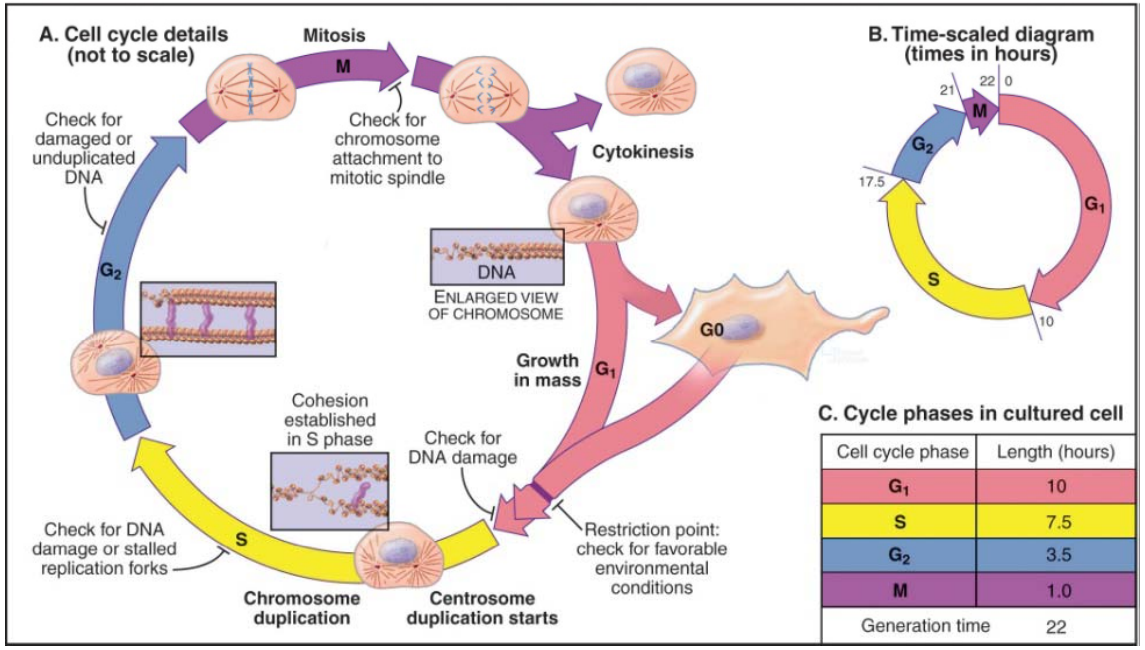
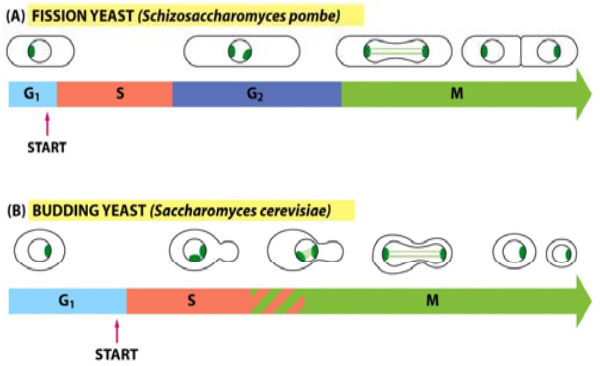
Overview of Cell Cycle (I)

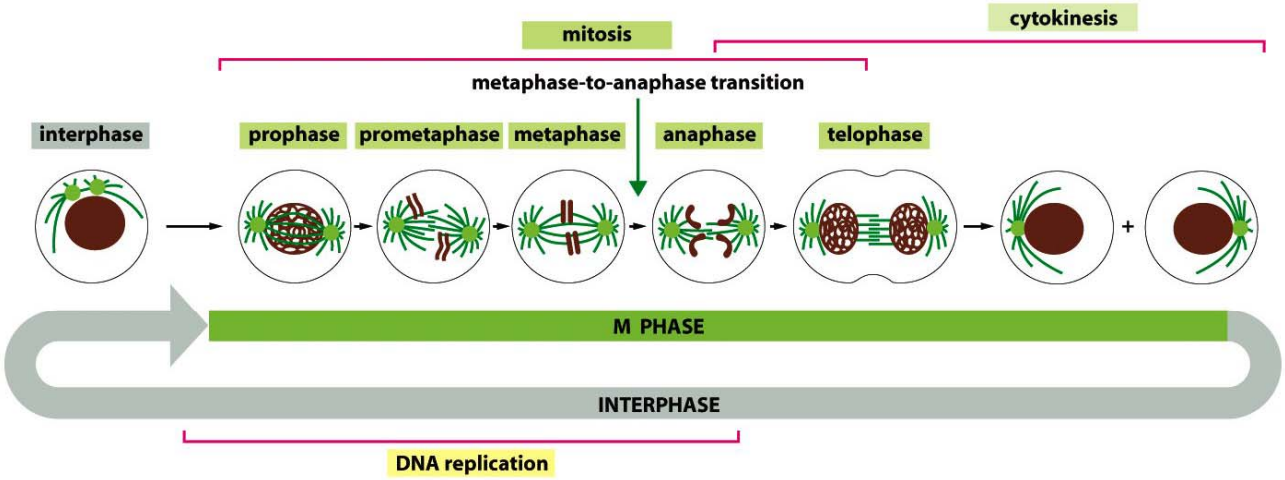
- The cell cycle is a series of events that leads to the replication and division of a cell.
- Two daughter cells inherit the same genetic information from the mother cell.
- Cell cycle must be tightly regulated.
- Basic mechanisms of cell cycle regulation are well conserved in eukaryotes.





Overview of Cell Cycle (II)

- Cell cycle is controlled by a series of biochemical switches (checkpoints).
- Multiple layers of regulation ensures fidelity of cell division by responding to internal and external signals.
- In addition to replicating their genome, most cells replicate their other organelles and macromolecules.
- Growth in cell mass must be regulated too.



- Overview of cell cycle
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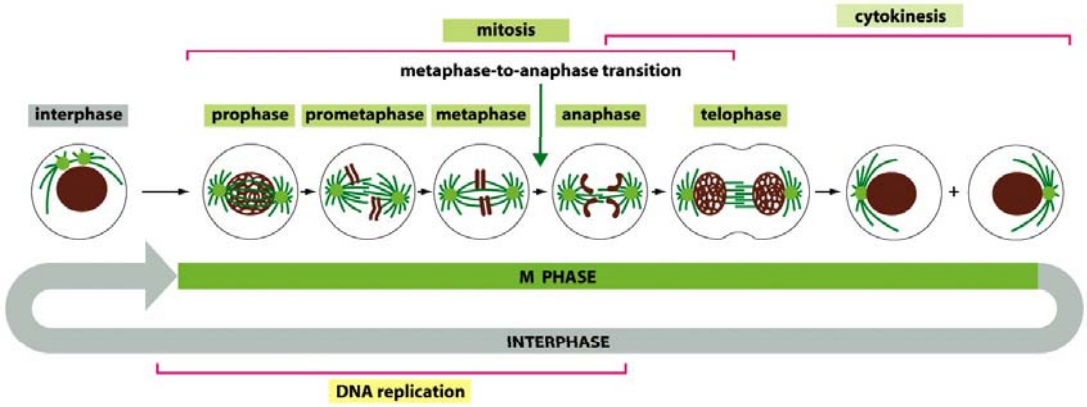
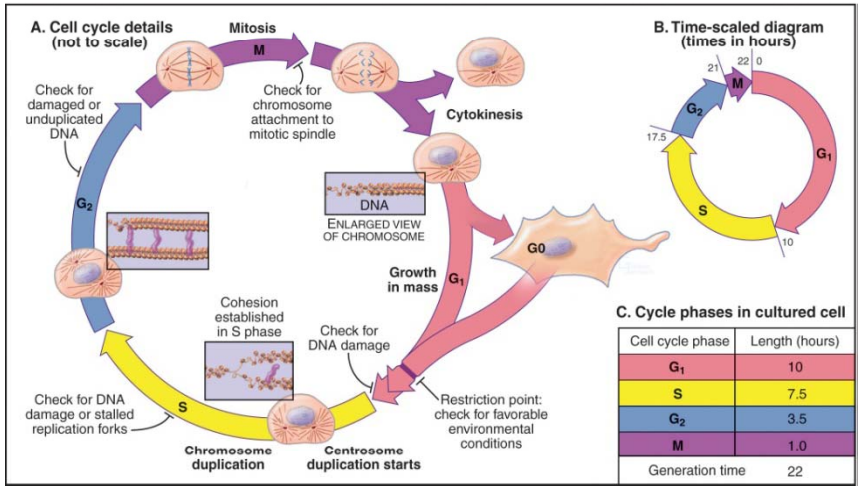
Phases of Cell Cycle (I)

- G_1 phase
  - Grow in cell mass
  - Genes required to activate cell cycle are turned off
  - Can be delayed
  - Can exit to G0
- G_0 and growth control
  - Cells no longer divide
  - Can exit from G_0
  - Cells are active in G_0

