BIO 3153 – Cell Biology

Practice Midterm Examination

(*Please note that the format for the Midterm that will be written in class has not yet been finalized*)

Part A

20 Multiple Choice Questions: (1 mark each for a total of 20 marks)

1. A single actin subunit that will be incorporated into a growing filament is called a:

   a. Monomer
   b. Dimer
   c. Homodimer
   d. Heterodimer
   e. Tetramer

2. Which of the following does not bind ATP?

   a. Actin
   b. α-tubulin
   c. β-tubulin
   d. Myosin II
   e. b and c

3. What is the last event that occurs immediately before microtubules fall apart during catastrophe?

   a. The availability of tubulin in the cytosol falls below the critical concentration.
   b. Hydrolysis of ATP to ADP and loss of the ATP cap.
   c. Hydrolysis of GTP to GDP and loss of the GTP cap.
   d. Conformational change of protofilaments into a curved shape.
   e. Rescue.

4. Which of the following is false?

   a. Thymosin sequesters actin and prevents growth of actin filaments.
   b. Profilin competes with thymosin and promotes assembly of actin filaments.
   c. Stathmin prevents dynamic instability of microtubules.
   d. α-actinin provides enough space for myosin bundles to slide between actin filaments.
   e. Filamin is an example of a gel-forming protein.

5. Nucleation of microtubules:

   a. occurs in the cytosol and involves the binding of Arp2/3 at an angle of 70°.
   b. is initiated by the PCM and is dependent on γ-tubulin (i.e. gamma-tubulin).
   c. can occur anywhere in the cell and requires formin.
   d. occurs only in the cytosol.
   e. None of the above.

6. Which of the following proteins are important for providing flexibility to the cortext of red blood cells?

a. Spectrin
b. Actin
c. Filamin
d. Fimbrin
e. Both a and b

7. Which of the following best describes the structure of the sarcomere? (Note α-actinin = alpha actinin)

a. Parallel rows of thin myosin filaments are separated by α-actinin; CapZ and tropomodulin prevent changes in myosin filament length; rows of thick filaments contain actin motors that bind to myosin in the presence of Ca^{2+}.
b. Parallel rows of thin actin filaments are separated by α-actinin; CapZ and tropomodulin prevent changes in actin filament length; rows of thick filaments contain myosin motors that bind to actin in the presence of Ca^{2+}.
c. Parallel rows of thin actin filaments are separated by α-actinin; CapZ and tropomyosin prevent changes in actin filament length; rows of thick filaments contain myosin motors that bind to actin in the absence of Ca^{2+}.
d. Parallel rows of thin actin filaments are separated by fimbrin; CapZ and tropomyosin prevent changes in actin filament length; rows of thick filaments contain myosin motors that bind to actin in the presence of Ca^{2+}.
e. None of the above.

8. In the myosin cycle, which of the following indicates the correct sequence of events? Each stage is separated by a " ; "

a. Attached (no ATP, locked); Cocked (hydrolysis, conformational change); Release (ATP bound, conformational change); Force generation (weak binding, dephosphorylation).
b. Attached (no ATP, locked); Release (ATP bound, conformational change); Cocked (hydrolysis, conformational change); Force generation (weak binding, dephosphorylation).
c. Attached (hydrolysis, conformational change); Release (ATP bound, conformational change); Cocked (no ATP, locked); Force generation (weak binding, dephosphorylation).
d. Attached (weak binding, dephosphorylation); Cocked (hydrolysis, conformational change); Release (ATP bound, conformational change);Force generation (no ATP, locked).
e. None of the above.

