Midterm I Review

What we will do today:

- Midterm format (brief).
- What happens after the Midterm
- Brief overview of lectures so far.
- Summary figure for intracellular control of the cell cycle.
- Tips for studying.
- Questions.

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Midterm Format

- Approximately 1 hour.
- There will be 2 parts to the exam:

  Part A: Multiple choice (20 x 1 marks)

  Part B: Long answer (2 x 10 marks)
  - About one page for each answer.
  - You may use point form.
  - Do not include excessive writing.
  - Diagrams may be included but they must be accompanied by text.

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After the Midterm

- Exams will be graded and returned to you.
- Please be patient.
- Questions should be directed to TAs:
  - A-K: Peter Zachar
  - L-Z: Sandra Noble

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Things to Consider...

Please note:

- The following are not *complete* answers, they are examples only.
- These are meant to *guide* you in your studies.

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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.

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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

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

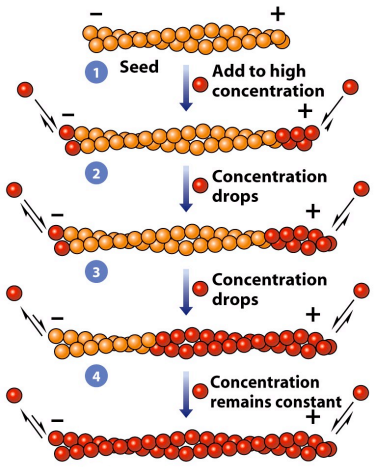

Figure 9-46b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

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Dynamic Instability – MTs

- [Tubulin]
- GTP cap
- Continuous transitions

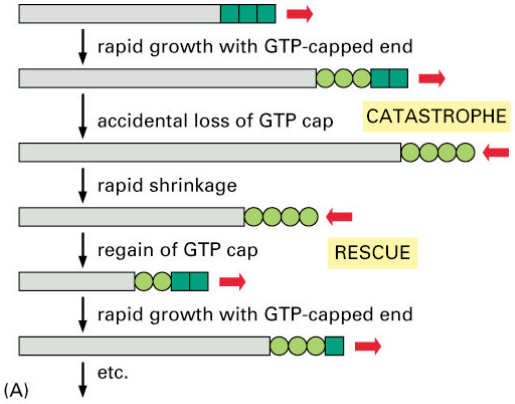

Figure 16–11 part 1 of 3. Molecular Biology of the Cell, 4th Edition.

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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...

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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.

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The Myosin Cycle

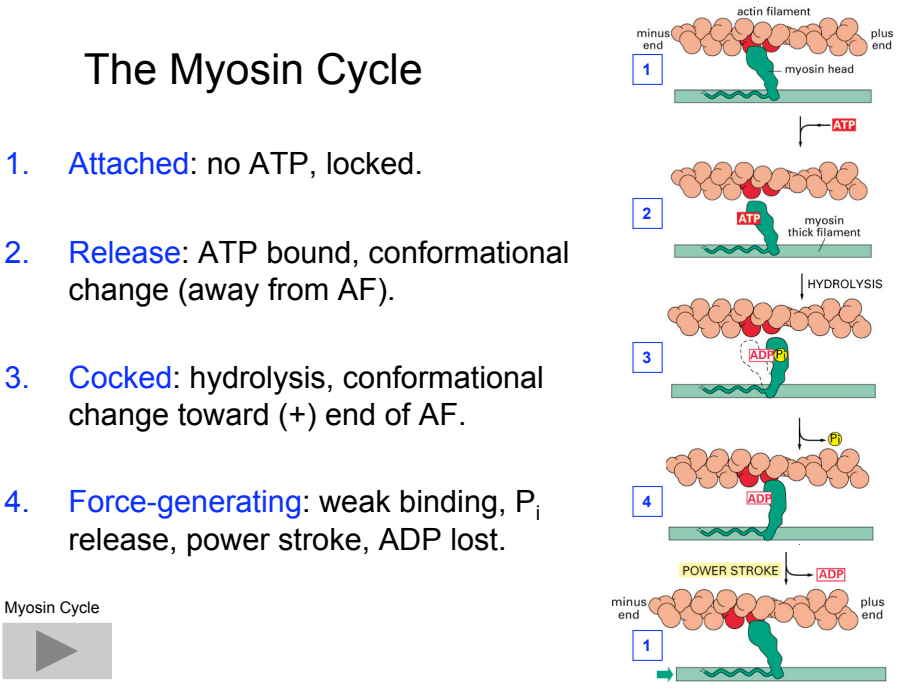
1. Attached: no ATP, locked.