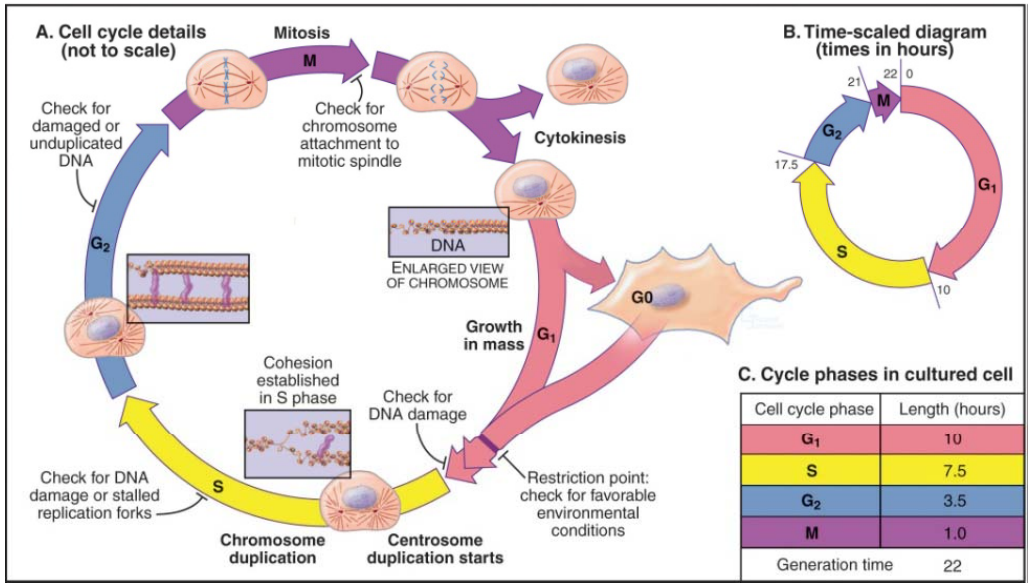




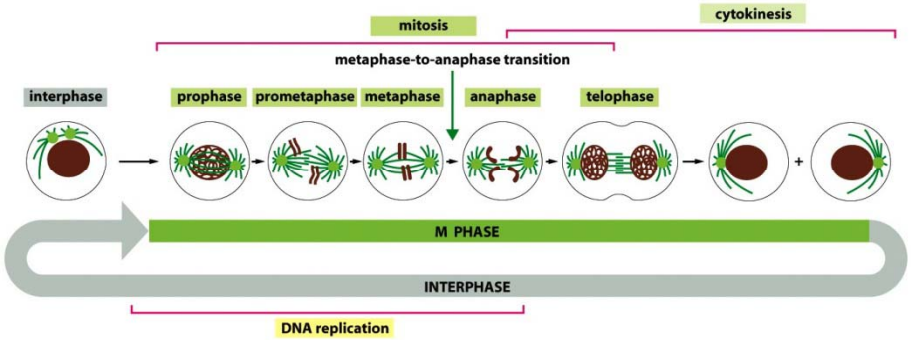
Phases of Cell Cycle (II)

- S phase
  - Centrosome replication
  - DNA replication
  - Sister chromatids are connected by cohesin

- G_2 phase
  - To check for unreplicated or damaged DNA
  - To prepare for mitosis (e.g. accumulation of enzymes)



Phases of Cell Cycle (III)



- M phase
  - → prophase:
    condensation of chromosomes; formation of two poles
  - → prometaphase
    nuclear envelope breakdown; bipolar attachment at kinetochores
  - → metaphase
    chromosomes aligned in the midzone
  - → anaphase
    sister chromatids separated; moving to the two poles
  - → telophase
    reformation of nuclear envelopes
  - → cytokinesis
    formation of contractile ring; separation of two daughter cells

- Overview of cell cycle
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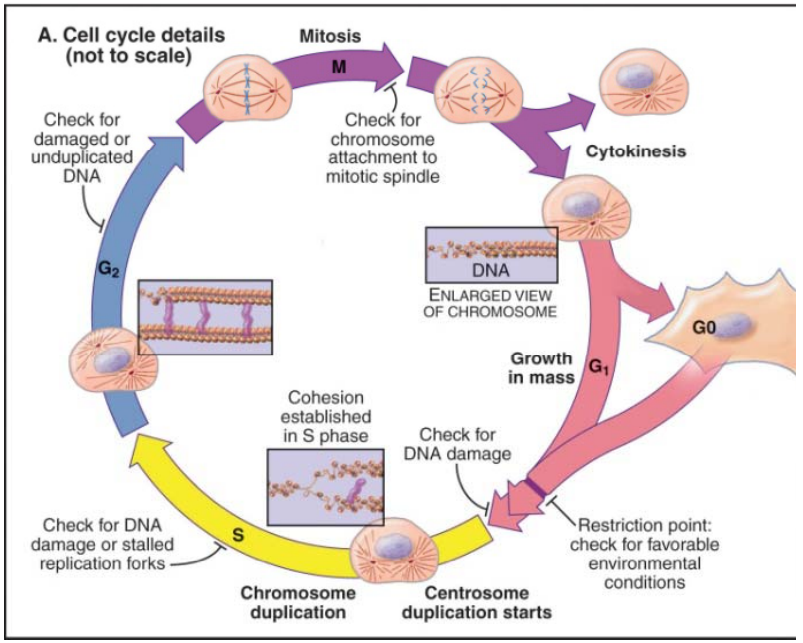
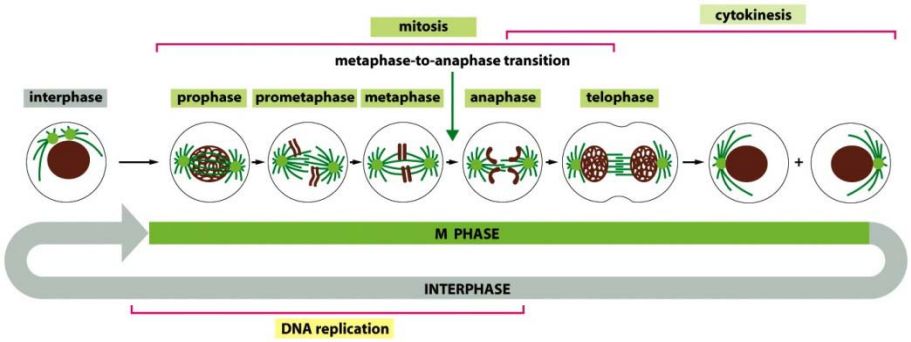
Checkpoints (I)

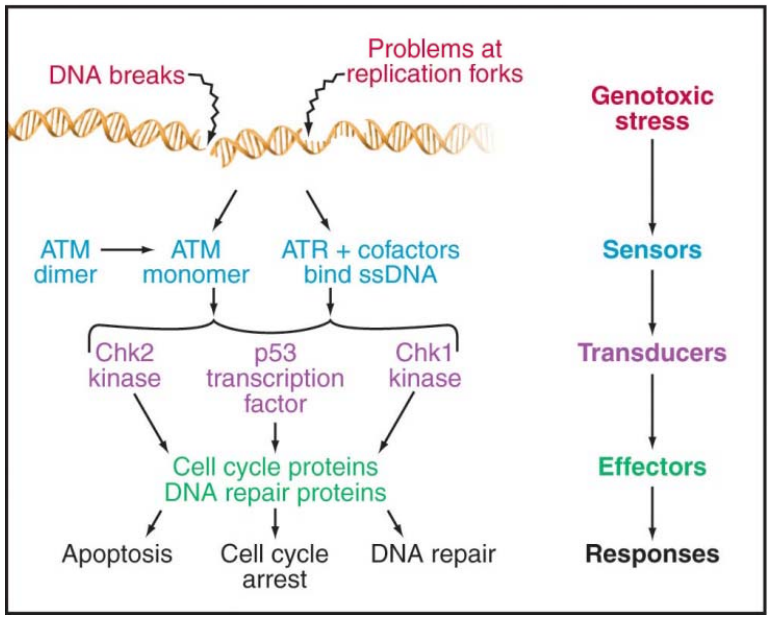
- Check points are biochemically implemented switches that control transition between cell-cycle stages
- Restriction point in G1 phase
- DNA damage checkpoint in G1, S, G2 phase
- DNA replication checkpoint
- Spindle assembly checkpoint





Checkpoints (II)

- DNA damage check point
- Sensing of DNA damage: ATM & ATR
- ATM & ATR activate Chk1& Chk2 and stabilizes p53.
- Cell-cycle progression is halted if DNA damage is detected.
- Cells can enter into several states
  - Cell death
  - Cell cycle arrest
  - Successful DNA repair; Resume cell cycle

