Midterm Review

What we will do today:

- Midterm format.
- What happens during/after the Midterm.
- Brief overview of lecture highlights.
- Summary figure for intracellular control of the cell cycle.
- Tips for studying / Sample question.
- Questions.
- Answers to “Things to Consider” are for your information. We will address these together if there is time.

Midterm Format

- Approximately 1 hour.
- Based upon lectures 1-10.
- There will be 2 parts to the exam:

  Part A: Multiple choice (20 x 1 marks)

  Part B: Long answer (3 x 10 marks)

  - About one page for each long answer.
  - You may use point form.
  - Do not include excessive writing.
  - Diagrams may be included but they must be accompanied by text.
  - Please write neatly!

During the Midterm

- Please be seated quickly.
- Notes, books etc. on the floor.
- Turn off and store devices.
- For questions, please approach the front.
- We will use computer scan sheets.
- Out of courtesy, remain seated for the last 15 min of the exam.

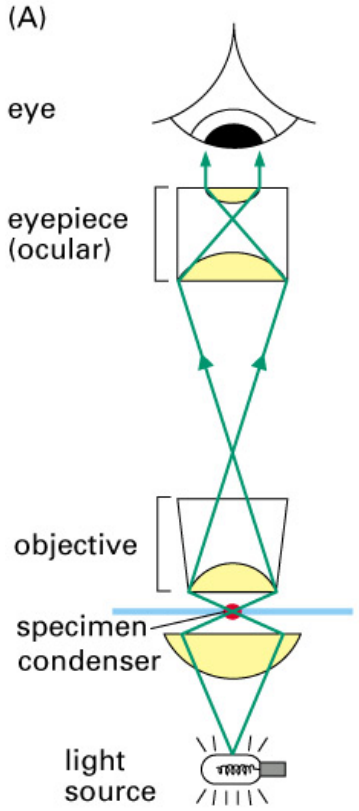
After the Midterm

- Exams will be graded as quickly as possible.
- Please be patient.
- Marks will be posted on the course website in approximately 2 weeks.
- We will hold exam viewing sessions in November.
- You will have an opportunity to meet with the TAs.

A brief overview…

The Light Microscope (LM)

- Utilizes basic light path.
- Used for live or fixed cells and tissue.
- Tissues: *upright microscope*.
- Isolated cells: *inverted microscope*.



An "upright" microscope

(B)

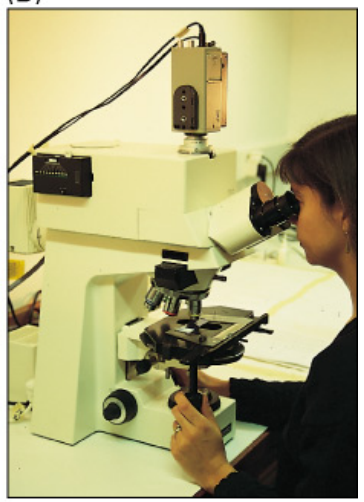
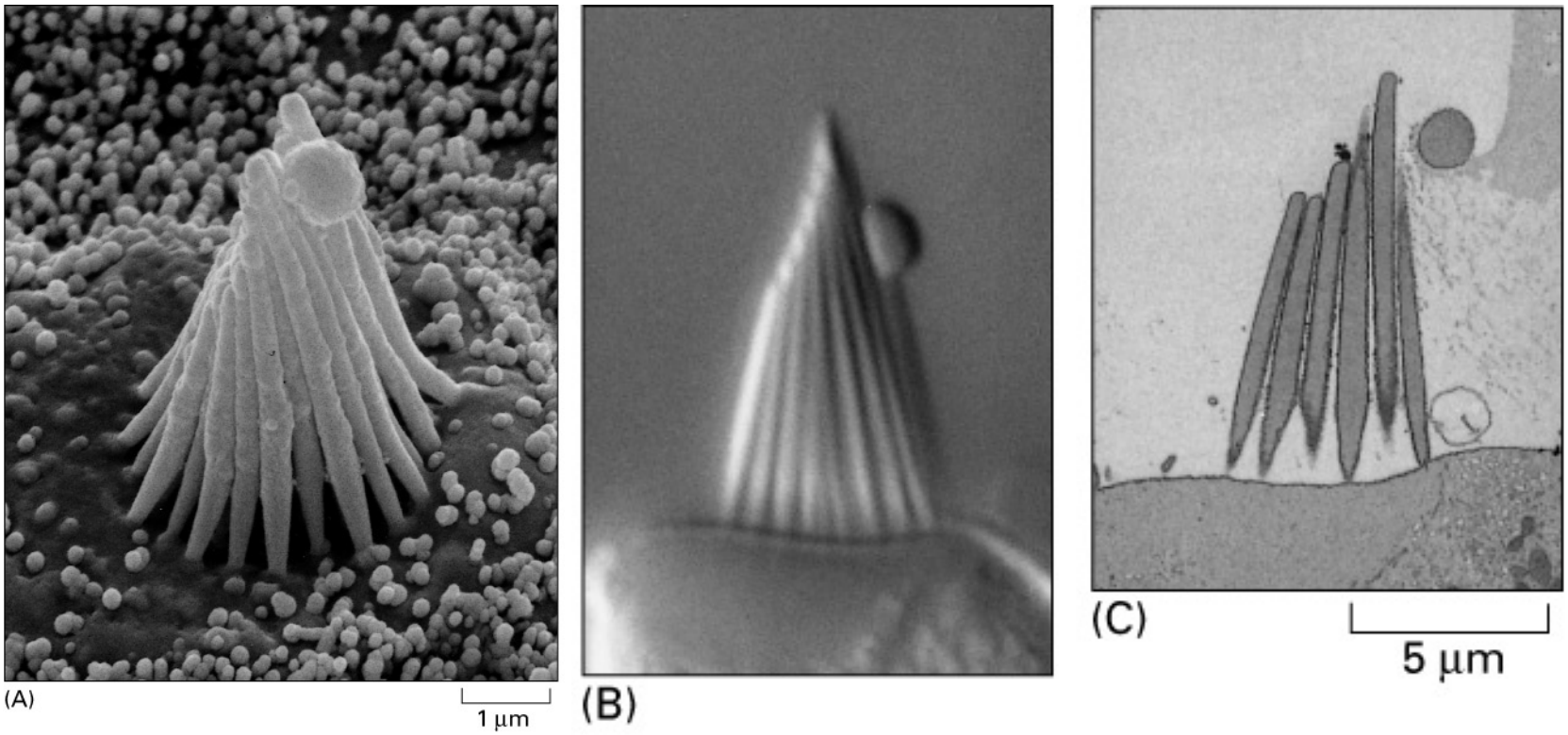


Figure 9–3. Molecular Biology of the Cell, 4th Edition.

EM and LM Compared

SEM DIC TEM



Stereocilia of hair cell from frog inner ear.

Molecular Biology of the Cell, 4th Edition.

Tubulin

- Heterodimer = 2 different proteins, but considered "1 subunit" of tubulin
- each binds GTP; hydrolyzed at only 1 site
- 13 protofilaments
- "plus" and "minus" end

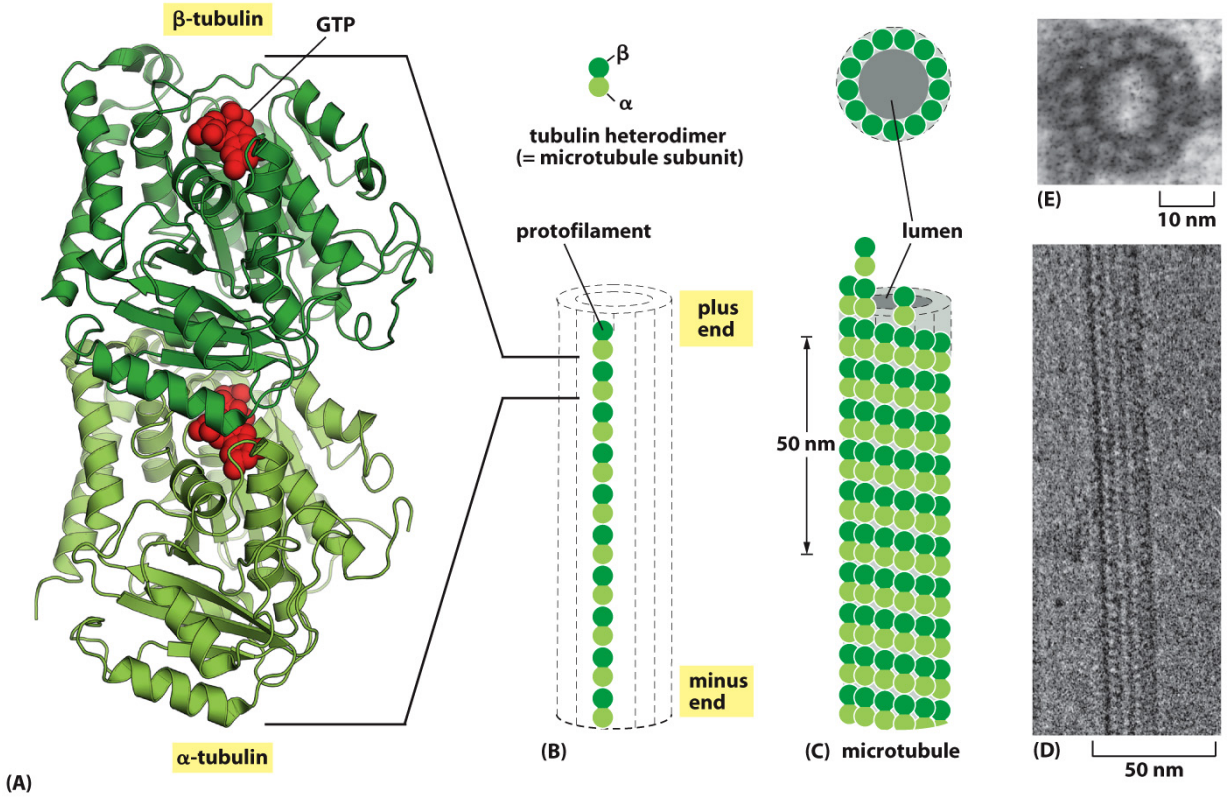


Figure 16-42 Molecular Biology of the Cell 6e (© Garland Science 2015)

Actin

- Monomer
- ATP
- “plus” and “minus” end

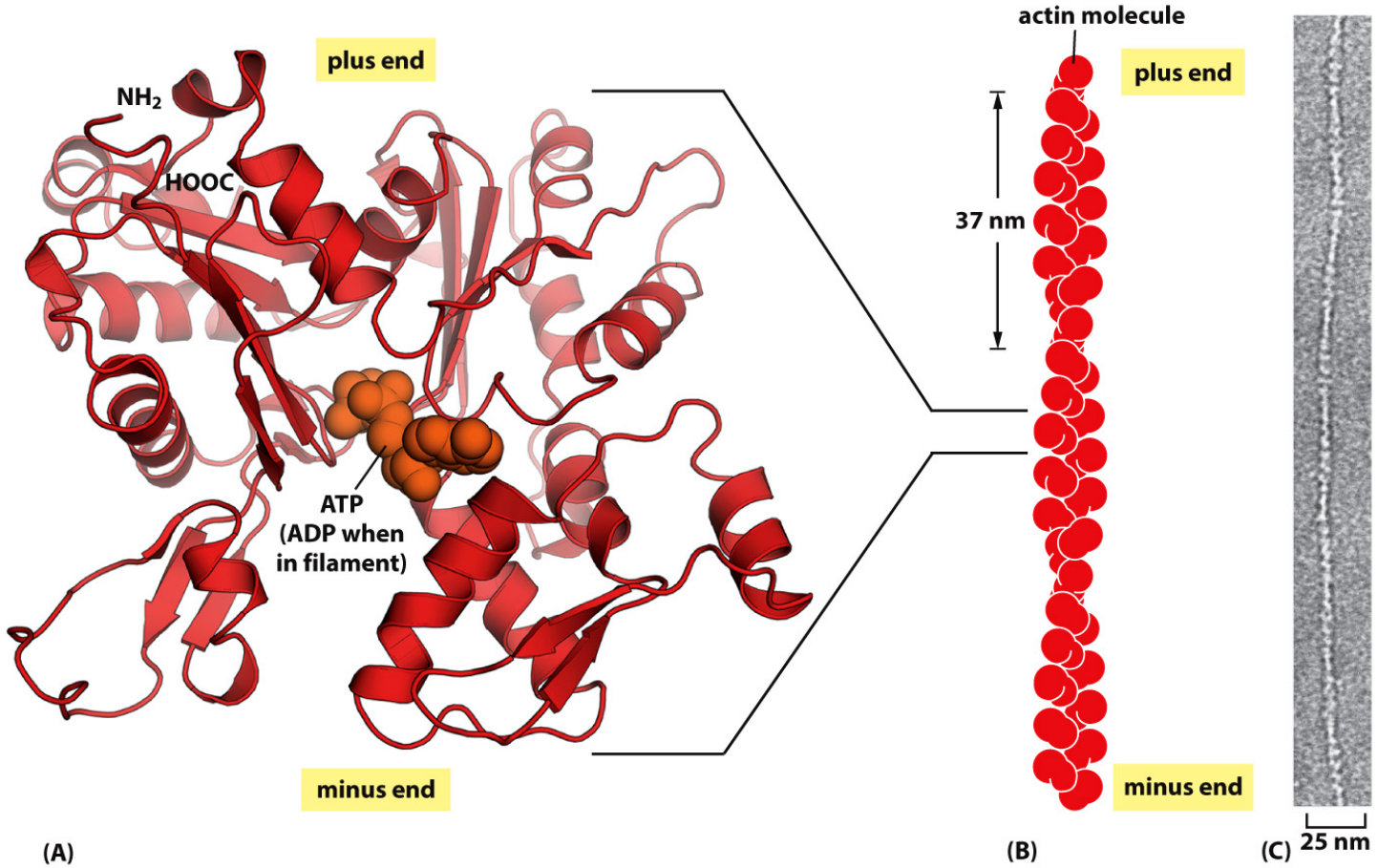
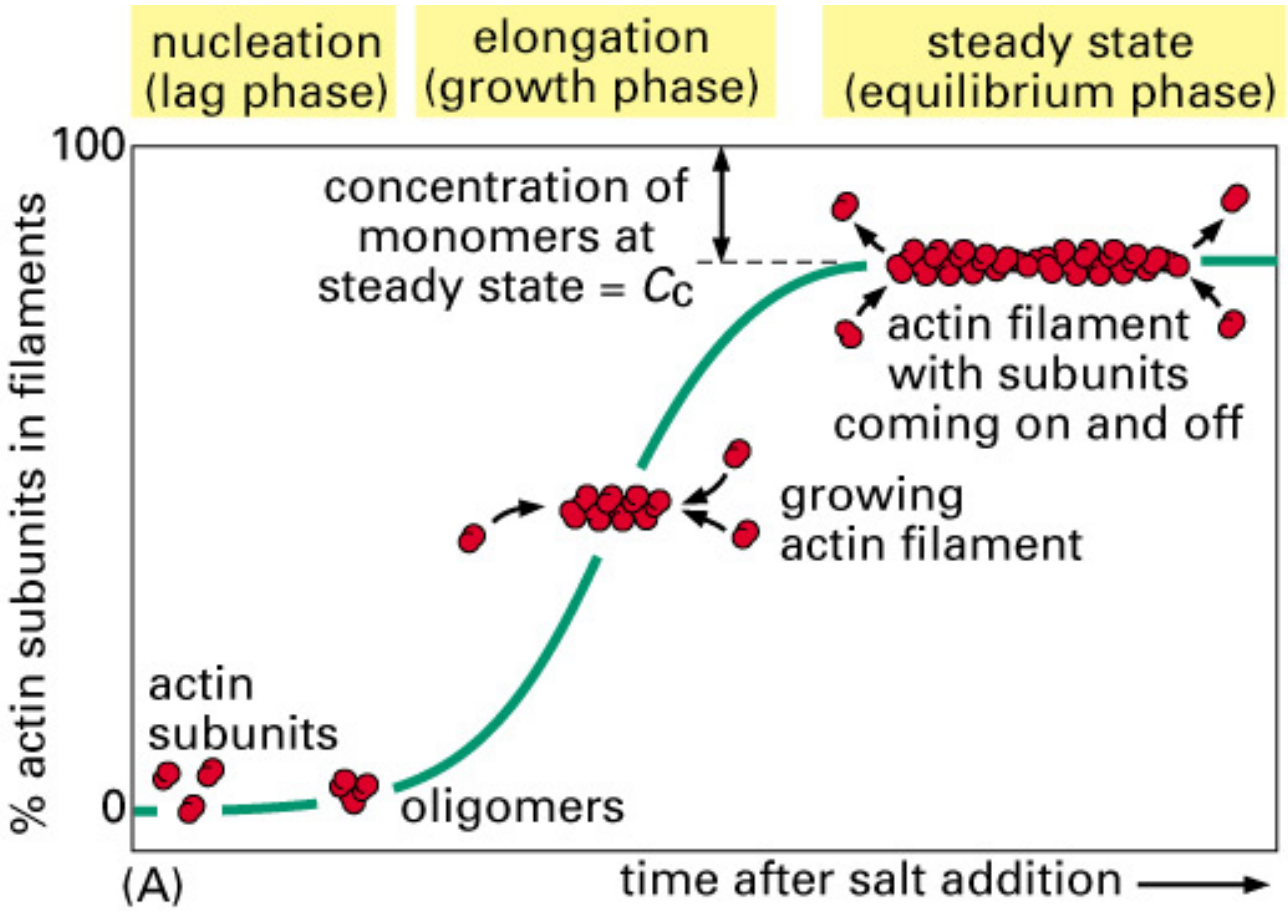