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.

Myosin Cycle



Kinesin Cycle

- *Processive* steps along MTs.

1. ATP binding of leading head induces conformational change in its linker region; trailing head advances.
2. ADP induces weak binding of leading head.
3. Hydrolysis of trailing head induces detachment; ADP dissociates from leading head. *(Repeat…)*

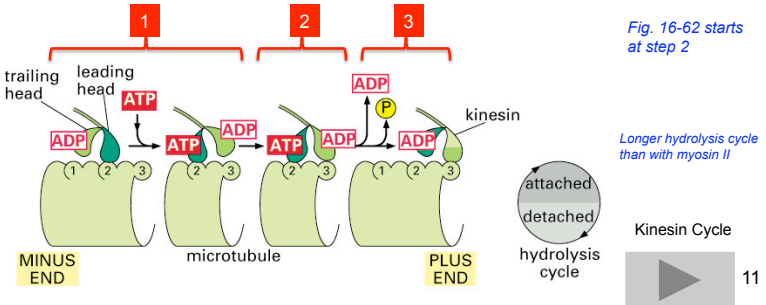


Fig. 16-62 starts at step 2

Longer hydrolysis cycle than with myosin II

Kinesin Cycle

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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.

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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...

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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...

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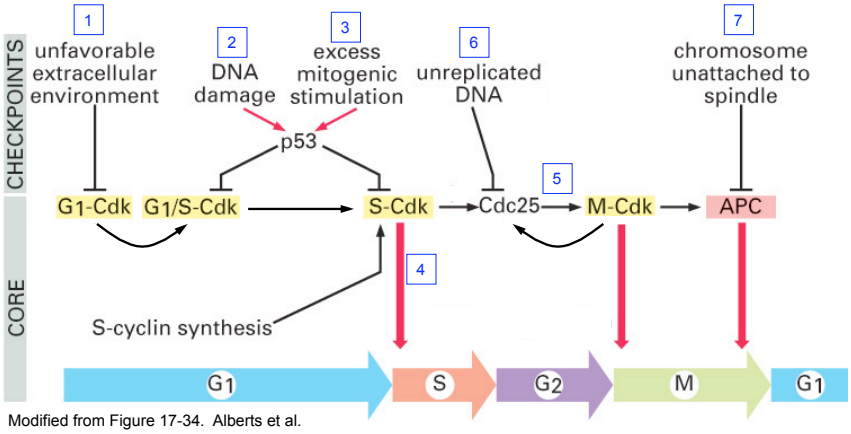
Review* of Intracellular Control of the Cell Cycle



Modified from Figure 17-34. Alberts et al.

1. **G1 Restriction Point**. Cell cycle proceeds only if mitogen present. Ras, MAP kinase, *myc*…

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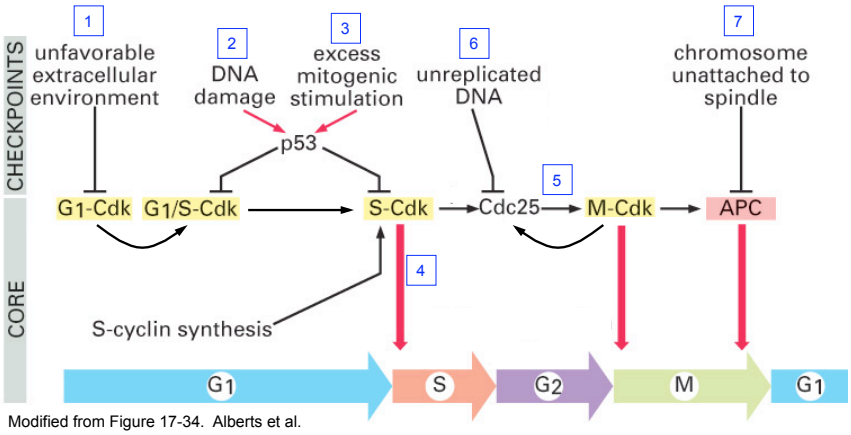
**Please note this is NOT an exhaustive review. We will only discuss a few points.*

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).

