Life Sciences 1a

Practice Problems 9

1. The following diagram from lecture shows a cartoon of how the activities of the Cdk1-cyclin complex and the APC complex change through the cell cycle.

![Diagram showing changes in Cdk1-cyclin and APC activities through the cell cycle.]

- a) Please indicate where mitosis and interphase would be occurring on the x-axis

- b) What causes the level of cyclin to change during the cell cycle?

- c) Does the level of Cdk1 (inactive plus active) dramatically change during the cell cycle? Briefly explain.

- d) What type(s) of feedback cause the increase in levels of active Cdk1-cyclin and active APC?

- e) If you injected an active Cdk1-cyclin complex into a cell in interphase what would you predict would happen?
2. A living cell from the lung epithilium of a newt is shown at different stages of mitosis in the figure below. Order these light micrographs into the correct sequence and identify the stage in mitosis that each represents.

3. Many anticancer drugs act on microtubules. It was, perhaps, initially surprising that high concentrations of some successful anticancer drugs (like nocodazole) inhibit microtubule polymerization and others (like taxol) stabilize microtubules by making microtubules with GDP-tubulin caps resistant to depolymerization. At lower concentrations, both classes of drugs appear to arrest cells in mitosis and induce apoptotic death of proliferating cells.

a) Why might high concentrations of nocodazole arrest cells in mitosis?

b) Why might high concentrations of taxol arrest cells in mitosis?
c) What would happen if cells treated with these drugs do not arrest at a particular stage of mitosis?

d) Why are cancerous cells more strongly affected by these drugs?

4. The complex kinetics of microtubule assembly makes it hard to predict the individual behavior of individual microtubules. Some microtubules in a population can grow, even as others shrink to nothing

a) Why can some molecules grow while others shrink?

b) Are the microtubules in a cell at steady state? Why or why not?

c) When an αβ-tubulin dimer adds onto a growing microtubule does it bind in a specific orientation? Why or Why not?
d) The images below were taken with an electron microscope and show two different types of microtubules labeled A and B. One image shows growing microtubules and the second shows catastrophically shrinking microtubules. Please match A and B with the proper microtubule dynamics and give a brief explanation for your choices.

![A](image1.png) ![B](image2.png)

e) Tubulin is distantly related to the GTPase family that includes Ras. The β-tubulin in an αβ-tubulin dimer hydrolyzes GTP at a slow intrinsic rate. When it is incorporated into a growing microtubule, however, its rate of GTP hydrolysis increases rapidly. What effect does this GTPase activity of tubulin have on microtubule dynamics?
5. Using zebrafish as a model to study human cancer, you discover \textit{bmyb}, a gene that encodes a transcription factor responsible for the regulation of the synthesis of cyclin B. You isolate a \textit{bmyb} mutant zebrafish that is unable to enter mitosis.

a) You analyze the mutant protein and align the protein sequence to the wildtype \textit{bmyb} as shown below. You find out that nearly all of the amino acids following the mutation in the \textit{bmyb} gene (black-shaded region of the mutant compared to gray-shaded region of the wildtype protein) are now different from the wild-type protein. How do you suspect that this occurred?

\begin{center}
\begin{tabular}{|c|c|}
\hline
\textbf{wildtype \textit{bmyb} protein} & \textbf{mutant \textit{bmyb} protein} \\
\hline
N & N \\
\hline
\hline
\end{tabular}
\end{center}

\begin{center}
\begin{tabular}{|c|c|}
\hline
 & C \\
\hline
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\end{tabular}
\end{center}

b) You next look to see what the levels of cyclin B look like in the \textit{bmyb} mutant and you find that the mutant cells have decreased levels of cyclin B. How is \textit{bmyb} responsible for the levels of cyclin B and why does a mutation in the \textit{bmyb} effect the expression of cyclin B?

c) What is the role of cyclin B in the cell cycle?

d) What should happen upon injection of cyclin B mRNA into the mutant embryos? Briefly explain.

e) What should happen upon injection of wildtype \textit{bmyb} mRNA into the mutant embryos? Briefly explain.

   ![Graph showing concentrations of proteins during the cell cycle](image)

a) Shown above

b) The concentration of the protein cyclin varies in a cyclical fashion during the cell cycle. Synthesis of the protein begins immediately after cell division and continues throughout interphase. [As cyclin accumulates it binds to Cdk1, activating the protein kinase activity of this enzyme and the active protein kinase activates the machinery that destroys cyclin.] At the end of mitosis cyclin gets tagged with ubiquitin (ubiquitin is attached to a lysine group in cyclin), additional ubiquitins get attached to the first ubiquitin and this ubiquinated cyclin gets directed to the proteasome where it is degraded.

c) No, the level of the protein Cdk1 does not change during the cell cycle. It is not synthesized and degraded in the same way as cyclin. Only its activity changes through its binding with cyclin and activation by other Cdk1-cyclin complexes.

d) Cdk1-cyclin complexes control their own activation through a positive feedback loop. As a little bit of activated Cdk1-cyclin is generated, it stimulates reactions that activate more Cdk1-cyclin. These newly activated complexes can in turn activate more Cdk1-cyclin and further accelerate the reaction. Active Cdk1-cyclin complexes increase the levels of active APC, but they do so in a delayed fashion to allow time for all of the Cdk1-cyclin complexes to become activated and to give cells time to enter mitosis. Since the Cdk1-cyclin activates the APC complex that will eventually result in the destruction of Cdk1-cyclin, it is an example of negative feedback [be generous on this part of the answer; it comes from the lecture notes and would be hard to infer the negative feedback from just the diagram].

e) It would cause the cell to enter mitosis.
2. A living cell from the lung epithilium.