Figure 16-11 Molecular Biology of the Cell 6e (© Garland Science 2015)

Nucleation


nucleation (lag phase)
elongation (growth phase)
steady state (equilibrium phase)
100
0
% actin subunits in filaments
concentration of monomers at steady state = C_C
actin filament with subunits coming on and off
growing actin filament
actin subunits
oligomers
(A)
time after salt addition

Treadmilling – Actin Filaments

Experiment:

1. Filaments added to ATP-actin.
2. [ATP-actin] high, addition occurs at both ends.
3. [ATP-actin] drops, addition greater at plus end.
4. Steady state.

   Treadmilling

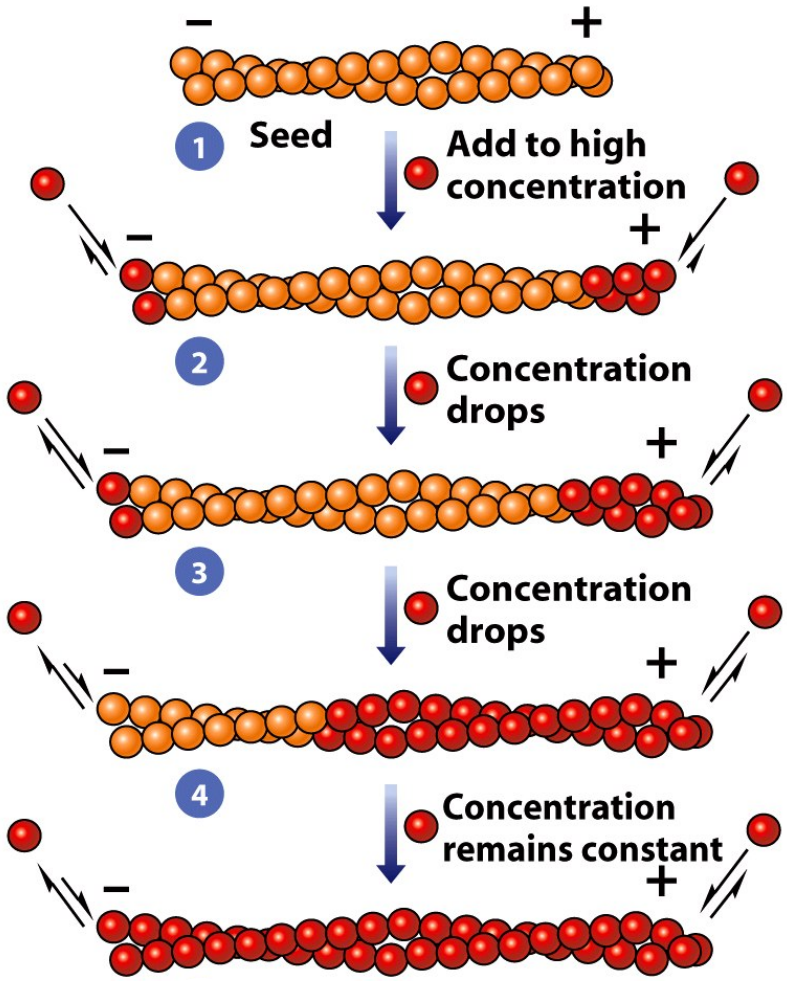


Figure 9-46b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Nucleation is not a factor here since pre-formed filaments were added to actin solution

Dynamic Instability – MTs

- [Tubulin] within critical values
- GTP cap
- Continuous transitions

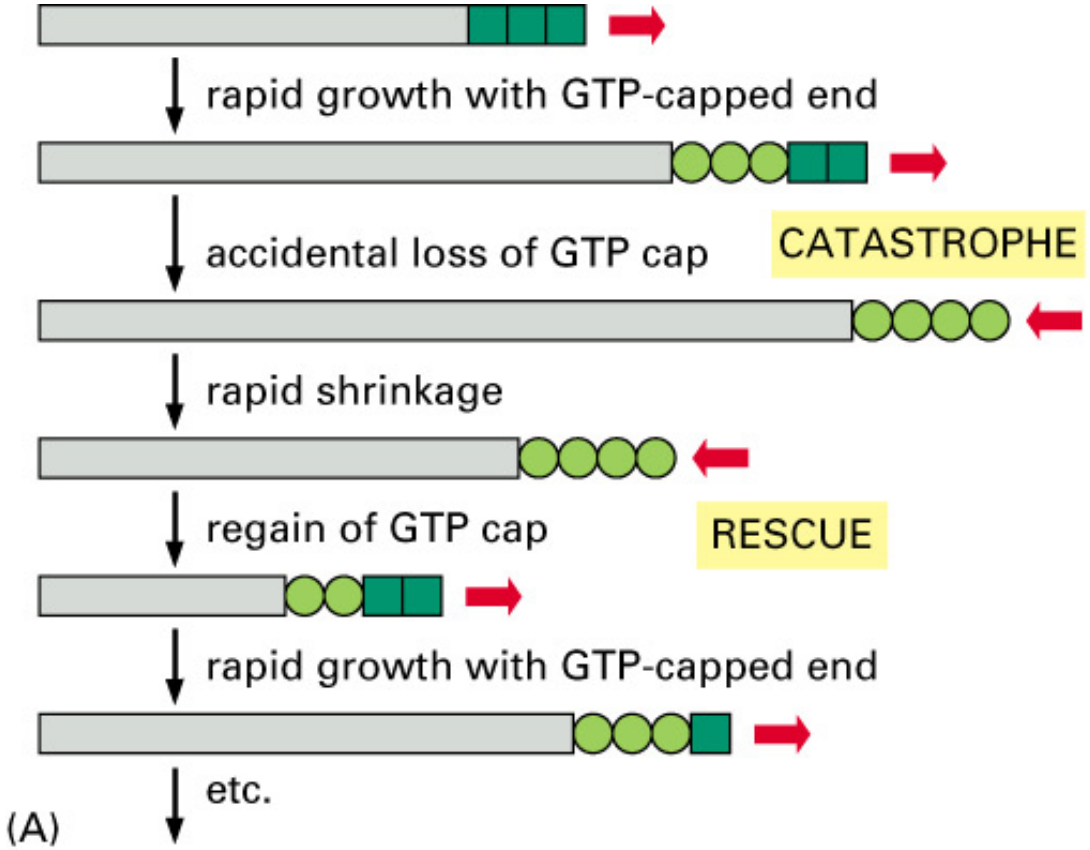


Figure 16–11 part 1 of 3. Molecular Biology of the Cell, 4th Edition.

Thymosin Sequesters Actin

- Thymosin sequesters, but profilin recruits monomers.
- Thymosin makes polymerization *less favourable*.
- Profilin *competes* with thymosin and promotes assembly.

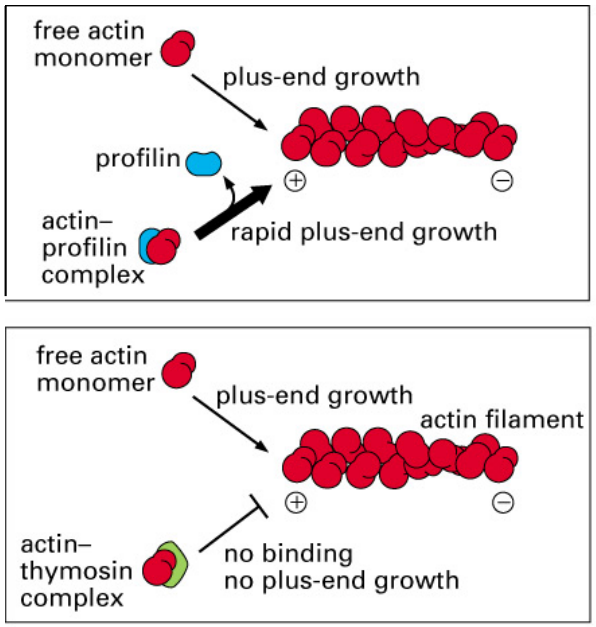


Figure 16–30 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

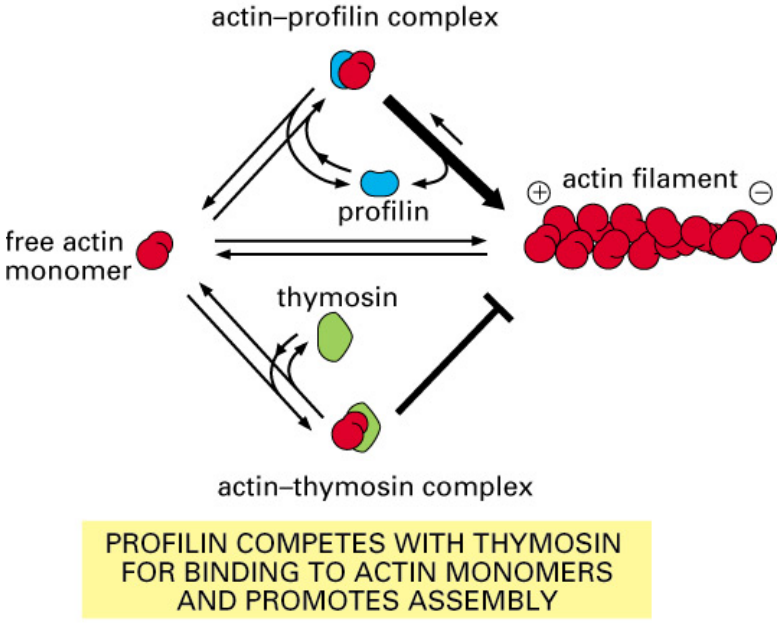


Figure 16–30 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

The Sarcomere

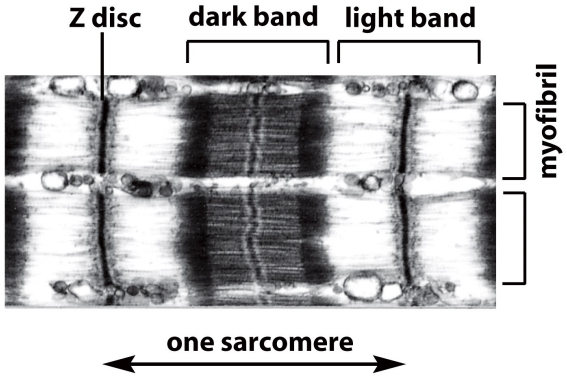


Figure 16-74b *Molecular Biology of the Cell* (2008)