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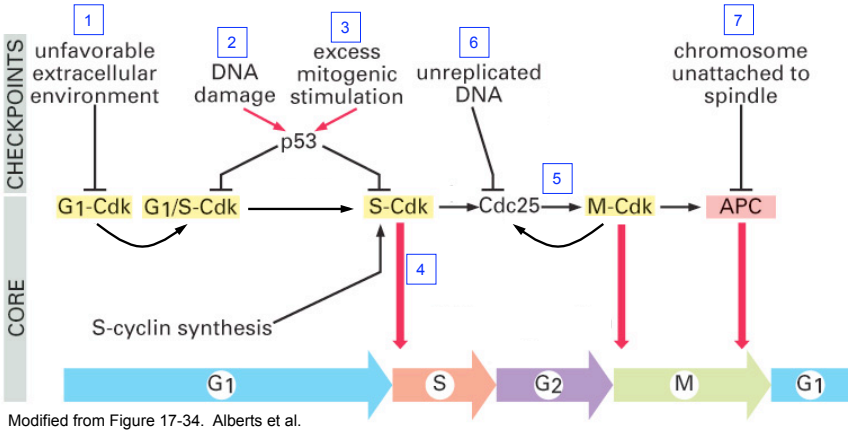
Review* of Intracellular Control of the Cell Cycle



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.

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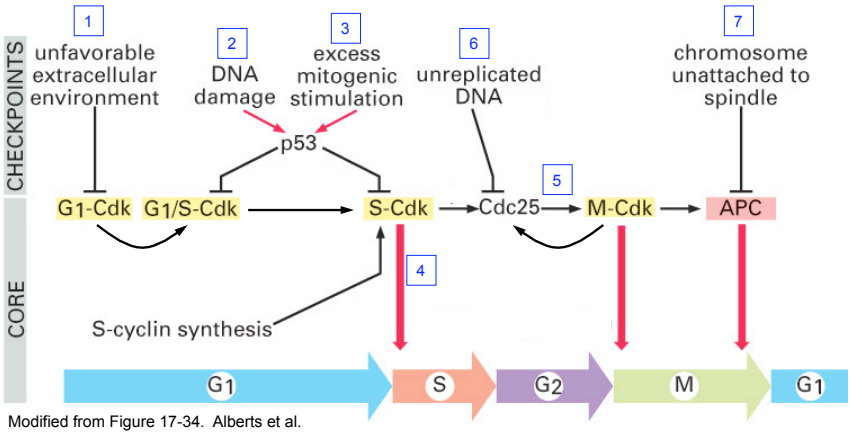
Review* of Intracellular Control of the Cell Cycle



Modified from Figure 17-34. Alberts et al.

4. **Initiation of DNA Replication**. Increased S-Cdk and assembly of pre-ORC leads to DNA replication.

We skipped over this, so briefly…

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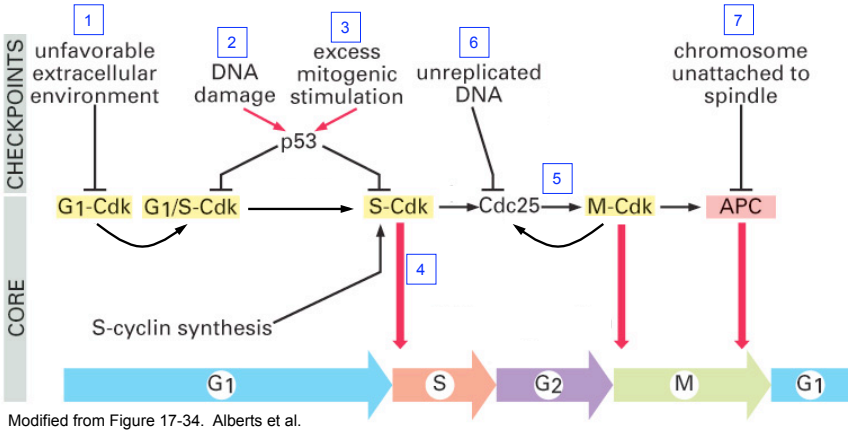
Review* of Intracellular Control of the Cell Cycle



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.