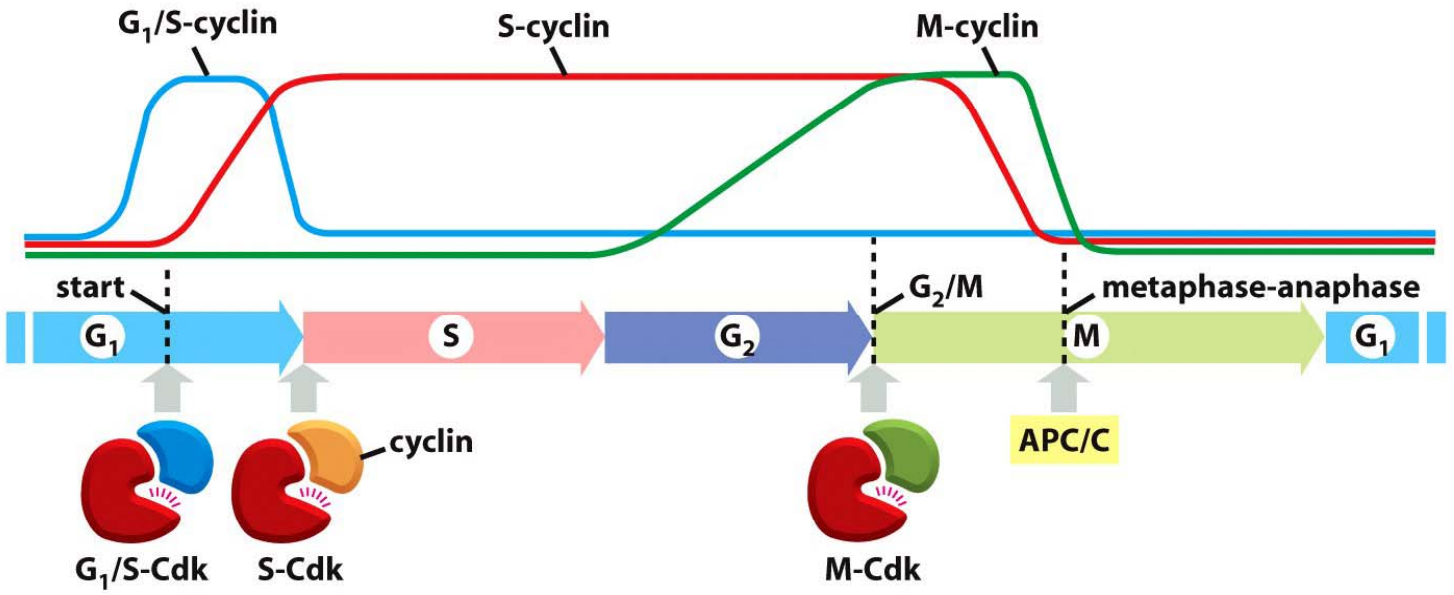


ATM: ataxia-telangiectasia mutated
ATR: ataxia-telangiectasia and Rad9 related

Cyclin and Cyclin-Dependent Kinase (I)

- Transition between different stages of the cell cycle are controlled by a network of kinases and phosphatases.

- Cyclin-dependent kinases play critical roles in regulating cell cycle.

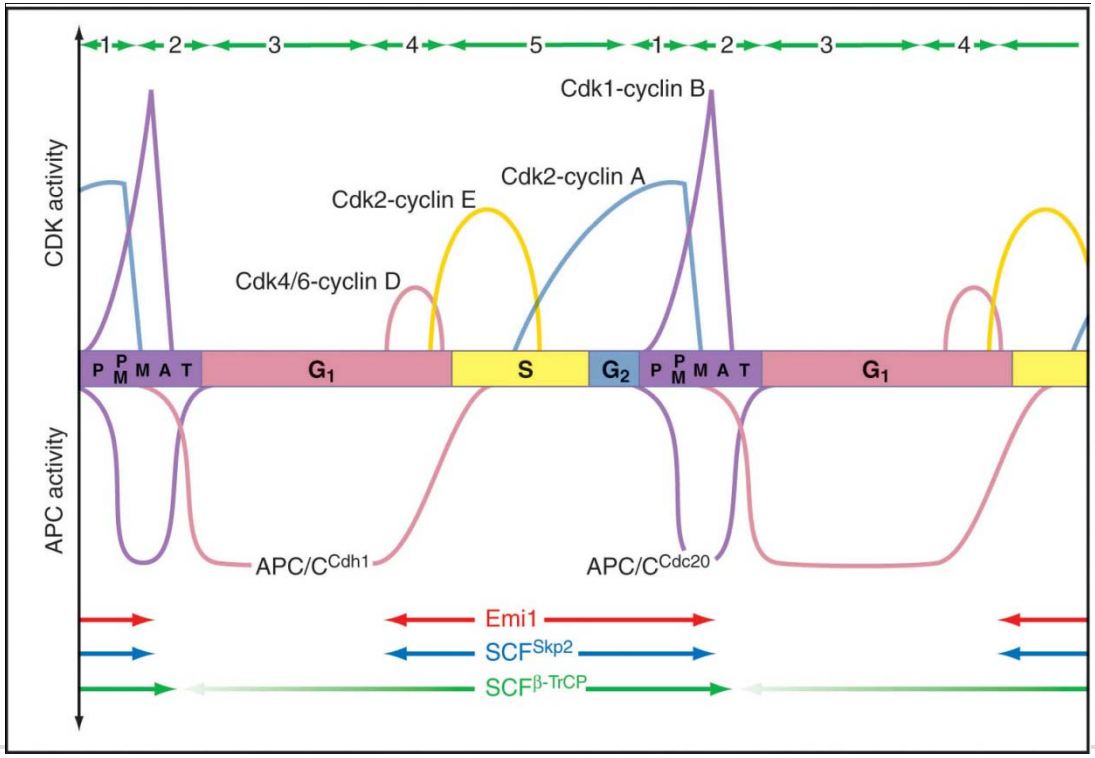


Cyclin and Cyclin-Dependent Kinase (II)

Table 17–1 The Major Cyclins and Cdks of Vertebrates and Budding Yeast

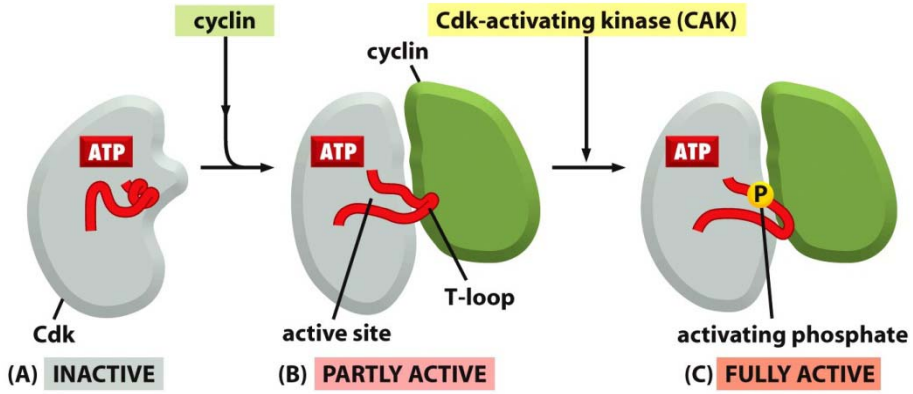
CYCLIN–CDK COMPLEX	VERTEBRATES		BUDDING YEAST	
	CYCLIN	CDK PARTNER	CYCLIN	CDK PARTNER
G_1-Cdk	cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G_1/S-Cdk	cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	cyclin A	Cdk2, Cdk1**	Clb5, 6	Cdk1
M-Cdk	cyclin B	Cdk1	Clb1, 2, 3, 4	Cdk1

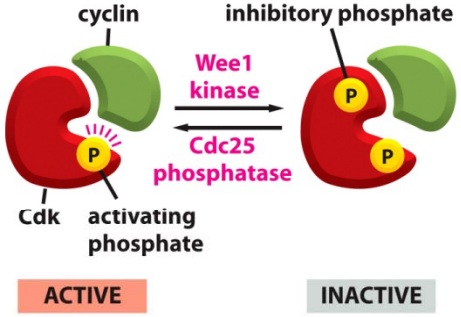
* There are three D cyclins in mammals (cyclins D1, D2, and D3).
** The original name of Cdk1 was Cdc2 in both vertebrates and fission yeast, and Cdc28 in budding yeast.

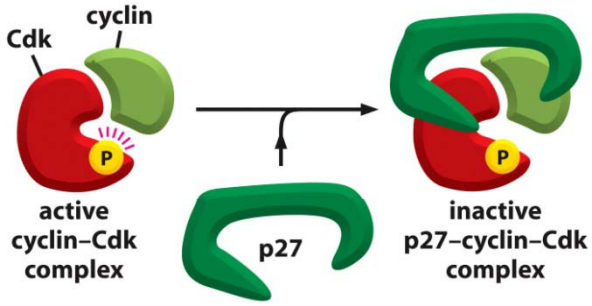


Cyclin and Cyclin-Dependent Kinase (III)

- CAK (cdk-activating kinase) actives cyclin-CDK complexes.
- Activated cyclin-CDK complexes can be inhibited by Cdk inhibitor proteins (CKI) or inhibitory phosphorylation.
- Redundant mechanisms used to ensure robustness and fidelity of cell-cycle control.