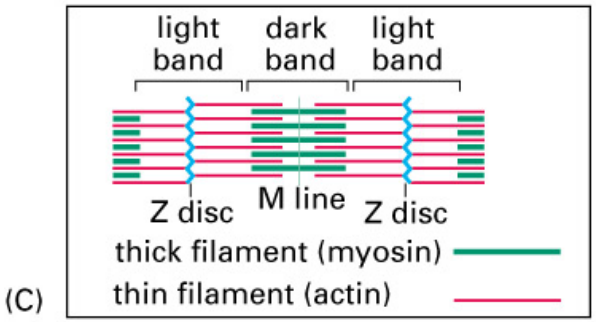


Figure 16–69 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

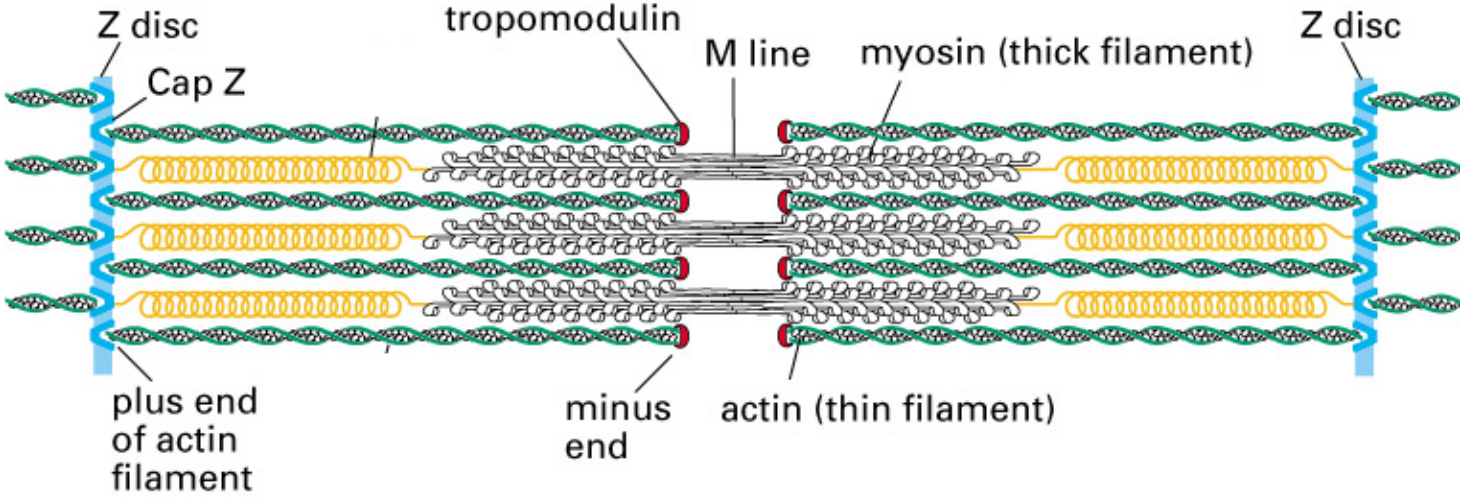


Figure 16–72. Molecular Biology of the Cell, 4th Edition.

Z disc = α actinin + CapZ

Ca^{2+}-dependence of Muscle Contraction

No Ca^{2+}, tropomyosin blocks myosin–AF binding

↓

Ca^{2+} released from SR and binds to troponin C

↓

Conformational change in troponin I

↓

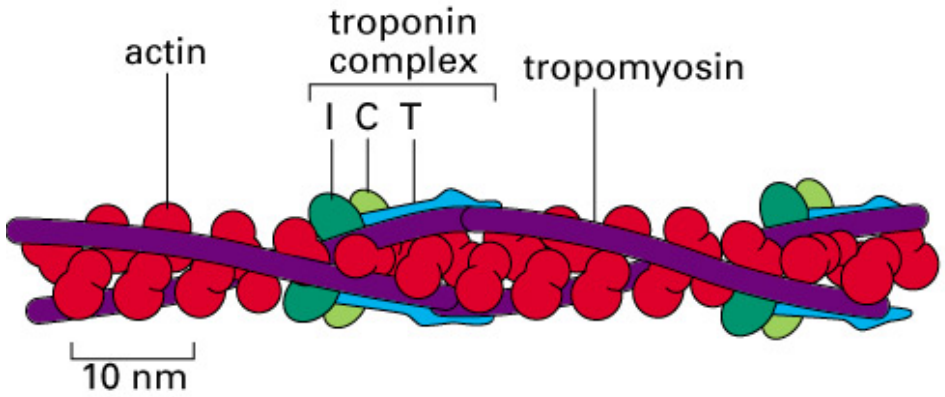
Tropomyosin (bound to Troponin T) moves and exposes binding sites

↓

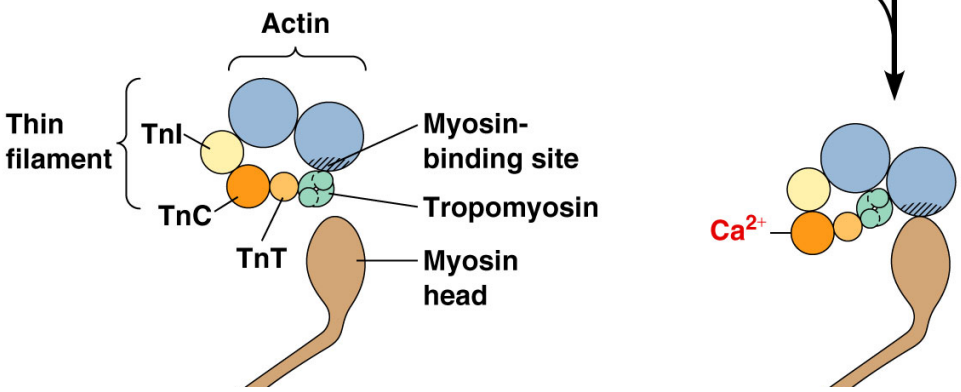
Myosin-AF binding

↓

Muscle contraction



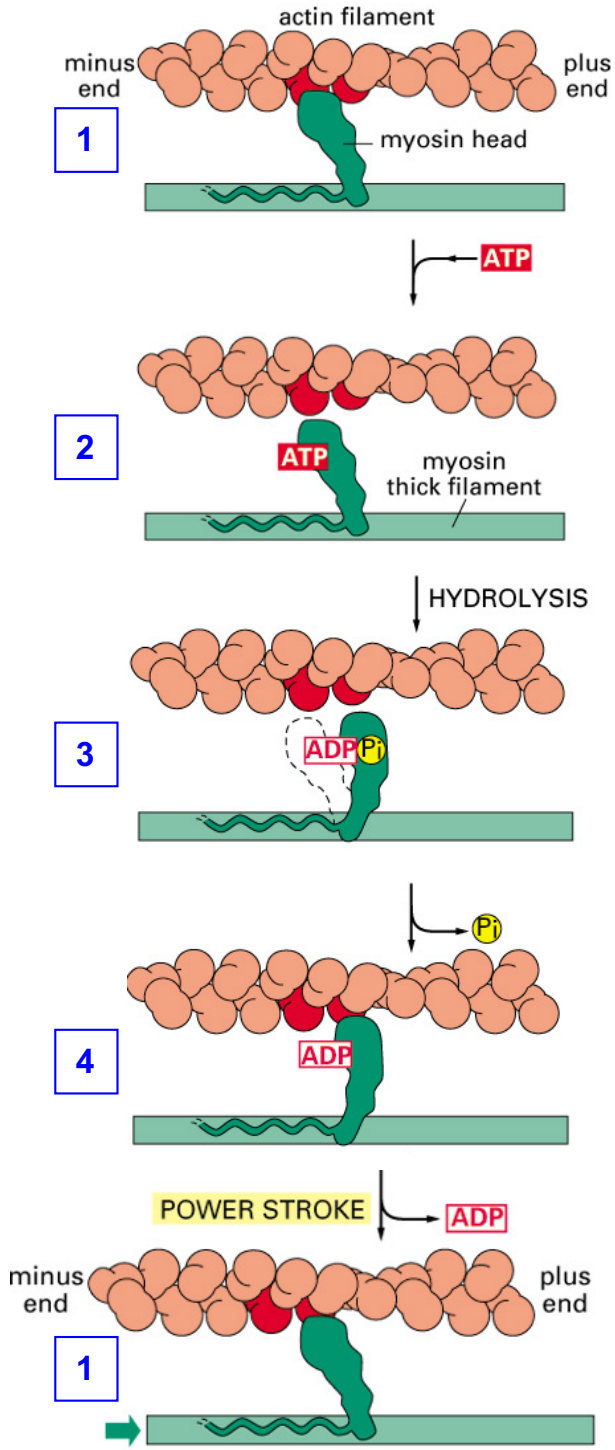
I = “inhibitory”
C = “calcium”
T = “tropomyosin”



Becker et al. World of the Cell

The Myosin Cycle

1. Attached: no ATP, locked.
2. Release: ATP bound, conformational change (away from AF).
3. Cocked: hydrolysis, conformational change toward (+) end of AF.
4. Force-generating: weak binding, P_i release, power stroke, ADP lost.



Cyclins and Cdks

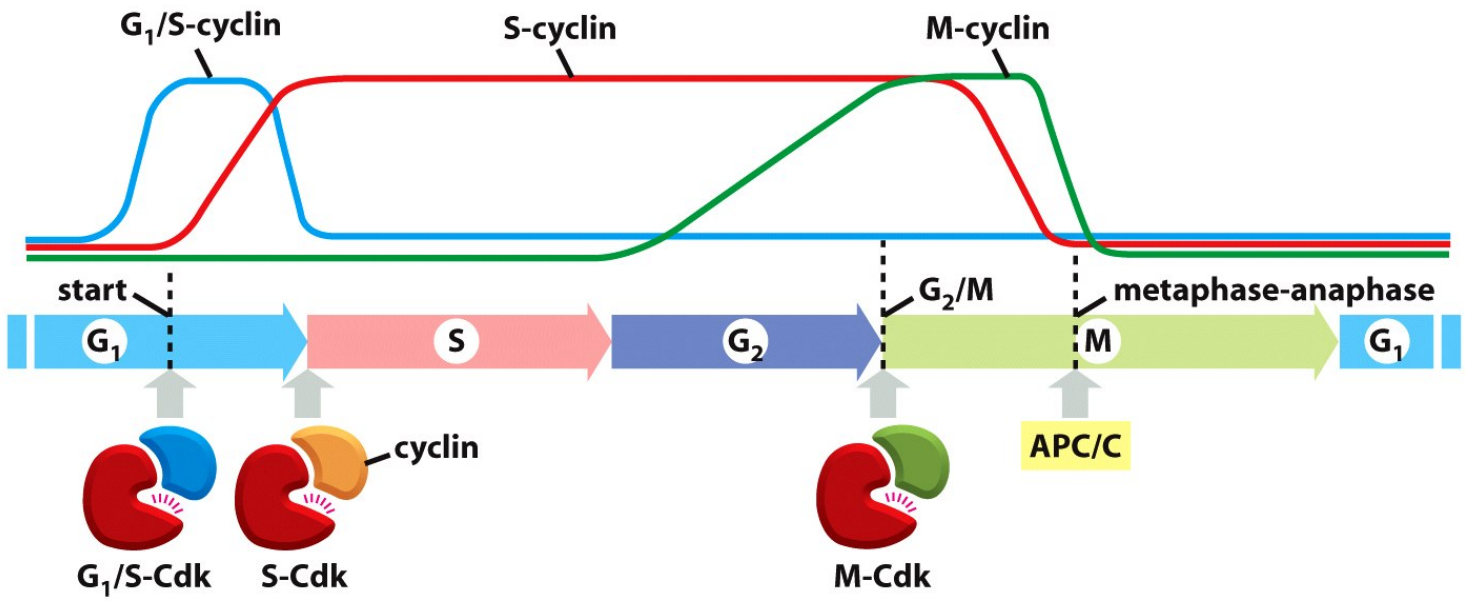


Figure 17-16 Molecular Biology of the Cell 5/e (© Garland Science 2008)