9. Which of the following statements is **incorrect**?

a. Kinesins transport cargo toward the axon terminal in neurons.
b. Dyneins transport cargo toward the cell body in neurons.
c. Myosin V binding to microtubules allows for transport in the cell periphery.
d. Cytoplasmic dynein binds indirectly to its cargo.
e. Myosins are a diverse family of motor proteins with conserved head regions

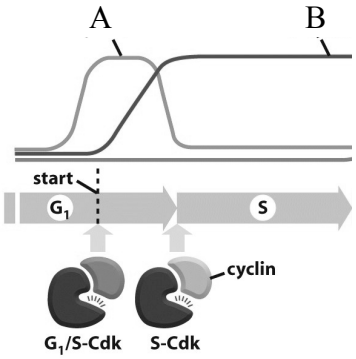
10. Which of the following best describe(s) the role of Ca^{2+} in the contraction of striated muscle?

a. Ca^{2+} is released from the Golgi apparatus.
b. Ca^{2+} binds to tropomodulin and induces a conformational change.
c. Ca^{2+} binds to troponin C and induces a conformational change.
d. Ca^{2+} binds directly to tropomyosin and exposes binding sites on actin filaments.
e. Both c and d.

11. Complete activation of a cyclin-dependent kinase (Cdk) occurs:

a. spontaneously.
b. only during M-phase.
c. only during S-phase.
d. after addition of CAK, and phosphorylation of Cdk by cyclin.
e. after addition of cyclin, and phosphorylation of Cdk by CAK.

12. In the figure to the right, the labels "A" and "B" are:



a. M-cyclin and S-cyclin, respectively
b. S-cyclin and G_1/S-cyclin, respectively
c. G_1/S-cyclin and S-cyclin, respectively
d. G_2/S-cyclin and M-cyclin, respectively
e. G_1/S-cyclin and M-cyclin, respectively

13. Which of the following events is not detected at a "checkpoint"?

a. DNA damage.
b. Unattached kinetochore.
c. Unreplicated DNA.
d. Initiation of DNA replication.
e. Both c and d.

14. Which of the following is **not** a characteristic of nerve growth factor?

a. It was discovered by Rita Levi-Montalcini.
b. It is a dimer.
c. Binding of NGF to a TrkA receptor induces autophosphorylation.
d. Binding of NGF to a TrkA receptor induces dephosphorylation.
e. It is an extracellular signalling molecule.

15. Which of the following choices best describes the correct sequence of events following recognition of NGF? Each stage is separated by a " ; "

a. NGF directly activates Ras; PI-3 kinase phosphorylates MAP kinase; Myc phosphorylates the ribosomal protein, S6.
b. NGF directly activates Ras; phosphorylation of MAP kinase; a gene regulatory protein translocates into the nucleus; Myc production is increased.
c. NGF binds to the extracellular domain of a TrkA receptor; receptor dimerization induces autophosphorylation; activated PI-3 kinase phosphorylates MAP kinase; Myc phosphorylates the ribosomal protein, S6.
d. NGF binds to the extracellular domain of a TrkA receptor; PI-3 kinase is activated; receptor dimerization induces autophosphorylation; PIP_2 is phosphorylated to PIP_3; TOR, S6K and S6 are sequentially phosphorylated; new proteins are synthesized.
e. NGF binds to the extracellular domain of a TrkA receptor; receptor dimerization induces autophosphorylation; PI-3 kinase is activated; PIP_2 is phosphorylated to PIP_3; TOR, S6K and S6 are sequentially phosphorylated; new proteins are synthesized.

16. A receptor tyrosine kinase:
    a. is an enzyme-linked receptor.
    b. dimerizes upon binding with an extracellular signalling molecule.
    c. is phosphorylated.
    d. can initiate signalling pathways that involve PI-3 kinase or Ras.
    e. all of the above.

17. A caspase is a protein that:
    a. has an aspartate residue at its catalytic site and cleaves other proteins at a cysteine site.
    b. activates essestial cellular proteins and promotes survival.
    c. is similar to the protease encoded by the gene, *ced-3*, in *Caenorhabditis elegans*.
    d. is deactivated following apoptosome formation.
    e. initiates both the intrinsic and extrinsic pathways of apoptosis.