Protein Degradation in Cell Cycle (I)

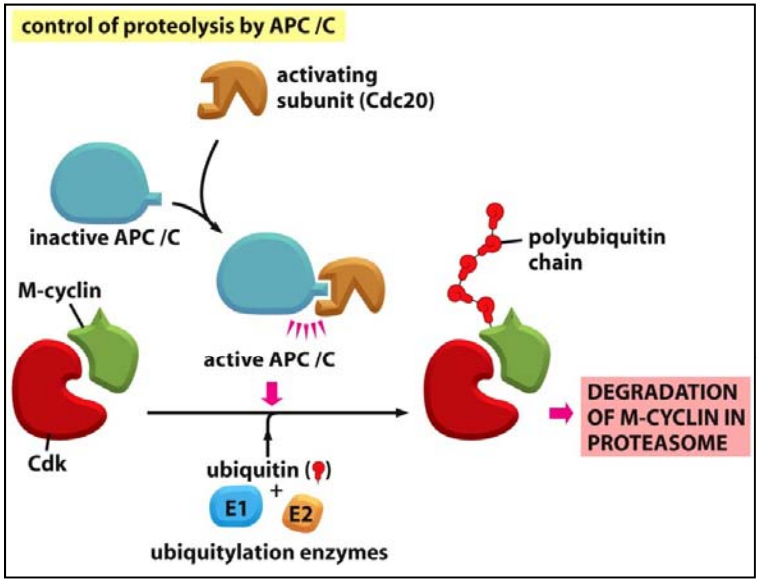
- Protein degradation is a critical cell cycle regulatory mechanism.
- Exit from mitosis requires CDK inactivation
- This is achieved by degradation of cyclins and securin (regulator of sister chromatid separation).
- Destruction of cyclins inactivate CDKs.
- Degradation of cyclin is performed in proteasome and requires ubiquitin enzymes (E1, E2, E3).

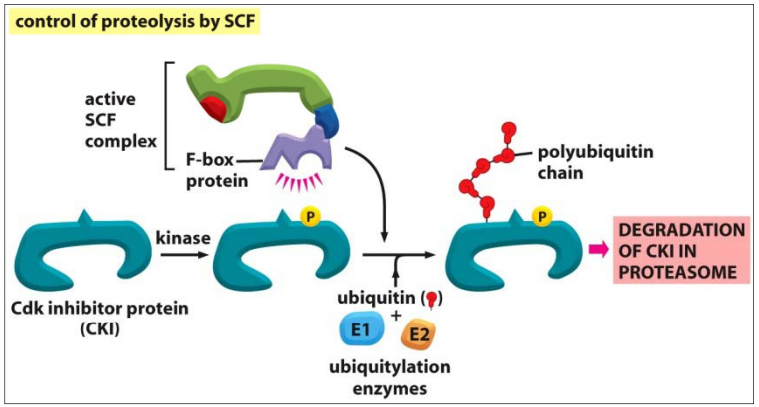
Protein Degradation in Cell Cycle (II)

- Example: a key regulator of cyclin degradation is APC/C (anaphase promoting complex/cyclosome).

- Two forms of APC/C
  - APC/C^{Cdc20}
  - APC/C^{Cdh1}

- APC/C^{Cdc20} is responsible for triggering metaphase-anaphase transition.





Summary of Cell Cycle Regulatory Proteins

Table 17–2 Summary of the Major Cell-Cycle Regulatory Proteins

GENERAL NAME	FUNCTIONS AND COMMENTS
Protein kinases and protein phosphatases that modify Cdks	
Cdk-activating kinase (CAK)	phosphorylates an activating site in Cdks
Wee1 kinase	phosphorylates inhibitory sites in Cdks; primarily involved in suppressing Cdk1 activity before mitosis
Cdc25 phosphatase	removes inhibitory phosphates from Cdks; three family members (Cdc25A, B, C) in mammals; primarily involved in controlling Cdk1 activation at the onset of mitosis
Cdk inhibitor proteins (CKIs)	
Sic1 (budding yeast)	suppresses Cdk1 activity in G_1; phosphorylation by Cdk1 at the end of G_1 triggers its destruction
p27 (mammals)	suppresses G_1/S-Cdk and S-Cdk activities in G_1; helps cells withdraw from cell cycle when they terminally differentiate; phosphorylation by Cdk2 triggers its ubiquitylation by SCF
p21 (mammals)	suppresses G_1/S-Cdk and S-Cdk activities following DNA damage
p16 (mammals)	suppresses G_1-Cdk activity in G_1; frequently inactivated in cancer
Ubiquitin ligases and their activators	
APC/C	catalyzes ubiquitylation of regulatory proteins involved primarily in exit from mitosis, including securin and S- and M-cyclins; regulated by association with activating subunits
Cdc20	APC/C-activating subunit in all cells; triggers initial activation of APC/C at metaphase-to-anaphase transition; stimulated by M-Cdk activity
Cdh1	APC/C-activating subunit that maintains APC/C activity after anaphase and throughout G_1; inhibited by Cdk activity
SCF	catalyzes ubiquitylation of regulatory proteins involved in G_1 control, including some CKIs (Sic1 in budding yeast, p27 in mammals); phosphorylation of target protein usually required for this activity

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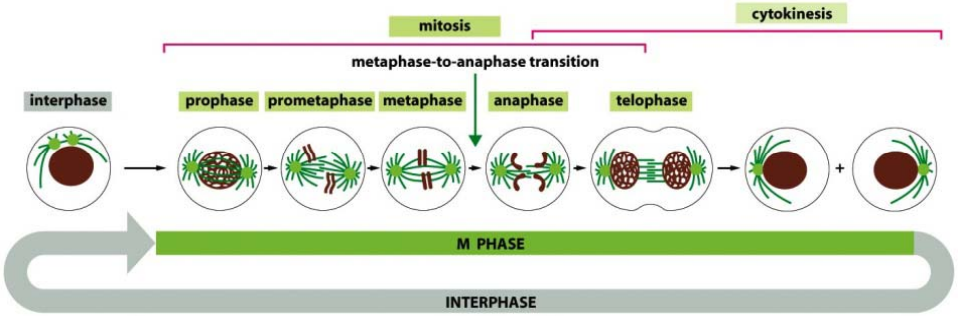
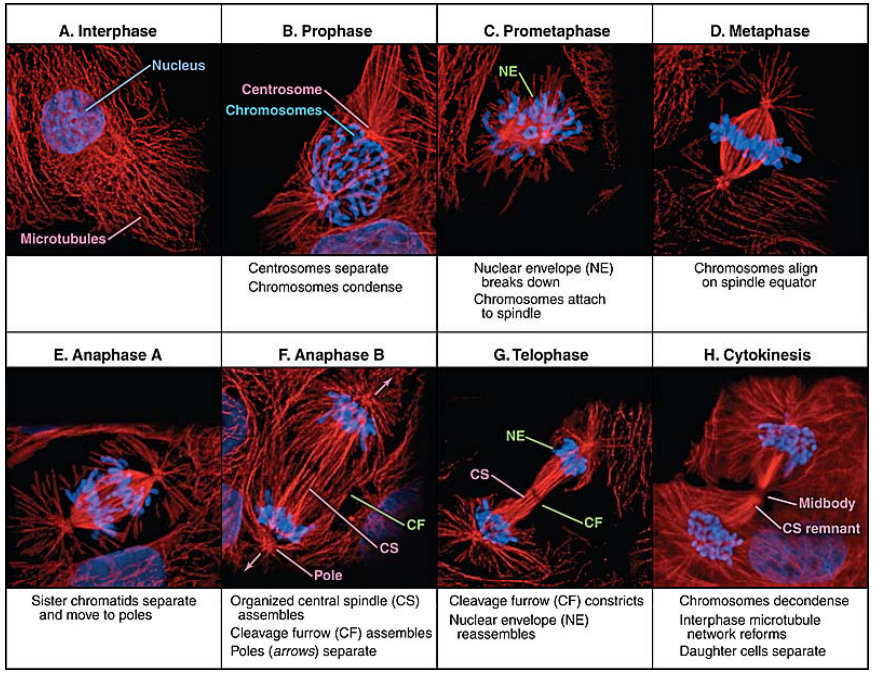
Overview of M Phase (I)

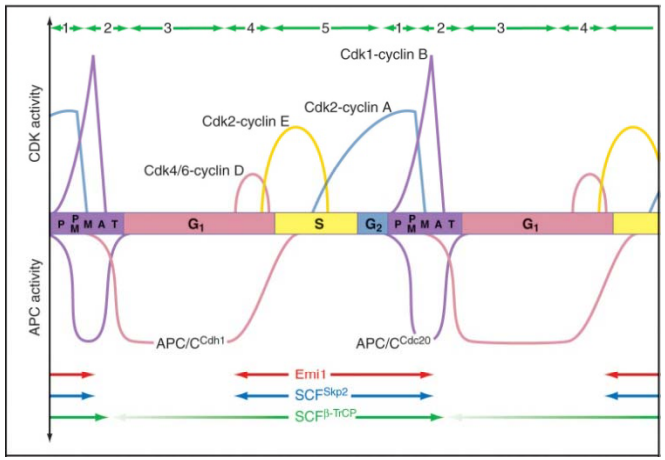
- In metaphase cells undergo dramatic and complex changes in its structure and organization. Only apoptosis is comparable.
- Mitosis is the most complex process in the cell cycle.