SCF and APC are Active During Different Stages

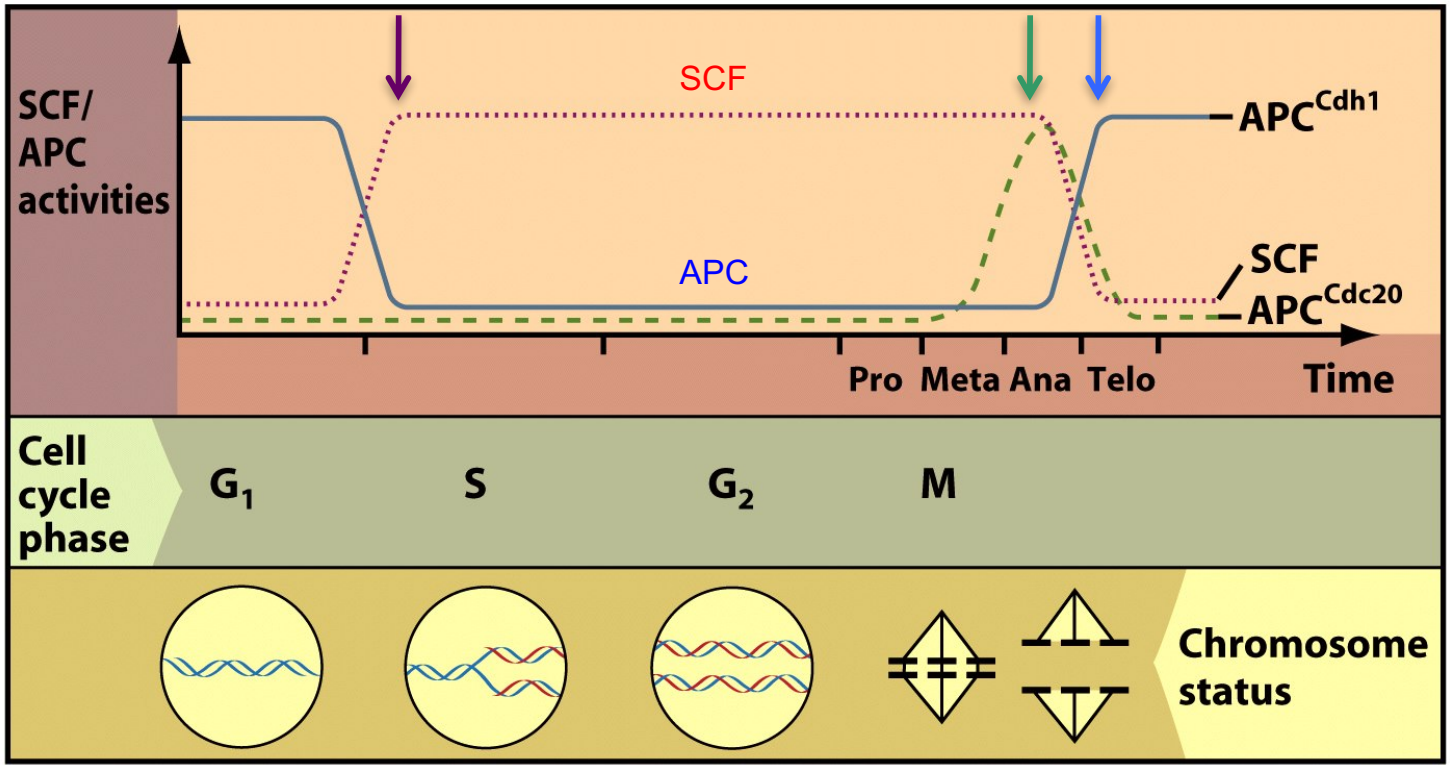
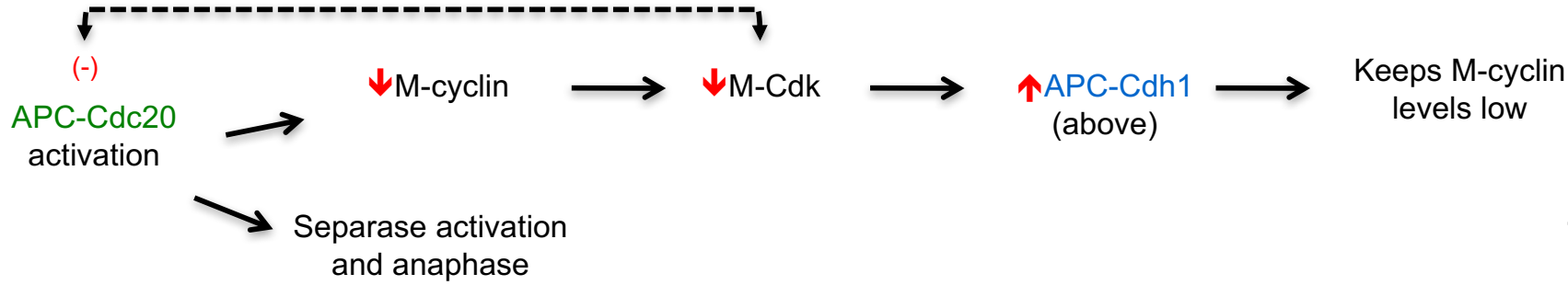


Figure 14-26a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)



Activation of M-Cdk Triggers Mitosis

Here, we are in the latter stages of G_2 on the brink of mitosis…

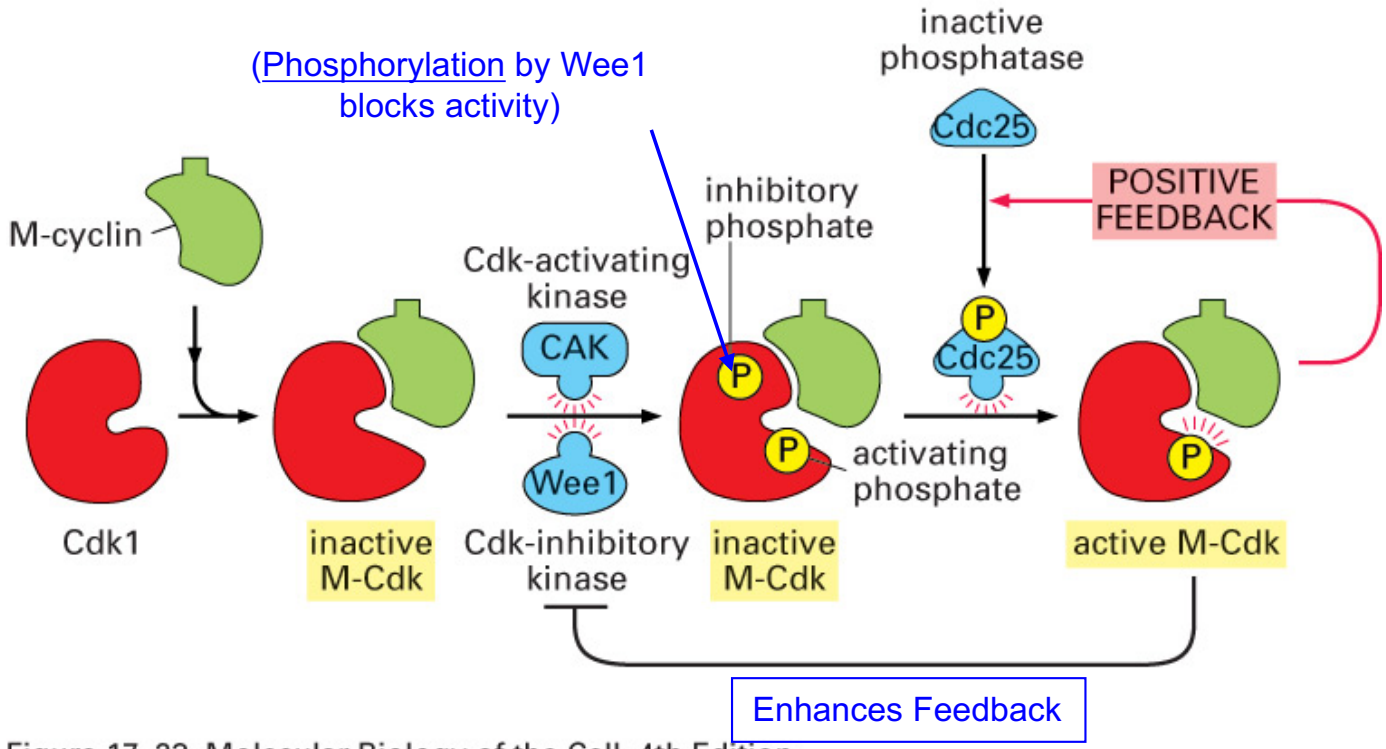
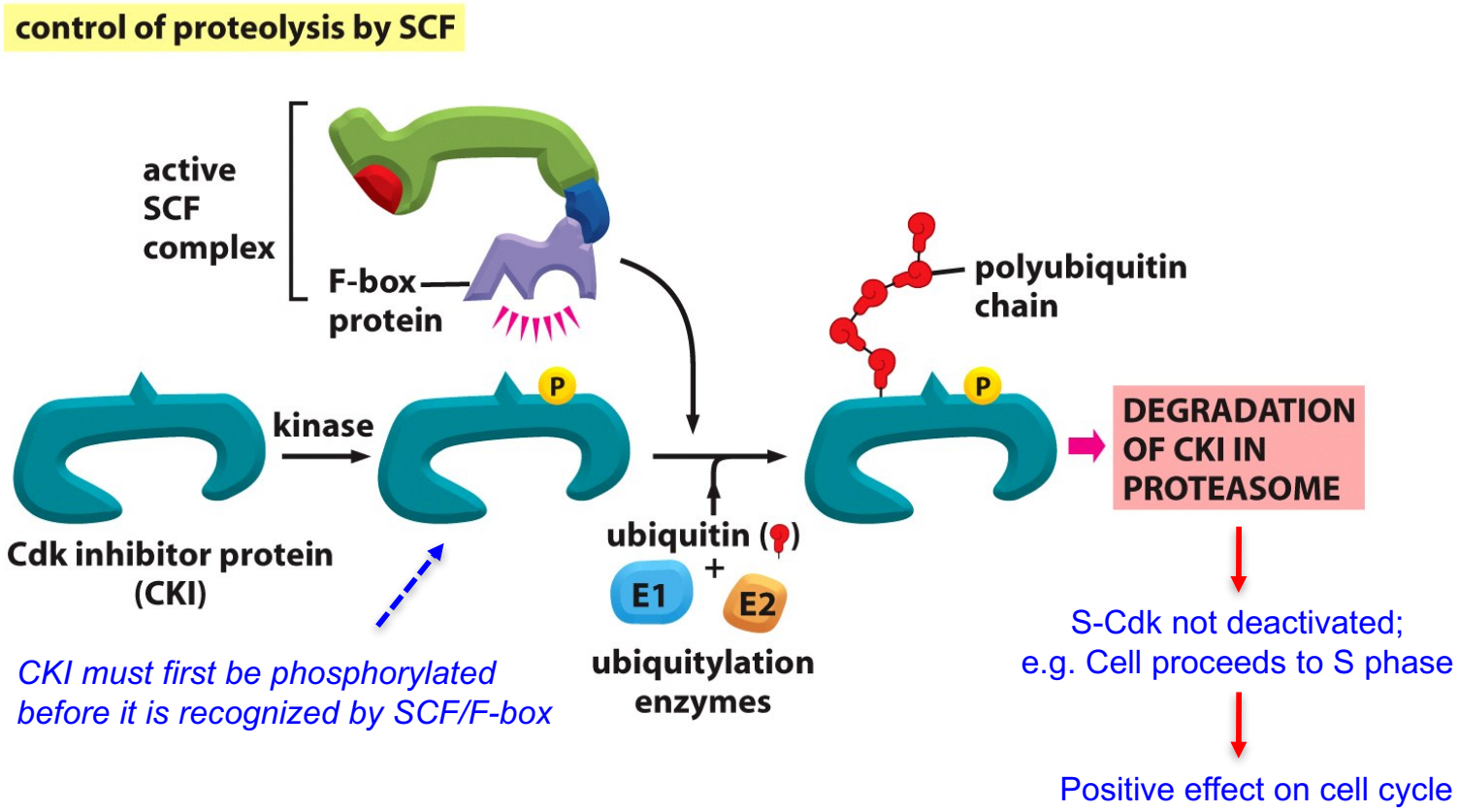


Figure 17–23. Molecular Biology of the Cell, 4th Edition.

SCF and APC are Ubiquitin Ligases

- SCF can lead to destruction of CKI.



Skp/**C**ullin/**F** box family of proteins; **A**naphase **P**romoting **C**omplex

“Checkpoints”

1. DNA replication checkpoint
2. Spindle attachment checkpoint
3. DNA damage checkpoints (several)

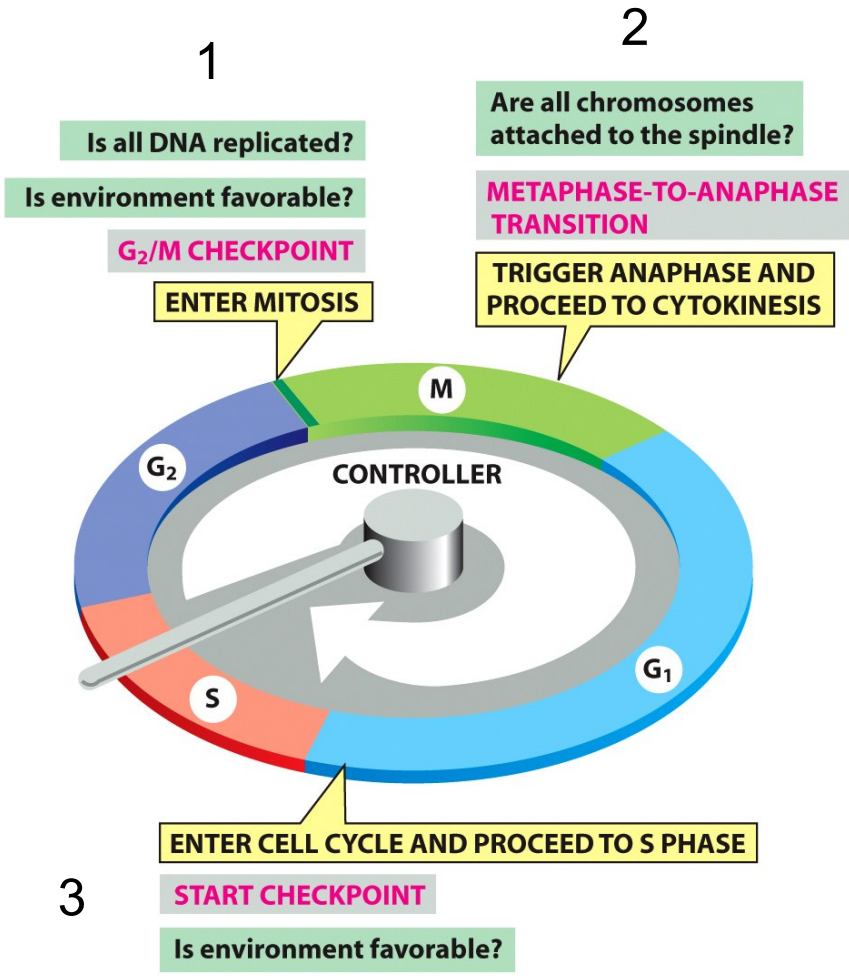
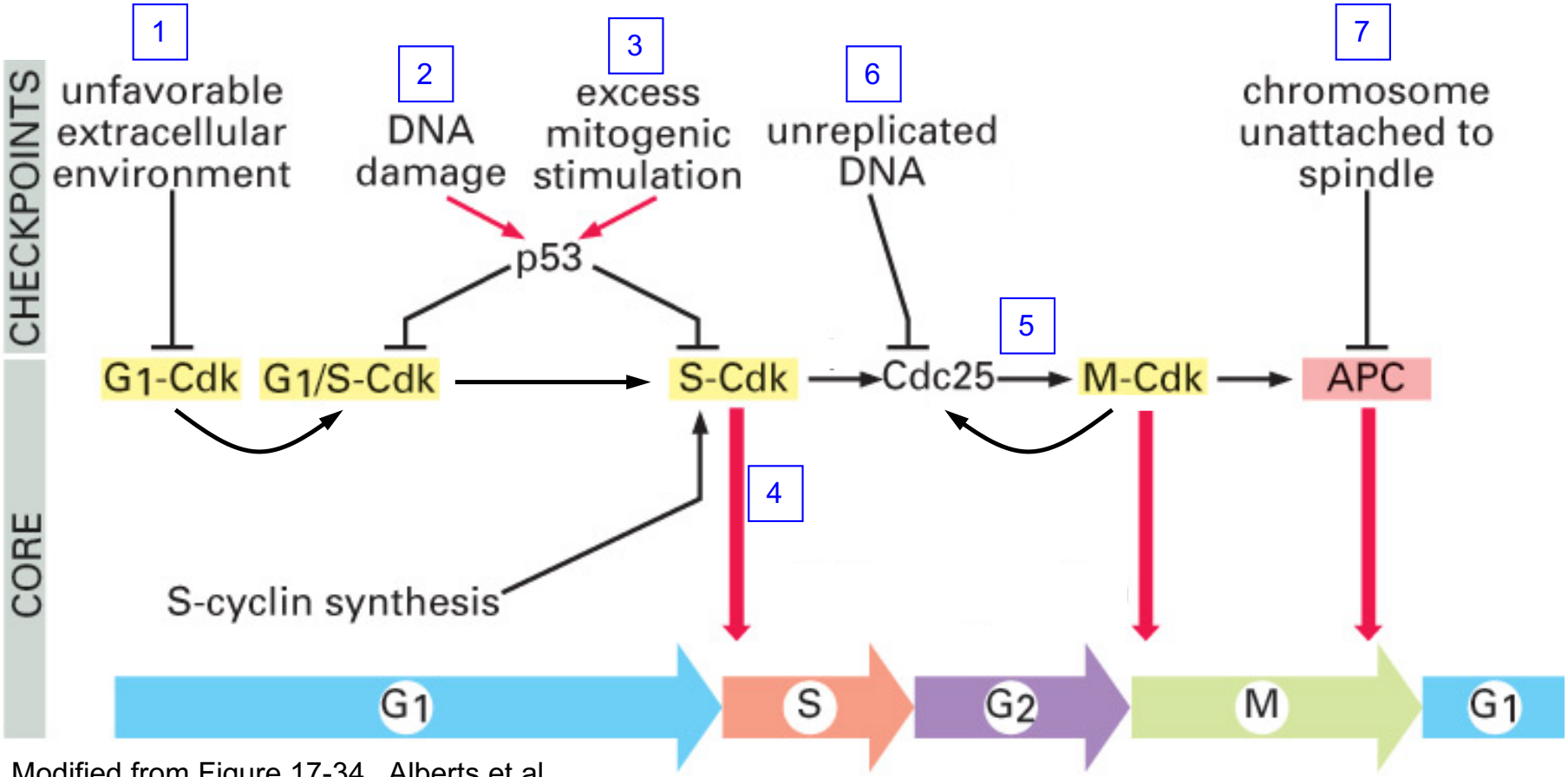