18. Which of the following best describes the sequence of events observed during apoptosis? Each stage is separated by a " ; "

    a. Chromatin condensation and shrinkage of the cytoplasm; detection of phosphatidylserine and phagocytosis by a phagocyte; nuclear and cellular fragmentation, blebbing.
    b. Detection of phosphatidylserine and phagocytosis by a phagocyte; chromatin condensation and shrinkage of the cytoplasm; nuclear and cellular fragmentation, blebbing.
    c. Nuclear and cellular fragmentation, blebbing; chromatin condensation and shrinkage of the cytoplasm; detection of phosphatidylserine and phagocytosis by a phagocyte.
    d. Chromatin condensation and shrinkage of the cytoplasm; nuclear and cellular fragmentation, blebbing; detection of phosphatidylserine and phagocytosis by a phagocyte.
    e. None of the above are correct.

19. Which of the following best describes only the intrinsic pathway of apoptosis?

    a. Release of cytochrome c and apoptosome formation.
    b. Release of cytochrome c and DISC formation.
    c. Binding of Fas ligand to Fas receptor.
    d. Inactive procaspases are cleaved and become active caspases.
    e. Active caspases are cleaved and become inactive procaspases.

20. Which of the following sequence of events best describes the formation of an apoptosome? Each stage is separated by a ";"

    a. Cytochrome c is released from the intermembrane space of the mitochondrion; a conformational change of Apaf1 reveals a CARD domain; dADP is exchanged for dATP and apoptosome formation is triggered.
    b. dADP is exchanged for dATP and apoptosome formation is triggered; a conformational change of Apaf1 reveals a CARD domain; cytochrome c is released from the intermembrane space of the mitochondrion.
    c. A conformational change of Apaf1 reveals a CARD domain; dADP is exchanged for dATP and apoptosome formation is triggered; cytochrome c is released from the intermembrane space of the mitochondrion.
    d. dADP is exchanged for dATP and apoptosome formation is triggered; cytochrome c is released from the intermembrane space of the mitochondrion; a conformational change of Apaf1 reveals a CARD domain.
    e. None of the above.

Part B

3 Long Answer Questions (10 marks each for a total of 30 marks).
Please note that the number of bullet points in each answer does not necessarily translate into an equal number of marks. In addition, you will not be required to draw figures or diagrams. These are included below only as a reminder.

1. 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 limitting in the cytosol, addition slows.

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).

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.

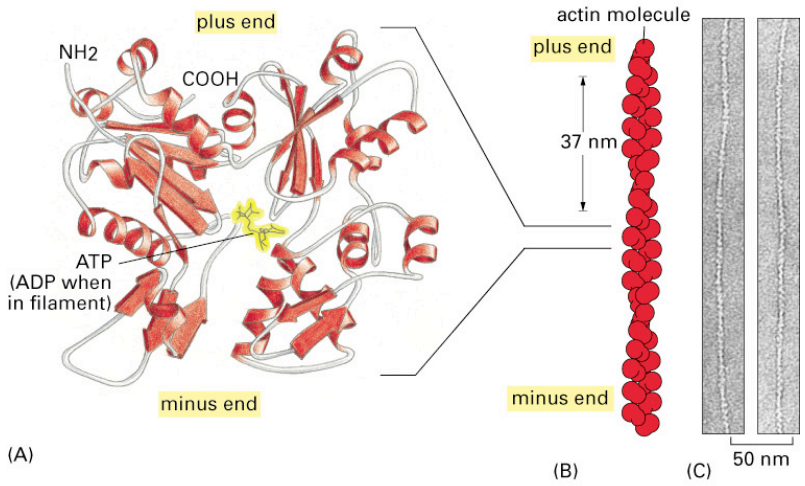


Figure 16–7. Molecular Biology of the Cell, 4th Edition.