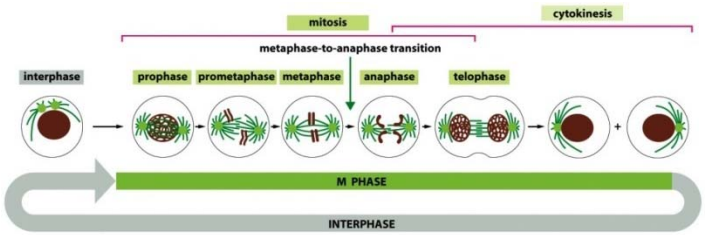
Overview of M Phase (II)

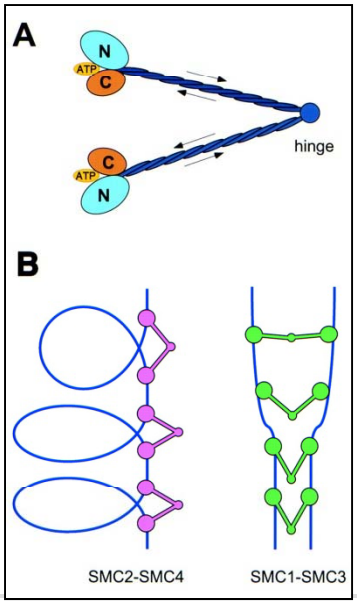
- Cell division is usually symmetric.
- An important exception is the asymmetric division of stem cells.
- Key regulators of metaphase include Cdk2-cyclin A, Cdk1-cyclin B and APC/C^{Cdc20}.



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

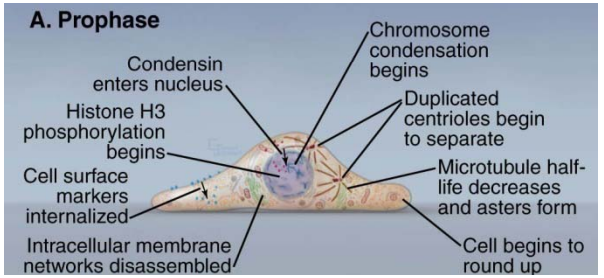


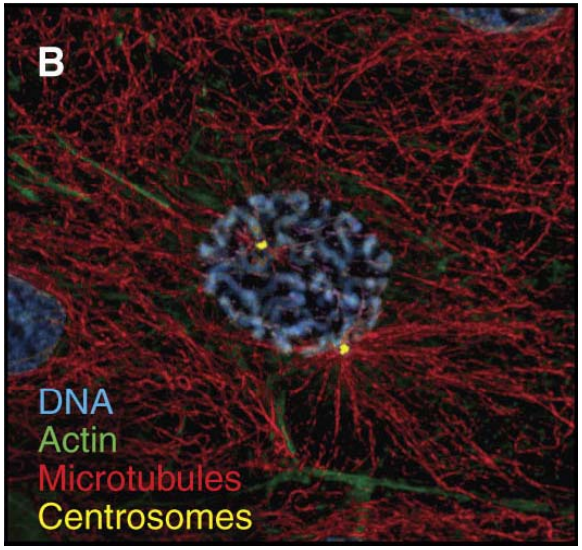


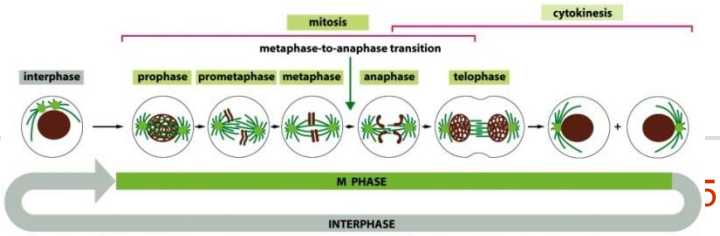
Hirano T, *Genes Dev.* 1999;13:11-19

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

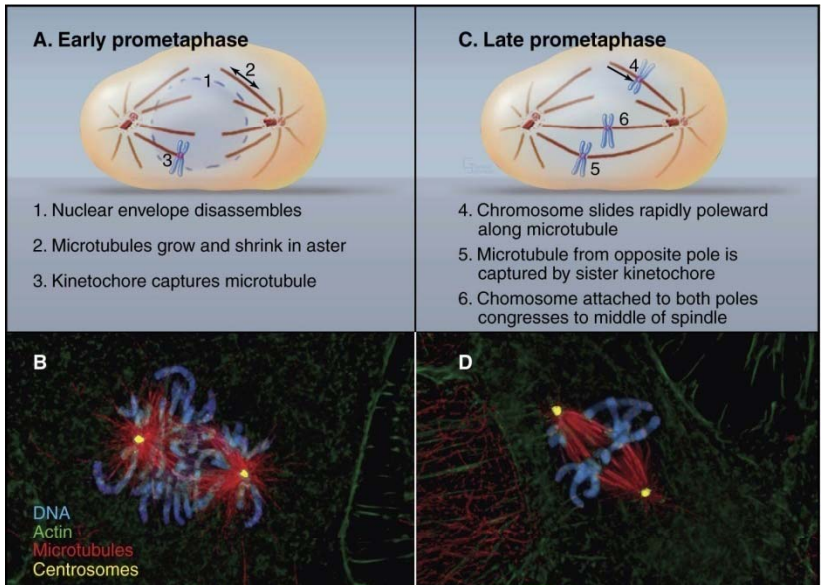
- Major events

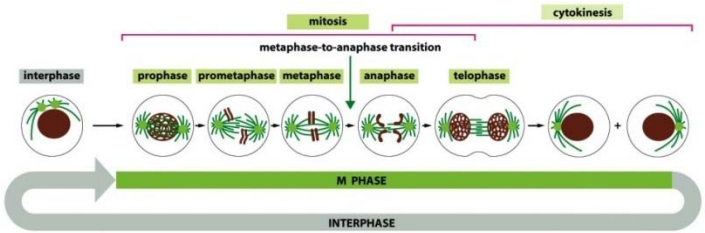
  - Nuclear envelope breaks down.

  - Capture of chromosomes by MTs.

  - Chromosomes establish bipolar attachment at kinetochores.

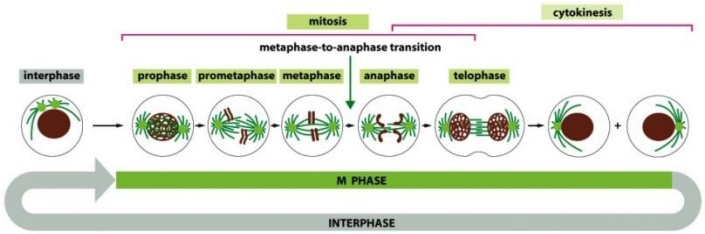
  - Correction of attachment errors under the control of spindle checkpoint molecules.

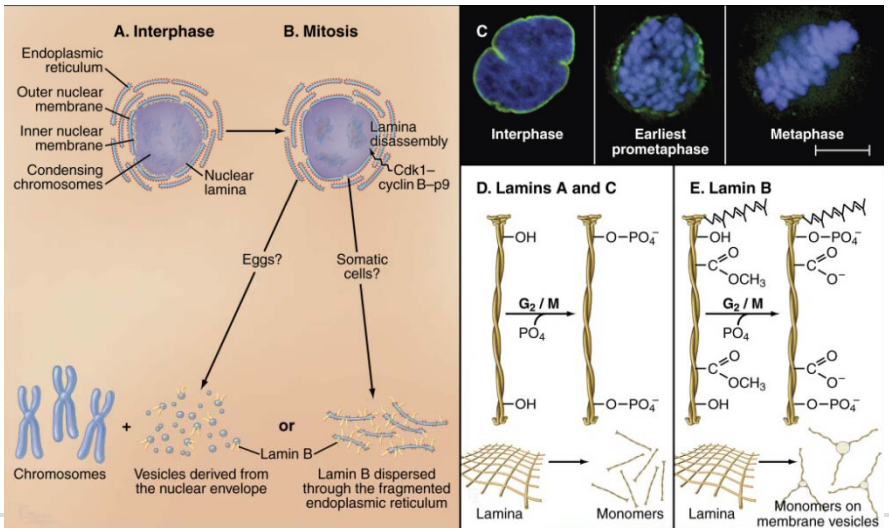




Prometaphase: Nuclear Envelope Breakdown

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

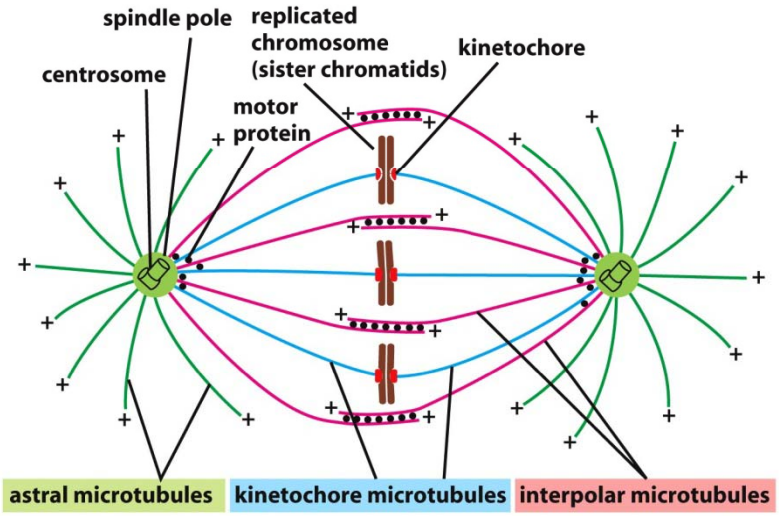


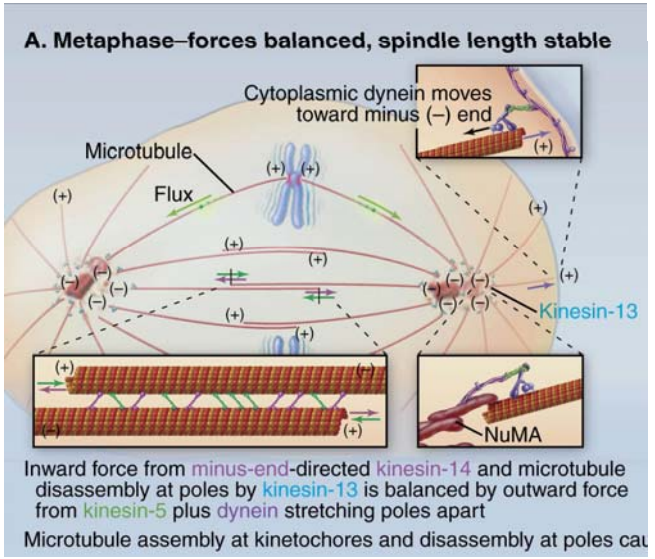


Prometaphase: Mitotic Spindle Organization (I)

