Name _____

7.06 Cell Biology
Exam #3

This is a closed book exam. You are allowed only two pages of notes, but not computers or any other types of electronic devices.

Please write your answers to the questions in pen (not pencil) in the space provided.

Be sure to put your first and last name on each page in case they become separated.

There are **12** pages to the exam. Make sure that you have a complete copy.

Remember that we will photocopy all of the exams before returning them to you.

Good luck.

Question 1. 6 points

Question 2. 20 points

Question 3. 12 points

Question 4. 10 points

Question 5. 12 points

Question 6. 15 points

Question 7. 25 points

Total. 100 points

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Question 1 (6 points)

You have identified a gene that encodes a candidate for a new Cyclin-Dependent Kinase Inhibitor (CKI). When you transfect and overexpress this gene in mammalian cells, the cells arrest: 1) They ***stop*** growing, 2) do ***not*** replicate their DNA, and 3) do ***not*** divide.

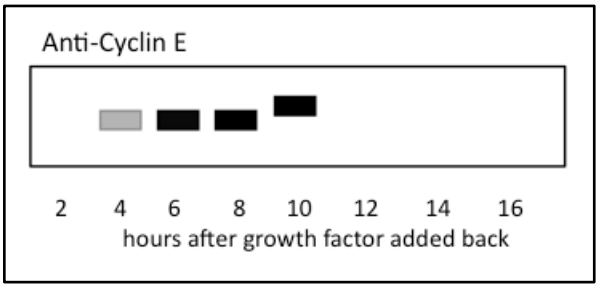
A. Which Cyclin/CDK complex is this CKI likely to affect? (2 points)

B. Using an antibody that can immunoprecipitate this CKI, how could you test your hypothesis from part A? What ***two*** criteria would you test to show that this is a CKI and that it acts on this Cyclin/CDK complex? What other reagents would you need to test these two properties? (4 points)

Question 2 (20 points)

A. What are two proteins phosphorylated by Cyclin E/CDK2 that can account for the role of Cyclin E/CDK2 in triggering the ***onset*** of S phase? (2 points)

B. You synchronize human Hela cells in early G1 by growth factor withdrawal. When growth factor is added back to the cells, they proceed synchronously through the cell cycle, spending 5 more hours in G1 before entering a 10-hour S phase. You observe the following levels of Cyclin E on a Western blot.



Explain why Cyclin E protein is undetectable after 10 hours. (2 points)

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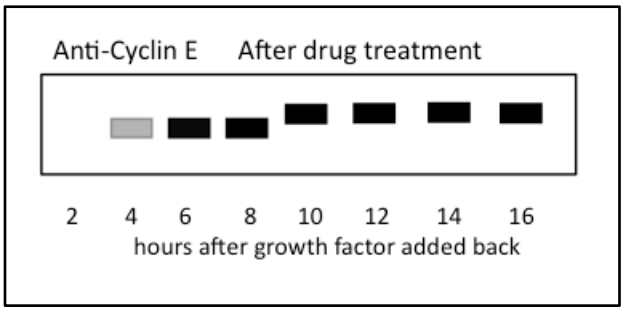
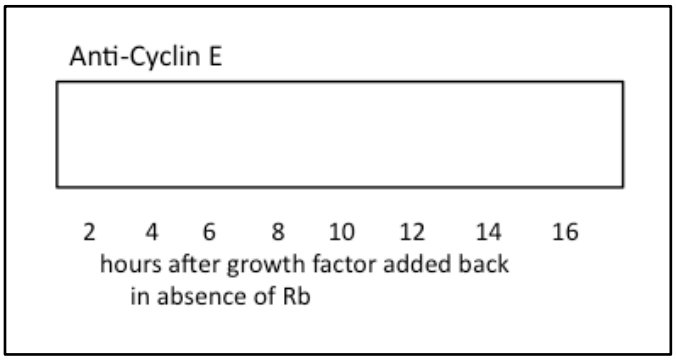
C. You do the same synchronization and release experiment, except this time you add a drug. Now you observe the following on a Cyclin E Western blot. (2 points)



What must the drug be targeting?

What is producing the minor mobility shift of this protein in the SDS-PAGE gel?