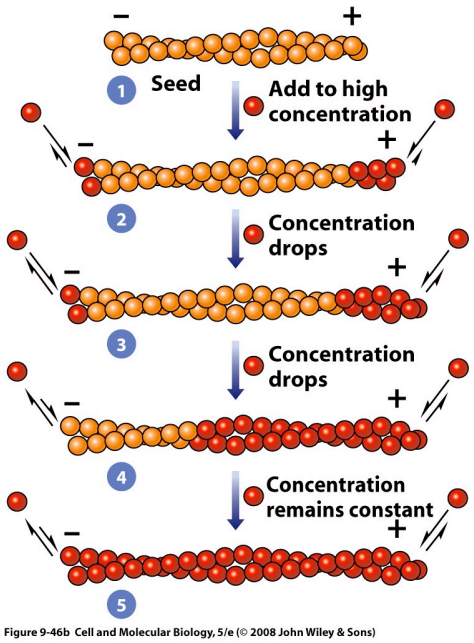
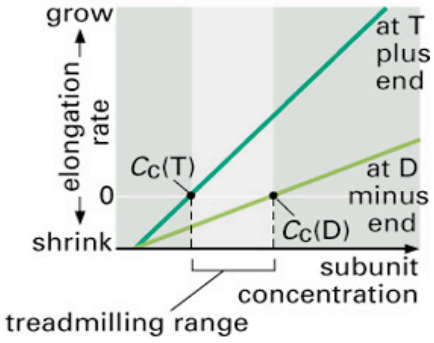


Figure 9-46b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)



For $C > C_C(T)$ but $< C_C(D)$, treadmilling occurs

(B)

2. Describe the mechanism through which APC (anaphase promoting complex) can initiate the destruction of M-cyclin. During which phase of the cell cycle does this occur, and what are the subsequent effects on the cell cycle? (10 marks)

Mechanism

- APC is a ubiquitin ligase typically kept low during other phases of the cycle, but it begins to increase around metaphase and anaphase.
- APC can be activated by the activating subunits, Cdc20 (i.e. APC^{Cdc20}) or Cdh1 (i.e. APC^{Cdh1}). Both forms should be stated (see pathway below and figure).
- Activated APC^{Cdc20} and APC^{Cdh1} recruit other ubiquitination enzymes (E1 and E2).
- These enzymes together mediate the addition of a polyubiquitin (or multiubiquitin) chain onto M-cyclin.
- Once tagged with a polyubiquitin chain, the protein is then sent to a proteasome where it will be destroyed by a protease enzyme within its core, and broken down into amino acids.

Which phase?

- This process takes place during M-phase of the cell cycle. More specifically, M-cyclin is degraded around the metaphase-anaphase portion of mitosis.

"Subsequent effects on the cell cycle"

- The end result of destruction of M-cyclin (and therefore inactivation of M-Cdk) is that the cell will be permitted to exit M-phase and continue through the cell cycle. (i.e. there is a positive effect on the cell cycle.)
- Furthermore, the activity of APC^{Cdh1} will continue to keep M-cyclin levels low until the end of G1 phase. (It might also be stated that this in fact creates G1 phase.)