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

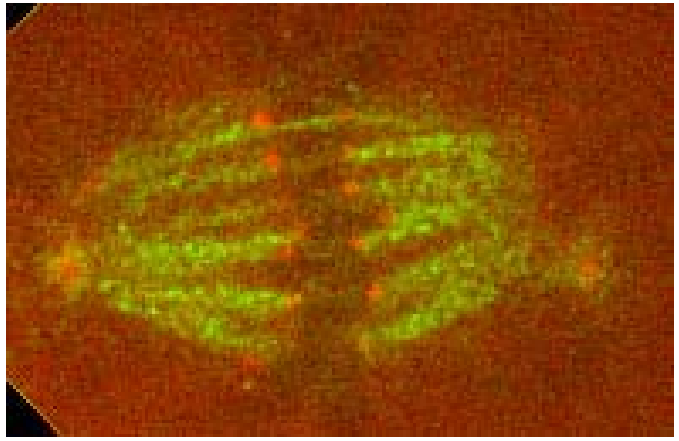
- Molecular motors play critical roles in maintaining the dynamic architecture of the mitotic spindle.



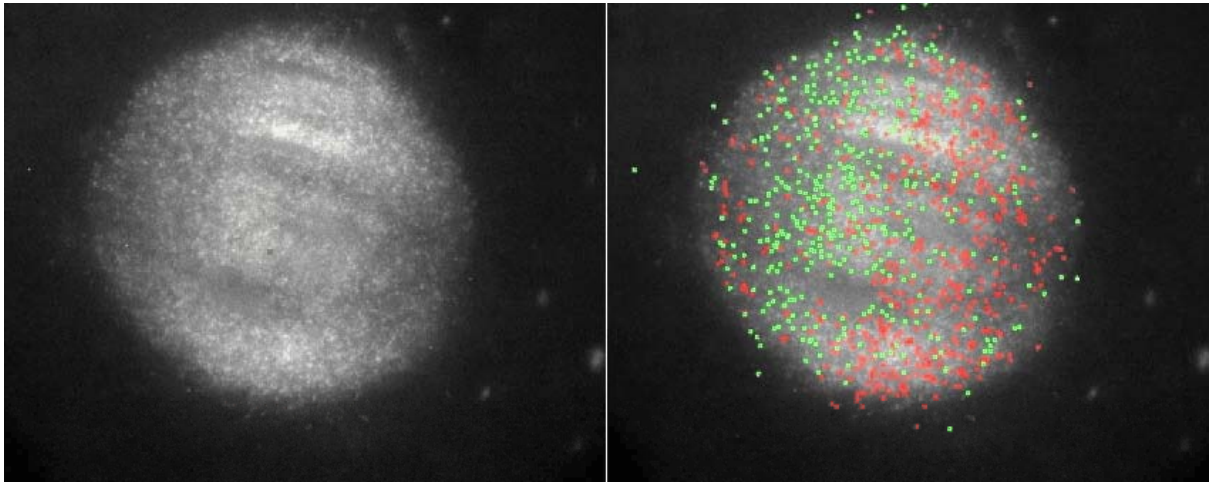


Prometaphase: Mitotic Spindle Organization (II)

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



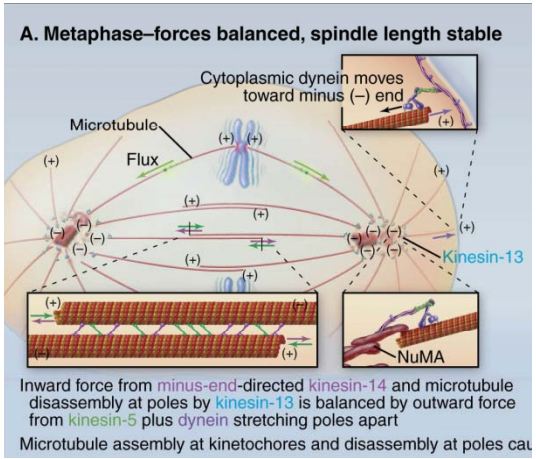
mitotic spindle



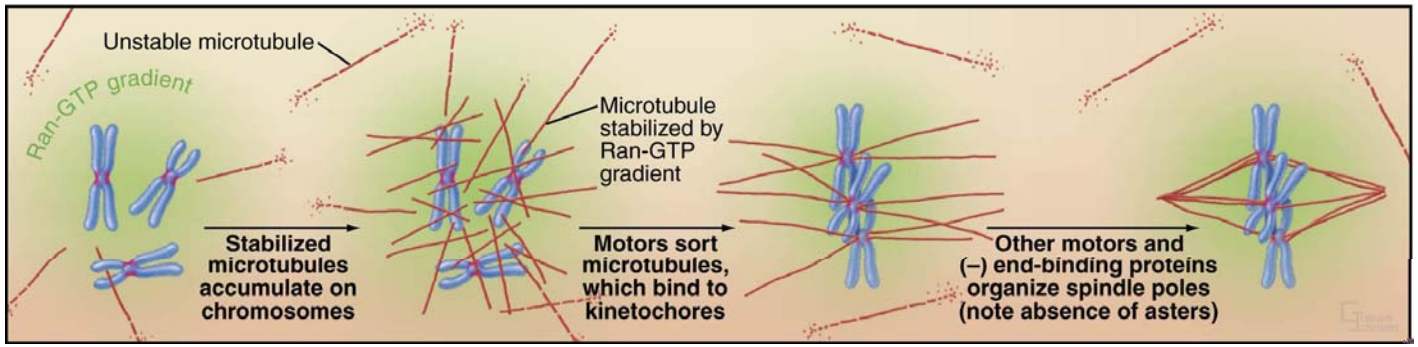
Yang et al., *J. Cell Biology,* 182:631-639, 2008

Spindle Assembly: MT Organization (I)

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

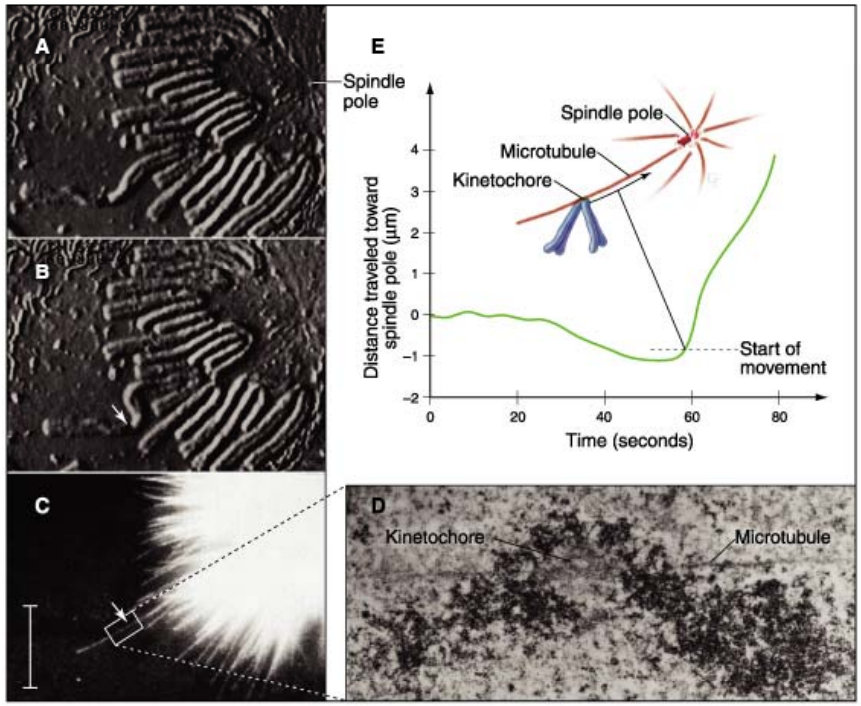


- Centrosome-independent spindle assembly depends on a Ran-GTP gradient.