Fig. 17-14. Alberts, 5th ed.

Review* of Intracellular Control of the Cell Cycle

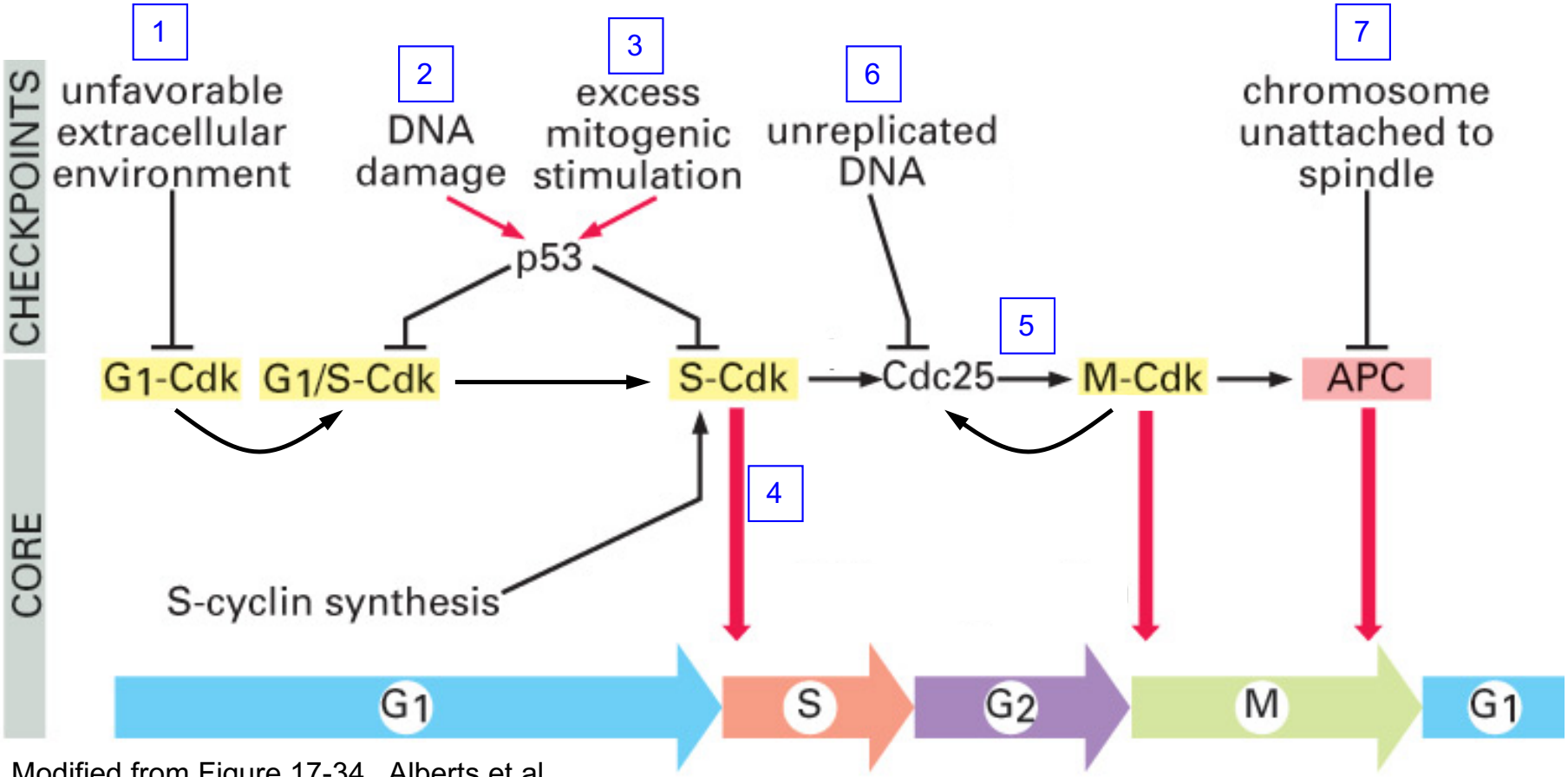


Modified from Figure 17-34. Alberts et al.

1. **G1 Restriction Point**. Cell cycle proceeds only if mitogen present. Ras, MAP kinase, *myc*… This brings cell out of G_0.

*Please note this is NOT an exhaustive review. We will only discuss a few points.

Review* of Intracellular Control of the Cell Cycle

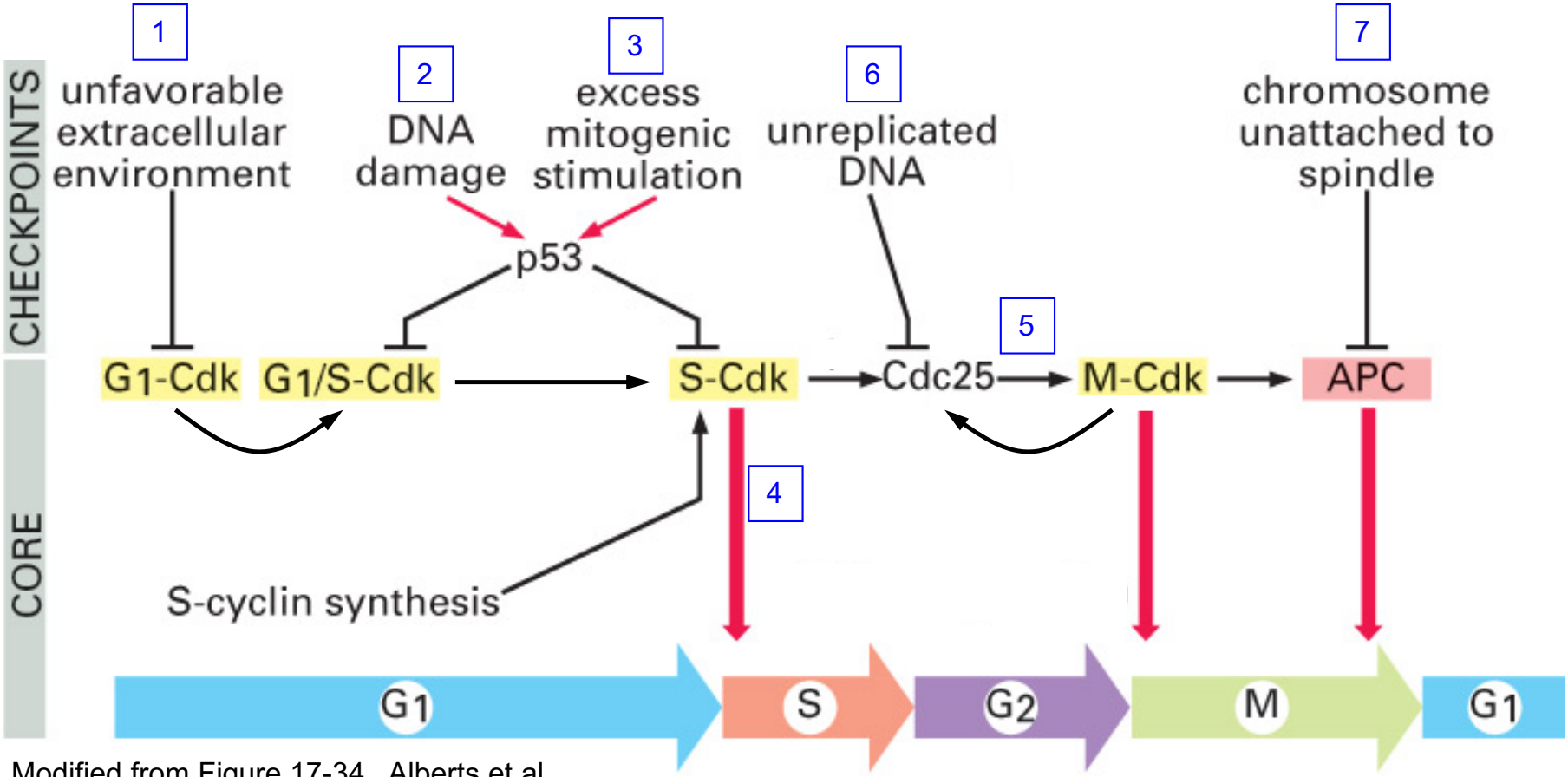


Modified from Figure 17-34. Alberts et al.

2. **DNA Damage Checkpoint**. Stabilization of p53 leads to inactivation of S-Cdk via p21 (a CKI).

*Please note this is NOT an exhaustive review. We will only discuss a few points.

Review* of Intracellular Control of the Cell Cycle

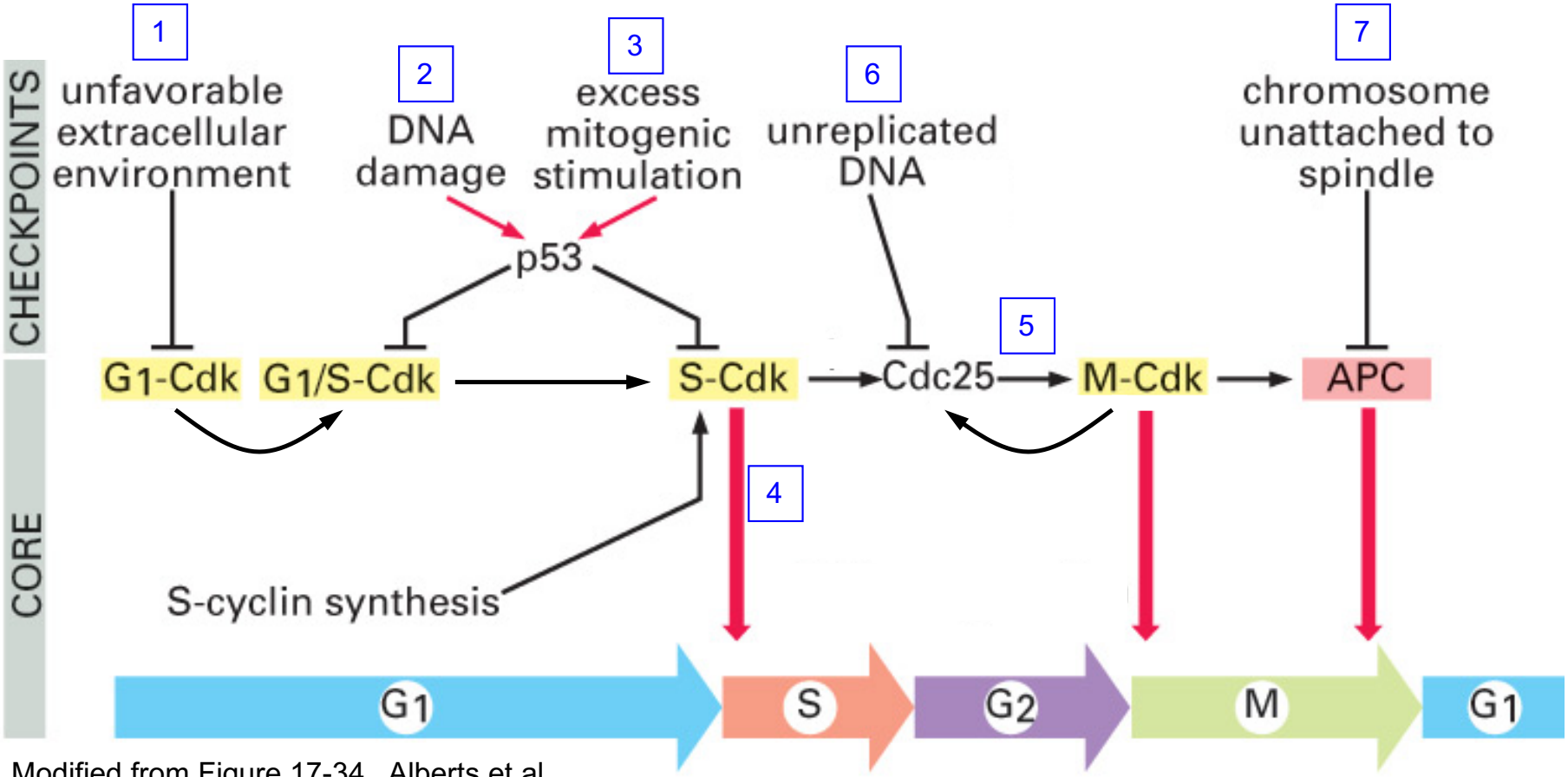


Modified from Figure 17-34. Alberts et al.