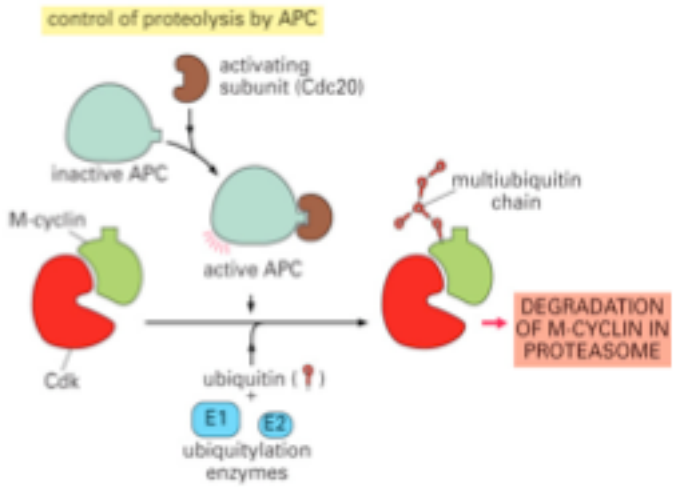


Figure 17-20 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

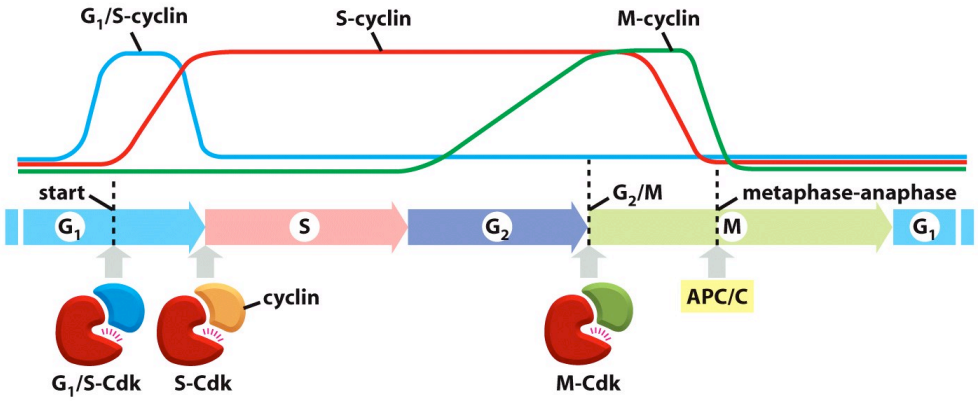


Figure 17-16 Molecular Biology of the Cell 5/e (© Garland Science 2008)

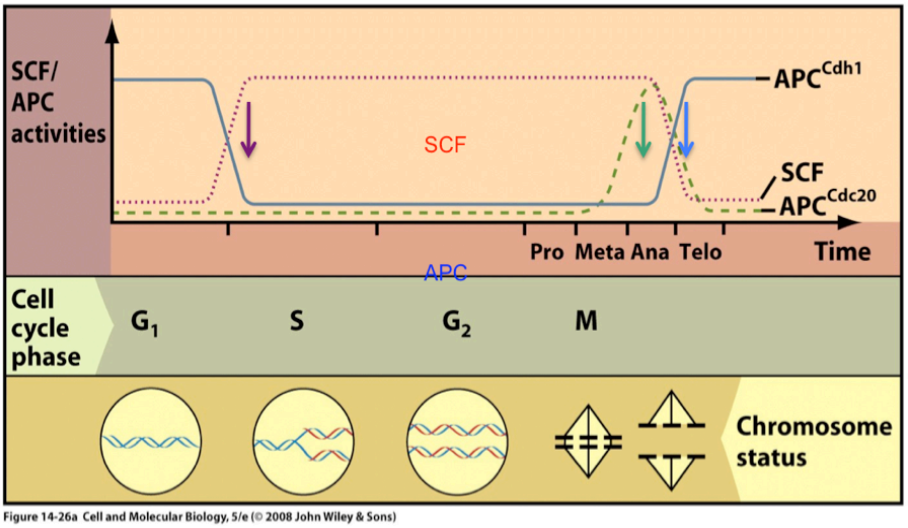


Figure 14-26a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

3. Describe the extrinsic pathway of apoptosis. Begin your answer with an event or "signal" that initiates this process, and end your answer with activation of the caspase cascade. (10 marks)

- In the extrinsic pathway of apoptosis, and extracellular signal initiates the process.
- The Fas ligand of a killer lymphocyte (killer T cell, Tc, lymphocyte also acceptable) binds to the Fas death receptor at the target cell destined for apoptosis.
- Both Fas ligand and Fas receptor are transmembrane proteins.
- Upon binding of ligand to receptor, activation of the death receptor recruits adaptor proteins, called FADD (Fas-activated death domain) because procaspases do not bind directly to the Fas receptor.
- Procaspases are then recruited and the DISC (death-induced signalling complex) forms.
- Procaspases are then aggregated in close proximity to one another.
- There is a background (or low) level of active caspases (with catalytic activity at cysteine sites/residues) that cleaves procaspases at aspartate sites/residues.
- This reaction initiates the caspase cascade.