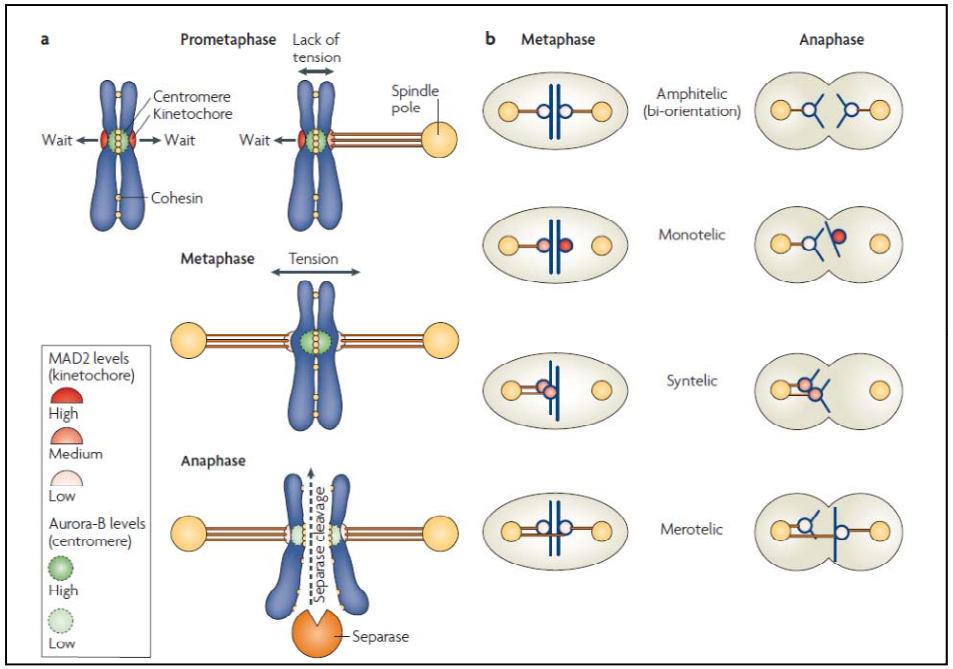
Spindle Assembly: Bipolar Attachment (II)

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



Error Correction & Spindle Checkpoint

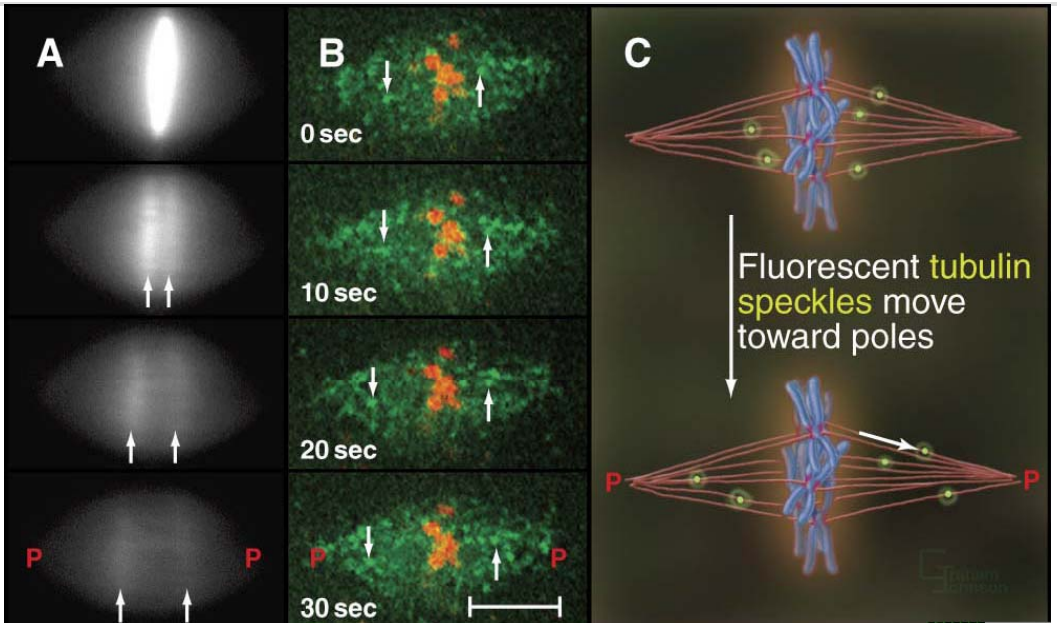
- Tension between sister chromatids is essential to error detection and correction.
- MAD1/2 & Aurora-B kinase play critical roles in spindle checkpoint.

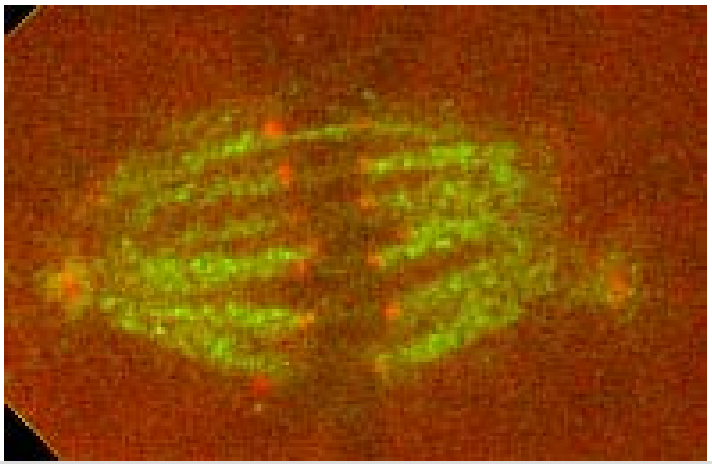


Musacchio & Salmon, *Nat. Rev. Mol. Cell Biol.* 8:319, 2007.

Metaphase

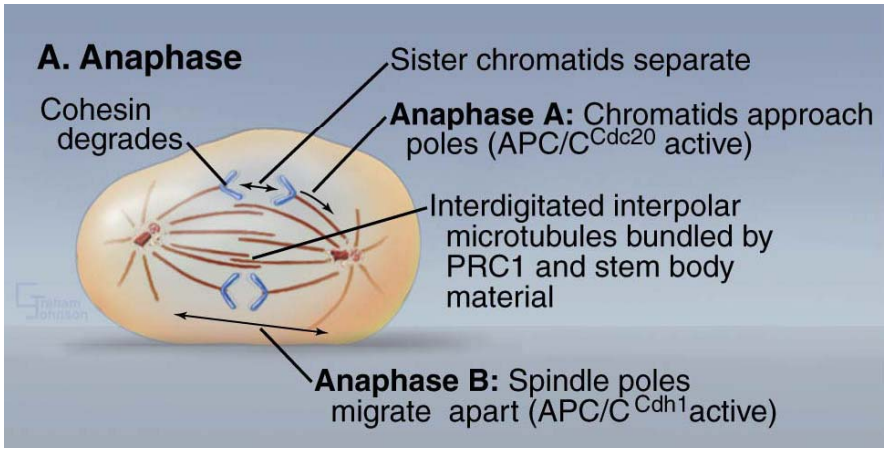
- Chromosomes become aligned at the metaphase plate.
- Microtubules undergo constant poleward flux.
- Chromosomes oscillation during metaphase.
- APC/C promotes the degradation of securin.

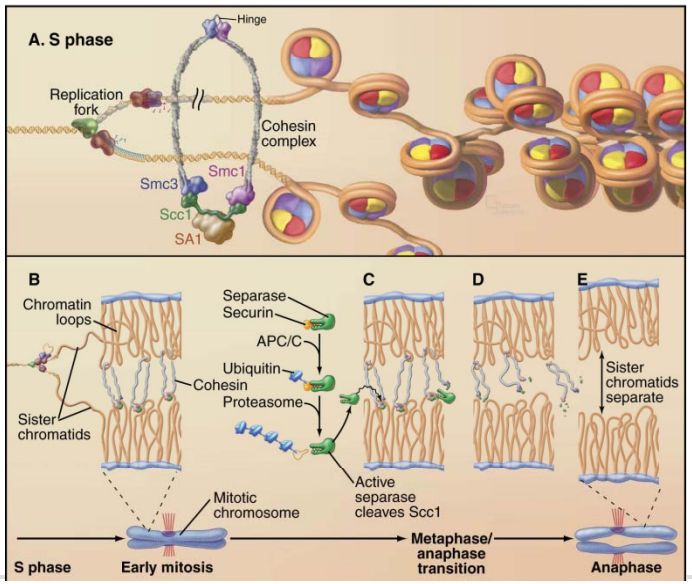




Anaphase A

- 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.



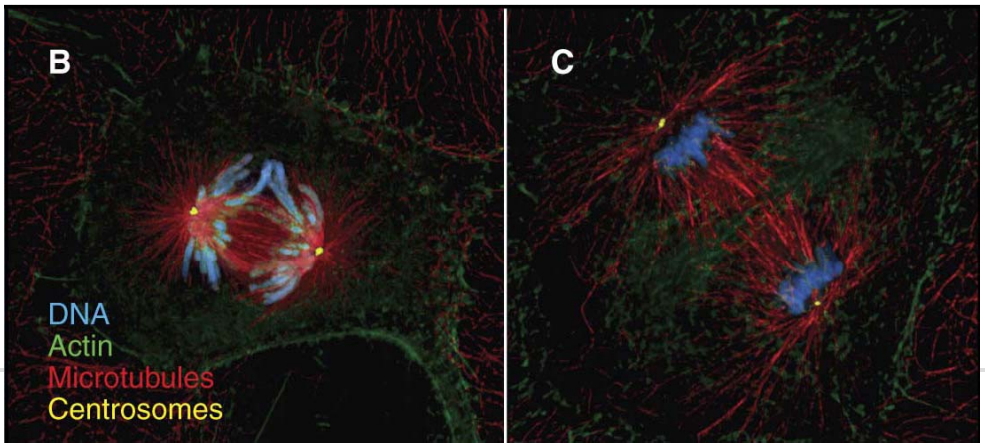


Anaphase B

- Spindle elongation pushes spindle poles apart in Anaphase B.