3. **Excess Mitogenic Stimulation**. Excess Myc production removes Mdm2 by p19 and stabilizes p53. Cell cycle arrests.

*Please note this is NOT an exhaustive review. We will only discuss a few points.

Review* of Intracellular Control of the Cell Cycle

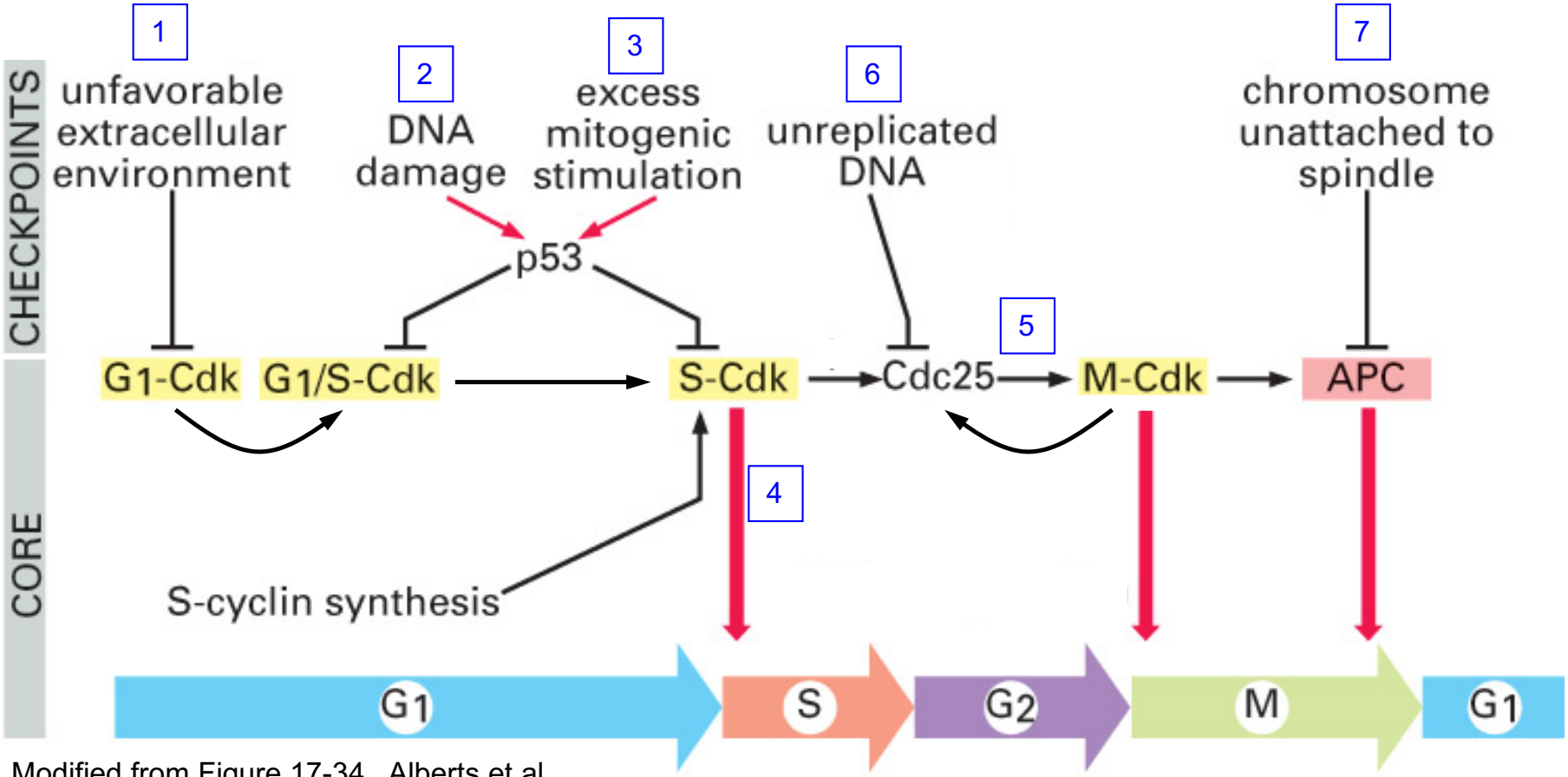


Modified from Figure 17-34. Alberts et al.

4. **S-phase and DNA Replication**. Increased S-cyclin (and active S-Cdk), and levels of M-cyclin are kept low because of APC.

*Please note this is NOT an exhaustive review. We will only discuss a few points.

Review* of Intracellular Control of the Cell Cycle

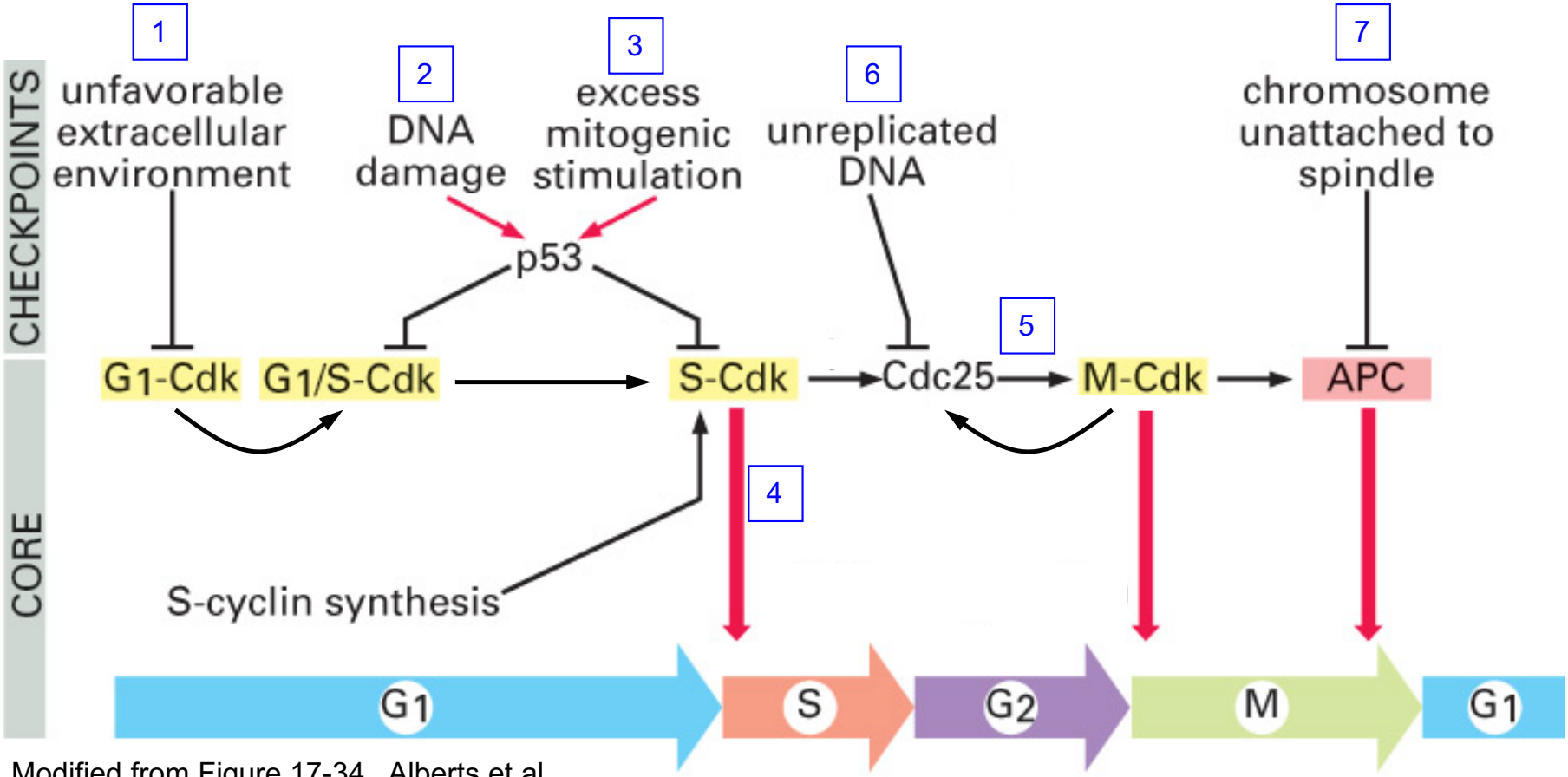


Modified from Figure 17-34. Alberts et al.

5. **Entry into Mitosis**. S-Cdk activates Cdc25, which will in turn remove inhibitory phosphate from M-Cdk and trigger entry into mitosis. Note that M-Cdk feeds back positively on Cdc25.

**Please note this is NOT an exhaustive review. We will only discuss a few points.*

Review* of Intracellular Control of the Cell Cycle

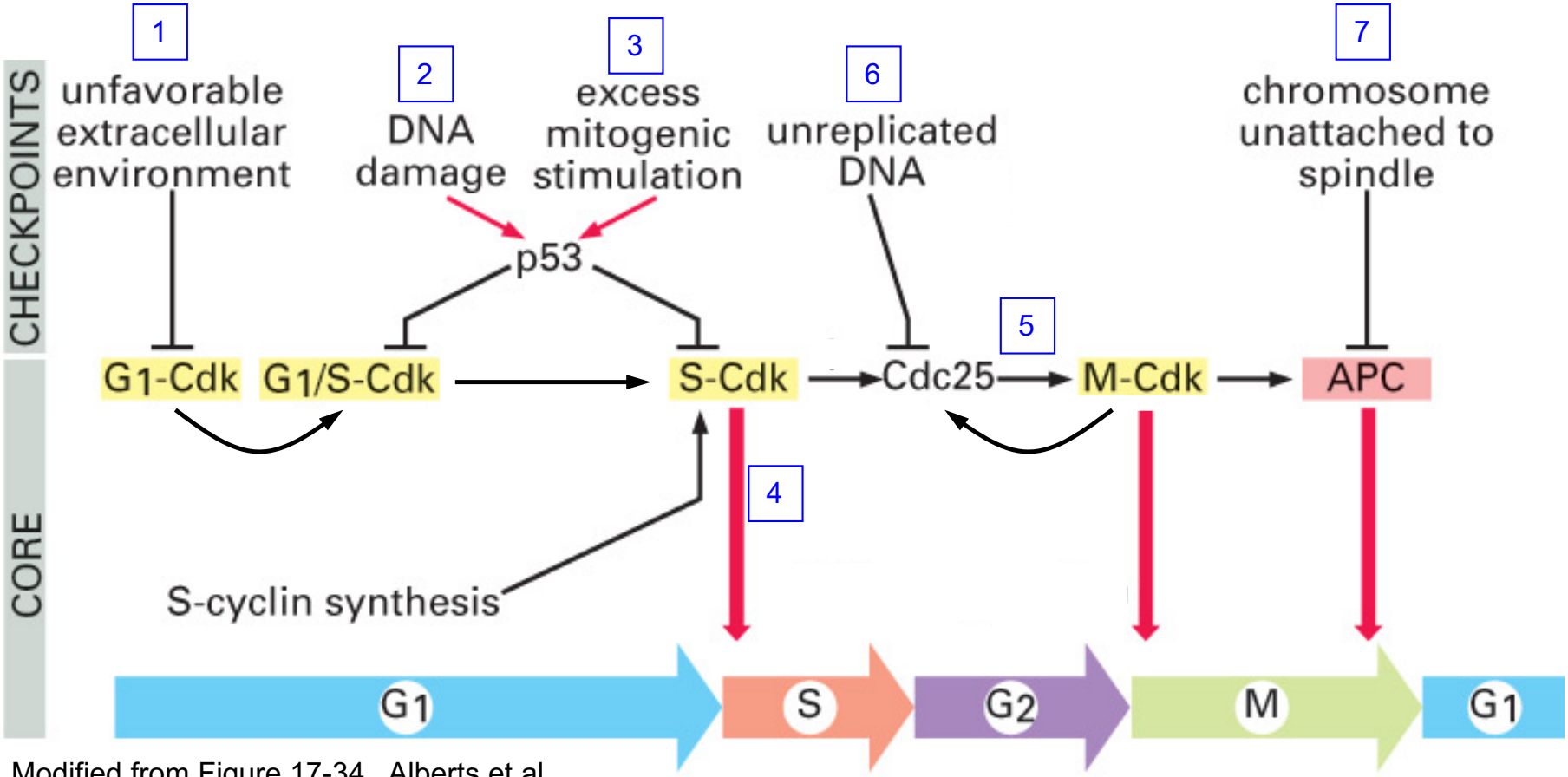


Modified from Figure 17-34. Alberts et al.

6. **Unreplicated DNA Checkpoint**. If unreplicated DNA present, Cdc25 inhibited and M-Cdk not activated. Entry into mitosis does not occur.

**Please note this is NOT an exhaustive review. We will only discuss a few points.*

Review* of Intracellular Control of the Cell Cycle



Modified from Figure 17-34. Alberts et al.