The correct order (and stage) are listed as follows: E (prophase), D (prometaphase), C (metaphase), A (anaphase), F (telophase), and B (cytokinesis).

3. anticancer drugs

a) Mitosis requires that the microtubules be dynamically growing and shrinking. High concentrations of nocodazole would block the polymerization of microtubules so there would no microtubules to find and attach to the centromeres. The failure of centromeres to attach to microtubules would prevent the chromosomes from being separated and arrest mitosis. [The spindle checkpoint would be activated, which in turn prevents the activation of the anaphase promoting complex (APC), the event that triggers anaphase and exit from mitosis-from Alberts p. 624].

b) Taxol stabilizes microtubules, so that all of the tubulin monomer in the cell would be polymerized to form microtubules. Once formed, these microtubules are stable rather than dynamic, so the cell loses its ability to use the microtubules to fish for the chromosomes. As a result many of them are not captured by microtubules so the chromosomes cannot be properly segregate and mitosis is arrested. [The spindle checkpoint is also activated as described above].

c) If cells do not arrest, cytokinesis will occur even though the cell is unable to segregate its chromosomes into two identical sets. [This failure of chromosome segregation will produce tetraploid cells (when cell division fails but cells enter the next cell cycle) multinucleate cells (when the different chromosomes in a daughter cell end up too far apart to be incorporated into the same nucleus) or cells that have gained or lost chromosomes].

d) Cancerous cells are proliferating whereas most non-tumor cells are not, so cancerous cells would be more strongly affected. [Note: why cancer cells are more sensitive than other proliferating cells is not clear. One attractive idea is that they already have defects in spindle assembly and chromosome alignment that cause their genetic instability, but also make them substantially more susceptible to the effects of low concentrations of these drugs.]

4. kinetics of microtubule assembly

a) The growing ends are protected from disassembly by a GTP cap. As long it maintains this cap, a microtubule keeps growing, but if the hydrolysis of GTP on newly added subunits makes the cap disappear, the intrinsic tendency of the protofilaments of GDP-tubulin to splay apart cannot be resisted, and the microtubule begins to shrink rapidly.
b) The population of microtubules is at steady state. The fraction of the microtubules that are shrinking, the fraction that are growing, and the fraction of the tubulin inside the cell that is polymerized remain the same over time, as long as the cells stay in the same phase of the cell cycle. However, the paths for growth and contraction are different and energy is required to maintain this population of microtubules suggesting that it is not an equilibrium reaction.

OR

Individual microtubules are not in steady state since the rate of subunit addition exceeds that of subunit loss while they are growing, and vice versa as they are shrinking.

c) Yes it does, since the two ends of the molecule are chemically different, with the head of one dimer binding to the tail of the other.

d) Image A shows microtubules growing. When microtubules grow the tubulin subunits add on to a GTP cap, which acts like a ring on the top of a barrel and keeps the 13 protofilaments in a tight bundle. Image B shows microtubules shrinking. When the microtubule has lost its GTP cap, the GDP tubulin has a different structure from the GTP tubulin, which causes the protofilaments to splay out.

e) The GTP cap holds the microtubule together since GDP bound tubulin present in a microtubule is unable to free itself if it is being held in by other tubulin dimers above and below it. When GTP bound tubulin is incorporated into a microtubule it's as if a timer has begun - if another GTP bound tubulin is not placed above it before the GTP is hydrolyzed to a GDP, the shape of the tubulin at the end will change and promote disassembly.

The increase in the rate of GTP hydrolysis of a tubulin dimer incorporated into a microtubule makes the growth and shrinkage of microtubules more dynamic since it decreases the amount of time that a new GTP tubulin subunit has to add onto the polymer to maintain a GTP cap at the end.

[Note: GDP bound tubulin prefers to be a monomer rather than part of the polymer (microtubule). So the fact that the "timer" runs very slowly when GTP tubulin is a monomer promotes microtubule assembly].

5. bmyb

a) A frameshift mutation has occurred at the border between the white and gray/black region of bmyb. At the junction point, 1-2 bases have either been added or removed, resulting in a changed reading frame. This would result in all of the amino acids encoded after the mutation to be read in a different frame from the wildtype gene. It is also likely that a stop codon results earlier than in the wildtype gene – thus explaining why the mutant is shorter as well.

b) According to the introduction, bmyb is a transcription factor that regulates cyclin B mRNA synthesis. Thus it appears that bmyb is a transcriptional activator for
cyclin B synthesis. Without it, the RNA polymerase machinery cannot be properly recruited to the bmyb gene and cannot transcribe the gene.

c) Cyclin B is a mitotic cyclin that when bound to CDK, can lead to the production of an active Cyclin/CDK complex that is able to activate other proteins essential for cell cycle progression.

d) Cyclin B mRNA would be translated into cyclin B protein, and at least one round of the cell cycle could occur.

e) bmyb mRNA would also be translated into bmyb protein. This protein could then activate transcription of cyclin B and allow for cell cycle progression. Additionally dependent upon the stability of Bmyb mRNA and how Bmyb protein is regulated, it may be able to support multiple rounds of the cell cycle.