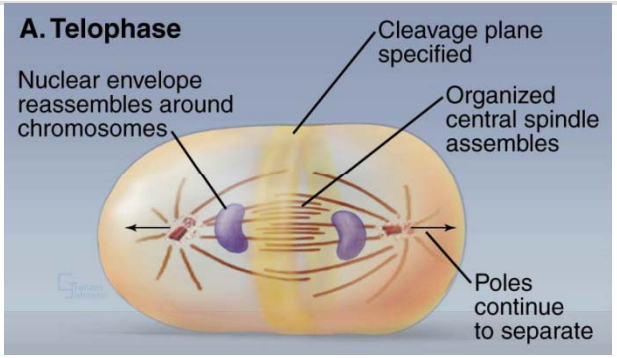
- Chromosome movement is driven by two factors
  - microtubule shortening and growth
  - microtubule flux

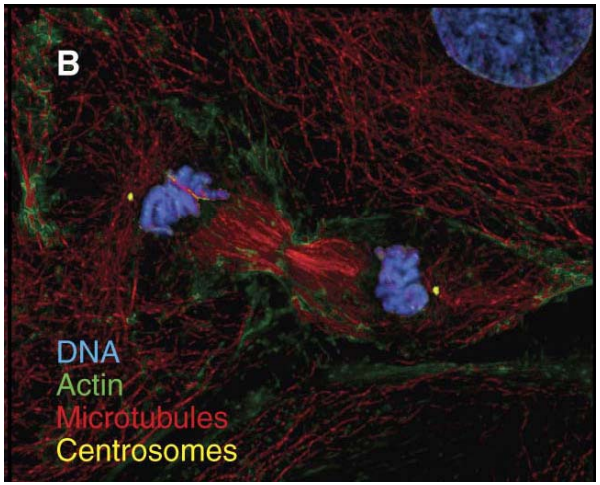
- Spindle elongation
  - Antiparallel sliding of microtubules
  - Microtubule growth
  - Spindle pole motility



Telophase

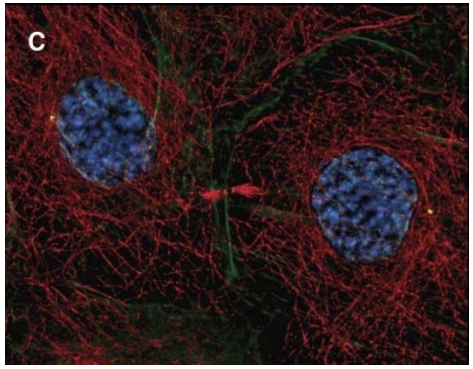
- Nuclear envelope starts to reassemble in late anaphase and is completed in telophase.
- Ran-GTP mediates nuclear envelope assembly.
- Nuclear lamina reassembles through recycling of disassembled lamin subunits.

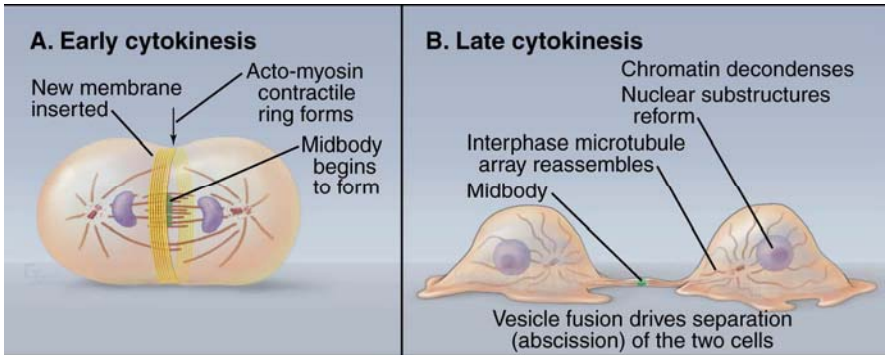




Cytokinesis (I)

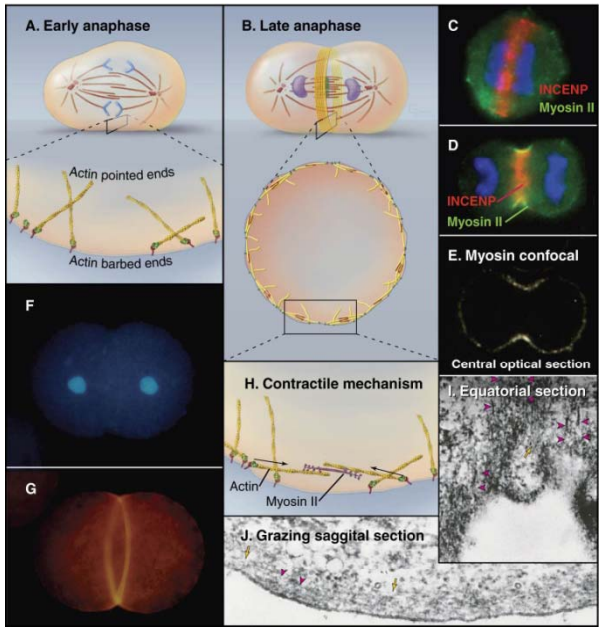
- Two daughter cells become separated through cytokinesis.
- Formation of the contractile ring requires actin and myosin-II.
- Separation of two daughter cells is accompanies by constriction and disassembly of the contractile ring.

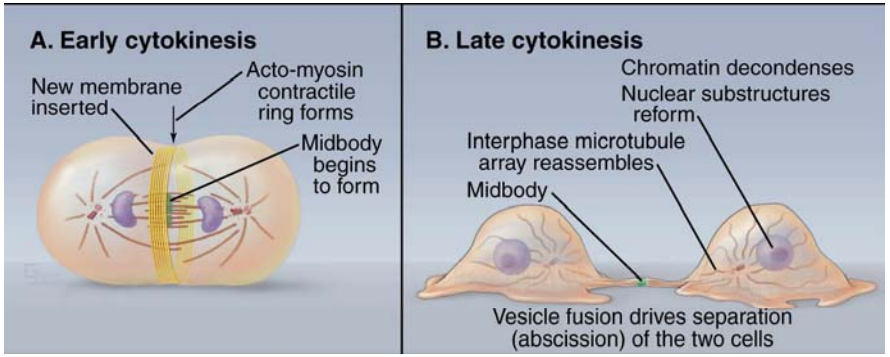




Cytokinesis (II)

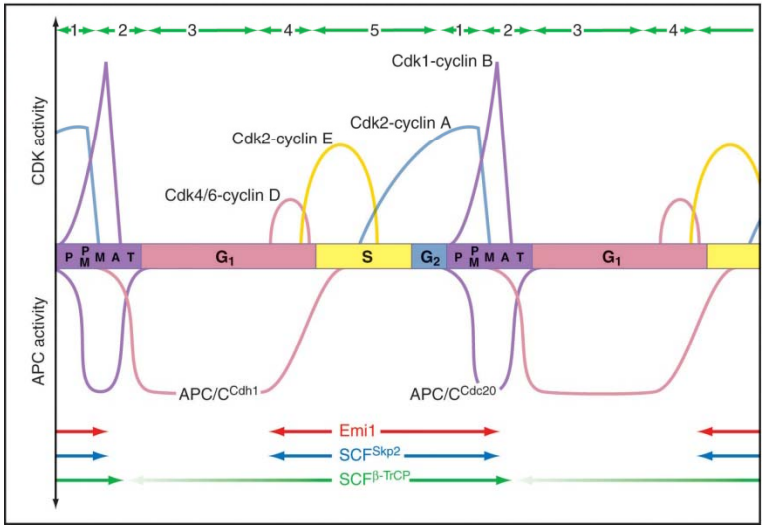
- Cytokinesis requires membrane addition and abscission.
- Secretory vesicles from the Golgi provides new membrane.
- Midbody contains many proteins involved in membrane trafficking.
- Intracelluar bridges may remain open to connect cells.





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.



- Overview of cell cycle
- Different phases of cell cycle
- Checkpoints; Cyclin and cyclin-dependent kinases
- A detailed look at M phase
- Quantitative system-level study of cell cycle
- Outlook

References

- Neumann et al, Phenotypic profiling of the human genome by time-lapse microscopy reveals cell division genes. *Nature*. 2010 464:721-727.

- John Tyson's lab
  http://mpf.biol.vt.edu/lab_website/index.php

- Bela Novek's lab
  http://www.bioch.ox.ac.uk/aspsite/index.asp?pageid=593

- Overview of cell cycle
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Outlook

- Rigorous training in cellular and molecular biology is essential to biomedical engineering education.
  - Like just about any field, the most effective way to learn biology is to actually do it.

- A follow-up course: 03-741 Advanced Cell Biology

- Other possible follow-up courses
  - Biophysics
  - Biochemistry
  - Molecular biology
  - Genetics
  - Computational biology

- Use many resources online
  - http://www.ibioseminars.org/
  - http://www.ibiomagazine.org/

Questions ?