7. **Spindle Attachment Checkpoint**. Unattached kinetochore leads to binding of Mad2 and prevents activation of APC by Cdc20. Chromatids not separated. (Recall that M-Cdk activates APC.)

*Please note this is NOT an exhaustive review. We will only discuss a few points.

Growth Factors

- Act through *receptor tyrosine kinase* (“enzyme-linked receptors”).
- Extracellular binding, transmembrane domain, and intrinsic enzyme acitivity (“autophosphorylation”).
- Ligand (dimer) binding → receptor dimerization and transfer of P_i from ATP by tyrosine kinase (TK domain).
- “*Autophosphorylation*” initiates intracellular pathways.

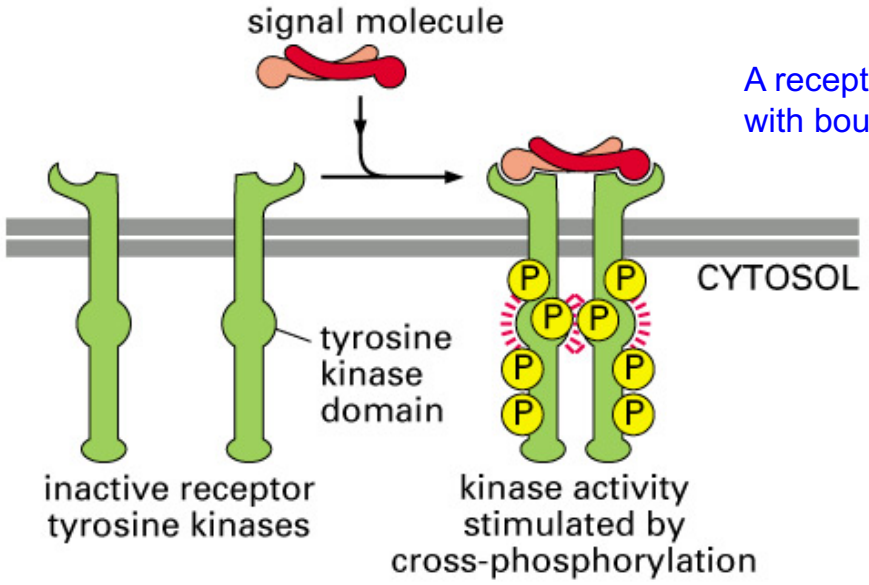


Fig. 15-51a. Alberts 4th ed.

A receptor tyrosine kinase with bound ligand (dimer)

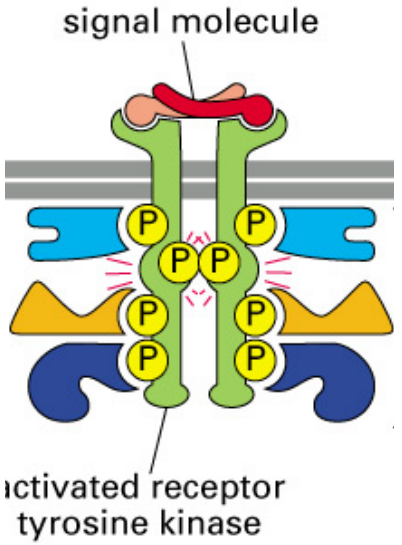


Fig. 15-52. Alberts 4th ed.

Extrinsic Pathway

- Extracellular signal (Fas ligand) activates death receptor (Fas protein).
- Recruitment of adaptor proteins and procaspase activation.
- Caspase cascade.

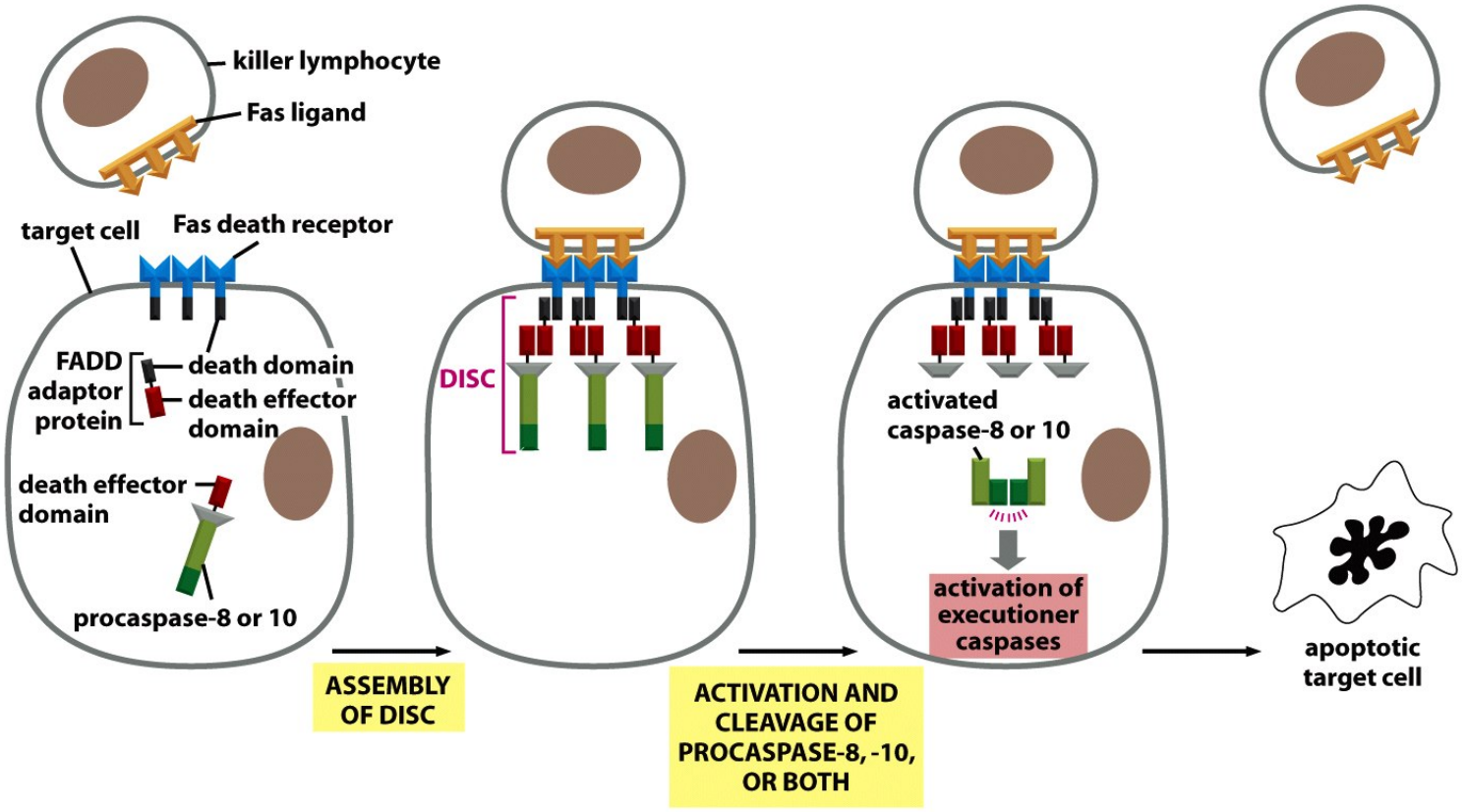


Fig. 18-6

FADD = Fas-associated death domain; DISC = death-inducing signalling complex

Intrinsic Pathway

- Formation of “apoptosome”.
- Caspase cascade.

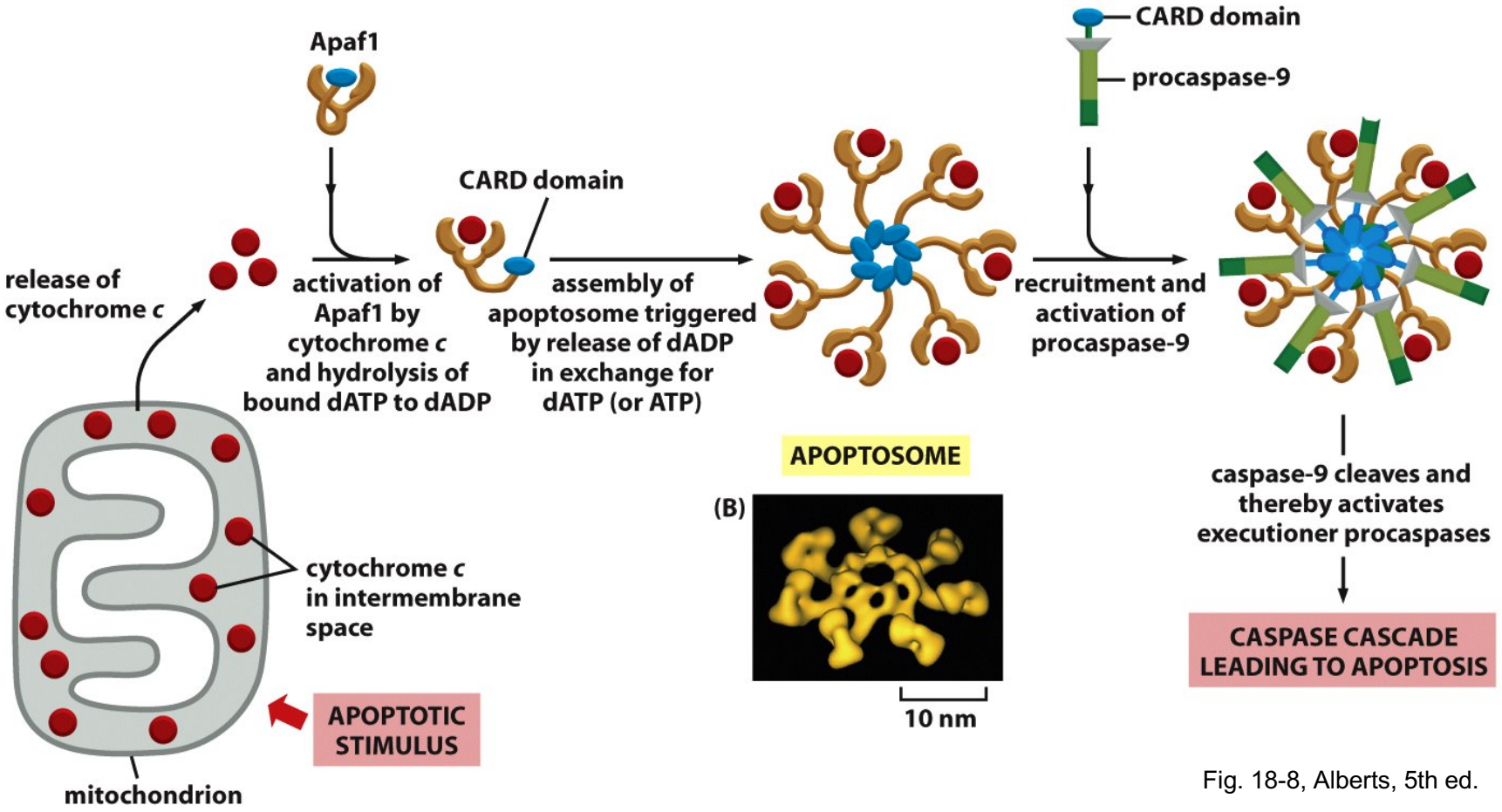


Fig. 18-8, Alberts, 5th ed.

Apaf-1 = apoptotic protease activating factor-1; CARD = caspase recruitment domain

Components of the Mitotic Spindle

1. Microtubules
- Astral
- Kinetochore
- Overlap

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

3. Chromosomes (chromatids)

4. Centrosome
- Centrioles
- PCM

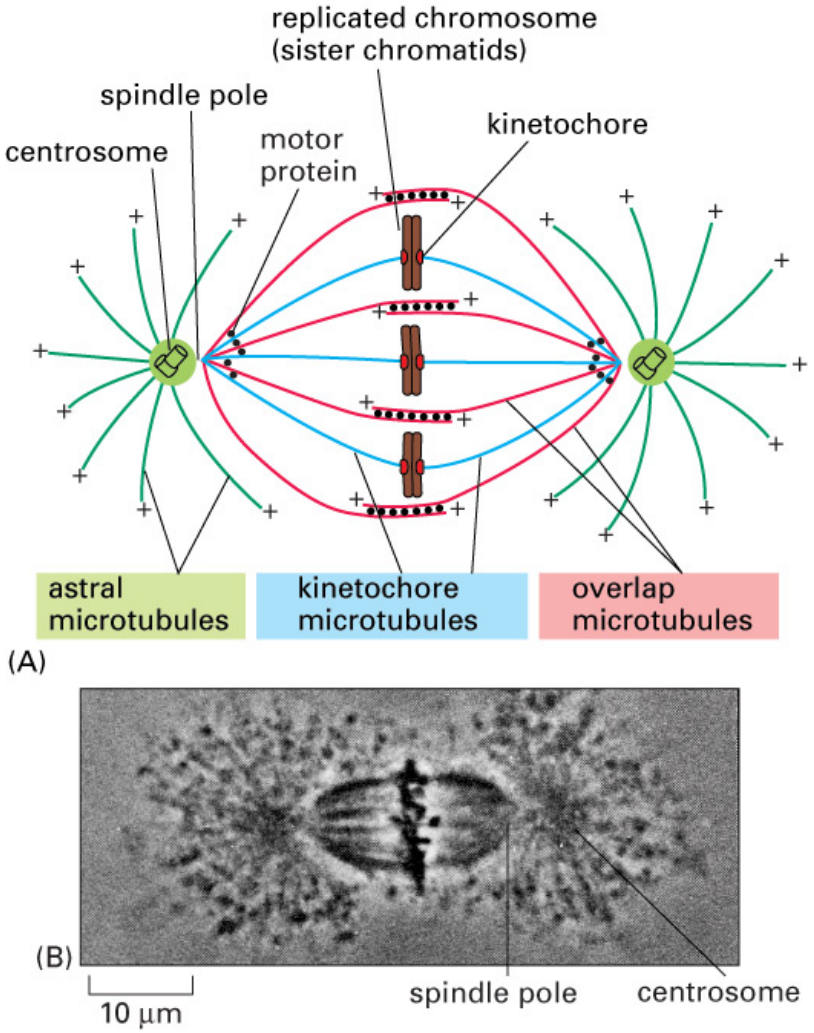


Figure 18–10. Molecular Biology of the Cell, 4th Edition.