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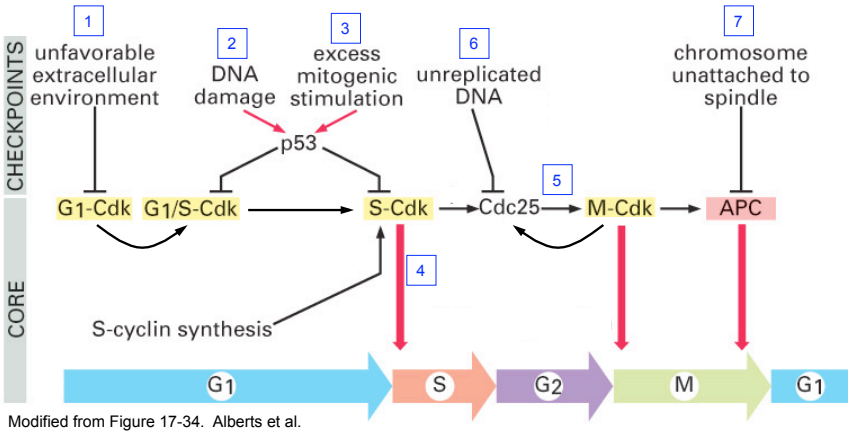
Review* of Intracellular Control of the Cell Cycle



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.

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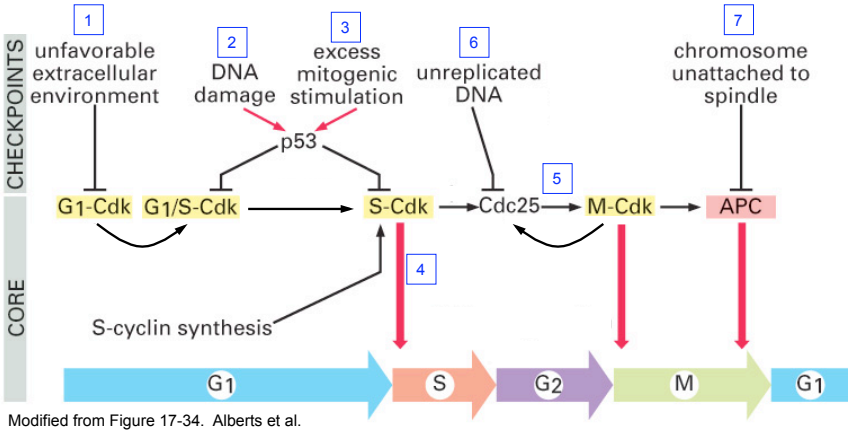
Review* of Intracellular Control of the Cell Cycle



Modified from Figure 17-34. Alberts et al.

7. **Spindle Attachment Checkpoint**. Unattached kinetochore prevents activation of APC by Cdc-20 and chromatids not separated.

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SCF and APC are Active During Different Stages

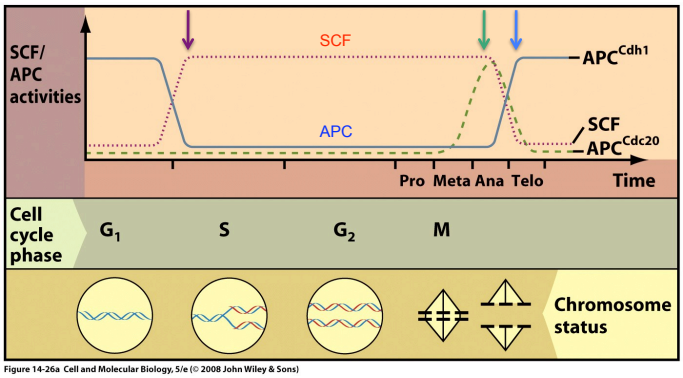

Figure 14-26a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

(-) APC-Cdc20 activation → ↓M-cyclin (slide 5 and 12) → ↓M-Cdk → ↑APC-Cdh1 (above) → Keeps M-cyclin levels low (slide 5)

(-) APC-Cdc20 activation → Separase activation and anaphase (slide 18)

See also Fig. 17-16, p1100

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Tips for Studying

1. Lecture material is most important.
2. Textbook important to reinforce understanding.
3. The previous figures are a nice summary.
4. Panels 16-1, 16-2, 16-3, and Table 17-21 are good as well.
5. Refer to sample midterm posted on website.

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Good luck!

Questions?

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