MT – Kinetochore Attachment

Motor proteins: balance at the MT-kinetochore interface.

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

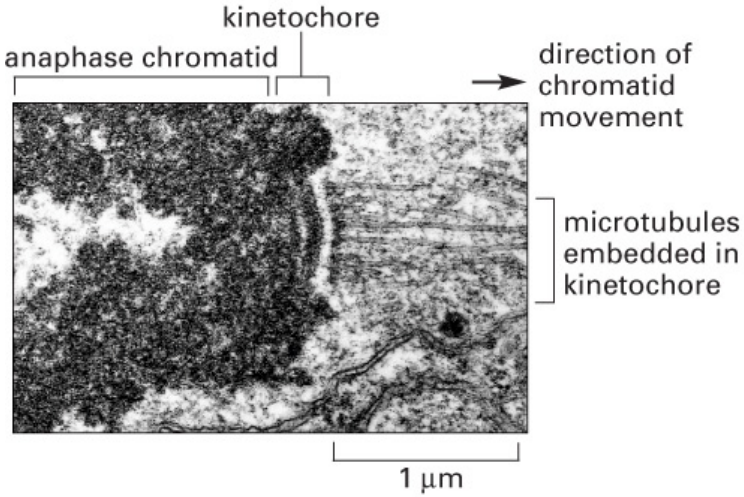
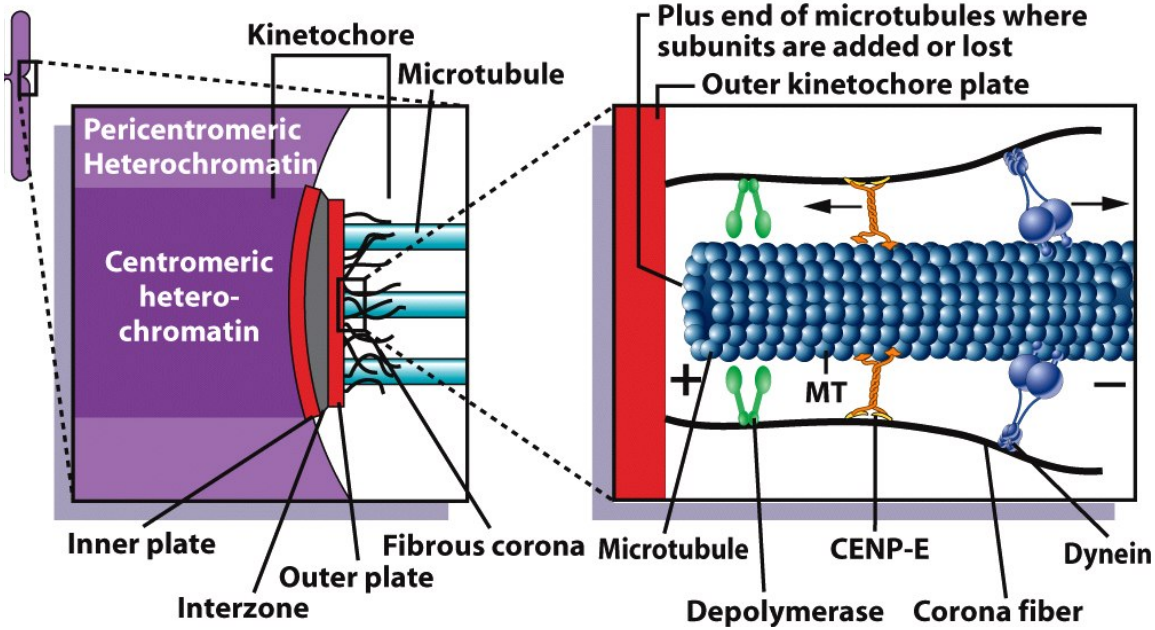


Figure 18–19. Molecular Biology of the Cell, 4th Edition.



(KRP/catastrophin)

(Karp 2008)

MT – Kinetochore Attachment

Motor proteins: balance by astral ejection force.

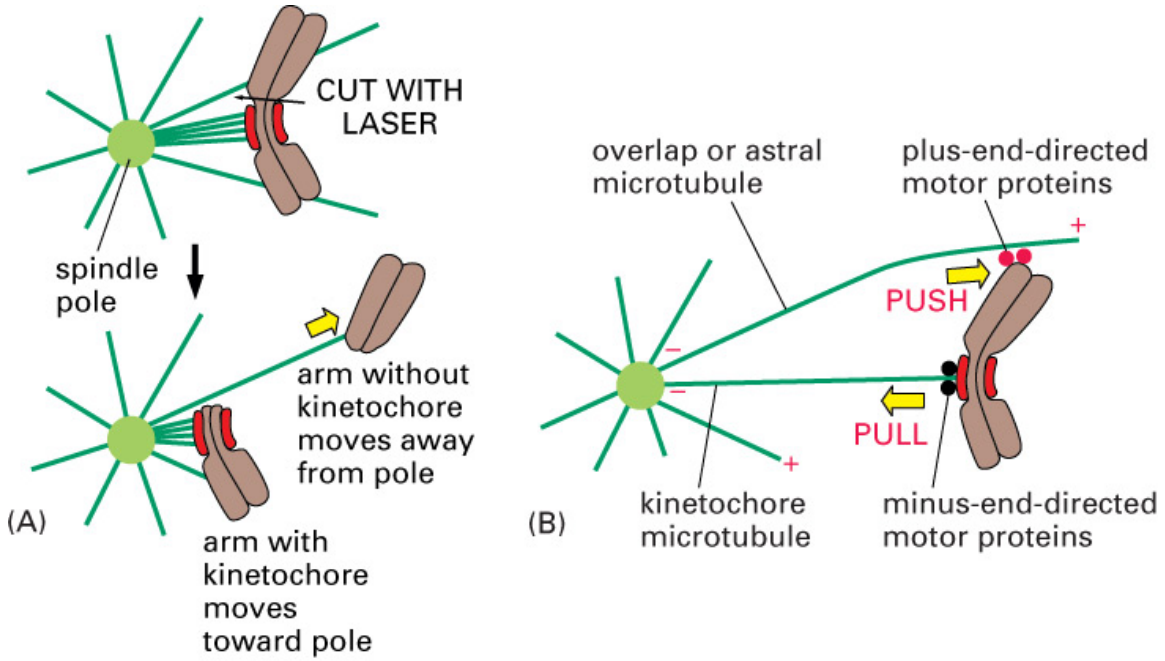
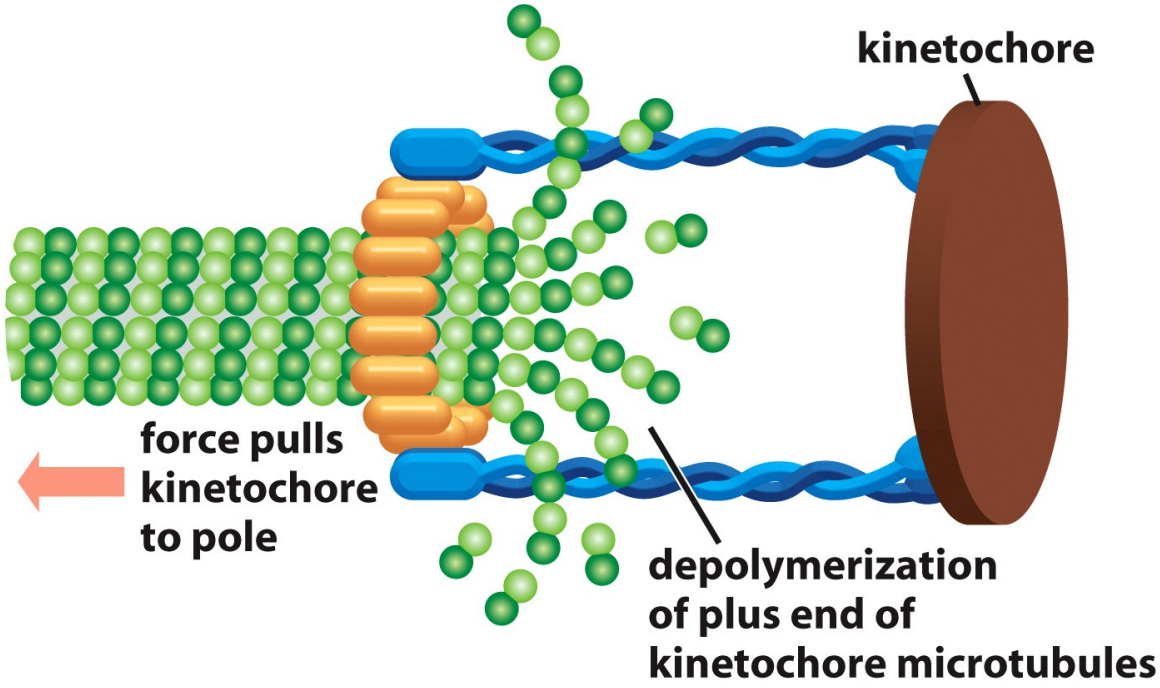


Figure 18–22. Molecular Biology of the Cell, 4th Edition.

2 Separation Forces

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



No additional energy required!

Figure 17-40

Tips for Studying

1. Lecture material is most important.
2. Textbook important to reinforce understanding.
3. The previous figure summarizing cell cycle control is a nice summary.
4. Panels in Alberts can also provide some good review.

Q: Describe growth and treadmilling in actin filaments. Assume the filaments have already been nucleated. Indicate the conditions required for treadmilling to occur, and give 2 examples of accessory proteins and how they regulate this process. (10 marks)

Growth (leading up to treadmilling)

- Actin subunits are monomers that have an ATP-binding site and orient themselves so as to produce a fialment with (+) and (-) ends. It is thermodynamcially favourable for the subunits to join (+) end to (-) end.
- Actin subunits bound with ATP will polymerize to form filaments and ATP hydrolysis will occur thereafter.
- If [actin-ATP] is high (i.e. above Cc for both (D) and (T) ends), subunits will be added to both (+) and (-) ends and the filaments will grow rapidly.
- At this point, the filament is not in equilibrium.
- As [actin-ATP] becomes limiting in the cytosol, addition slows.

Q: Describe growth and treadmilling in actin filaments. Assume the filaments have already been nucleated. Indicate the conditions required for treadmilling to occur, and give 2 examples of accessory proteins and how they regulate this process. (10 marks)

Treadmilling

- Treadmilling is the process of addition and removal of subunits to actin filaments in which there is no significant net growth or shrinkage.
- This is a state of equilibrium and is almost entirely dependent on the subunit (actin) concentration. Importantly, treadmilling will occur only if the concentration of actin subunits is within the "treadmilling range", or $Cc(T) < C < Cc(D)$.
- The treadmilling range is produced because of the different elongation rates at the "T" or "plus" end (where it is faster) and "D" or "minus" end (where it is slower) due to their specific thermodynamic properties, and these define two different critical concentrations: Cc(T) and Cc(D).

Q: Describe growth and treadmilling in actin filaments. Assume the filaments have already been nucleated. Indicate the conditions required for treadmilling to occur, and give 2 examples of accessory proteins and how they regulate this process. (10 marks)

Possible Examples, of which 2 should be provided (*only the first 2 will be marked, 1 mark each*).

- Thymosin sequesters actin and prevents addition to actin filaments.
- Profilin recruits actin and promotes growth.
- Arp complex (Arp2/3) reduces subunit loss (or “caps”) at the minus end during formation of actin filament branches.
- Cofilin destabilizes actin filaments and leads to loss of subunits (i.e. increases turnover).
- Tropomyosin will stabilize filament (e.g. in muscle) and reduce treadmilling.
- CapZ is a capping protein that binds to the plus end and reduces subunit addition. Also found in the sarcomere.
- Tropomodulin is a capping protein that binds to the minus end and reduces subunit loss.

Good luck!

Questions?

Things to Consider: Lecture 2

1. What are the primary differences between phase contrast and DIC?
   - Both exploit phase shift of light by sample, but only DIC utilizes separation and reconstitution of light by prisms. In addition, phase involves “phase rings”.

2. Think about appropriate applications in which you would use standard fluorescence, confocal and two-photon microscopy.
   - Single cells, tissue sections, thick tissues of live specimens.

Things to Consider

1. Can you differentiate between the processes of treadmilling and dynamic instability?
   - AFs or MTs; (-) or (+) end; ATP/GTP caps; dependence on subunits and ATP/GTP...

2. Think about the differences in assembly, growth and shrinkage between each of the 3 filament proteins that we discussed.
   - Subunits/components; structure; polarity; where does it occur...

Things to Consider

1. Think about how each accessory protein affects the stability of AFs and MTs
   - e.g. capping proteins, MAPs etc. See Table Ch. 16.

2. Many of the dynamic mechanisms that we discussed today do not apply to IFs. Why?
   - Because of the structure of IFs, they do not undergo dynamic changes like AFs and MTs.

Things to Consider

1. Structure is related to function. Think about the differences between types of motors at the molecular level that lead to differences in function.
   - Carry cargo vs. filament sliding; speed and force of movement; direction; myosin II vs. myosin V neck length.

2. Why isn't there a single motor protein that performs all of the roles discussed?
   - A diversity of function has evolved at the cellular level.

Things to Consider

1. There are many details that you've learned in these lectures. Try to piece everything together and think about where in the cell cycle everything fits, i.e. in 'chronological' order.
   - Progression through phases and checkpoints; see summary figure.

2. What roles do feedback mechanisms play in cell cycle control?
   - Rapid changes in activity ensure progression through cycle.

3. Remember the differences between extracellular and intracellular mechanisms of cell cycle control.
   - Both require some intracellular pathways; extracellular signals can influence cell cycle too...

Things to Consider

1. How do extrinsic and intrinsic pathways of apoptosis differ? Think about how the caspase cascade is initiated in each case.
   - Both require some intracellular interactions and procaspase activation; aggregation by transmembrane receptors, DISC vs. cytochrome c release and apoptosome...