D. On the blot below, draw what you predict you would observe if you inactivated *Rb* (using a homozygous conditional allele) at the same time as growth factor is added back. (4 points)



E. You have a human HeLa cell line in which both gene copies of *cyclin E* have a conditional allele that is inactive at 40°C, but active at 35°C. You can measure whether these cells undergo DNA replication based on BrdU incorporation. You synchronize the cells by growth factor withdrawal and release them back into G1 by adding back the growth factor, as in part (A). If you release them at 35°C, you observe BrdU incorporation after 6 hours. If you release them at 40°C andmaintain them at this temperature, you never observe BrdU incorporation. **Explain**. (2 points)

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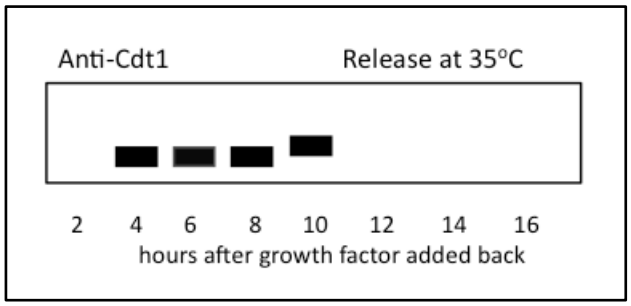
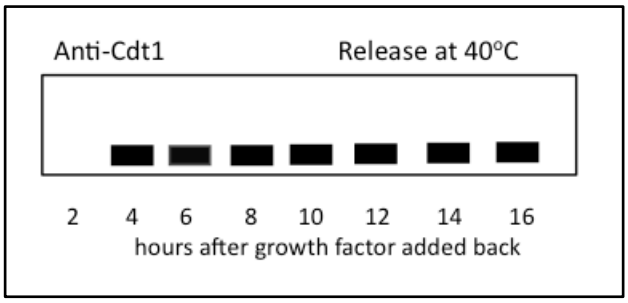
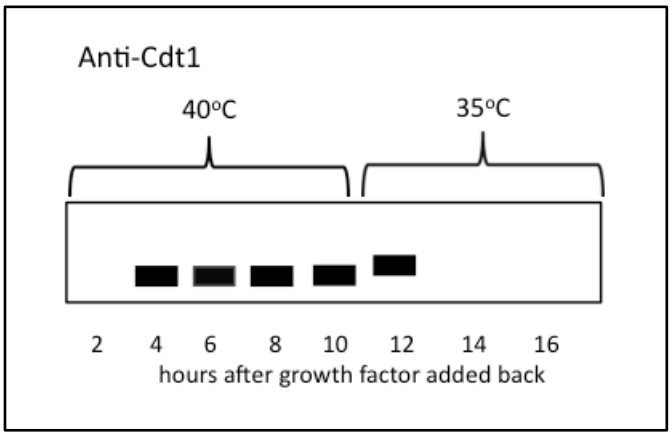
F. You do the same synchronization and release experiment with the *cyclin E* mutant HeLa cells described in (E), but now you monitor Cdt1 levels by Western blot.





Explain what is occurring with Cdt1, the difference between the two blots, and what you conclude about Cyclin E function. (4 points)

G. Now you synchronize and release the *cyclin E* mutant Hela cells at 40°C, but after 10 hours shift down to 35°C. You observe BrdU incorporation only after 10 hours and see the following on a Cdt1 Western blot. (4 points)



i) What does this tell you about when DNA replication starts under these conditions?

ii) What does this tell you about the timing of Cyclin E's control of S phase?

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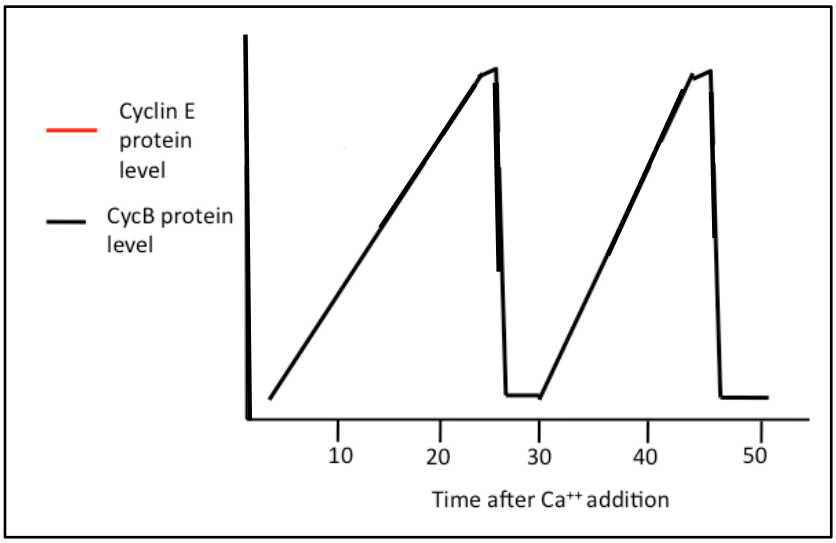
Question 3 (12 points)

Using extracts from Xenopus eggs that cycle, it is possible to observe rapid S-M cycles of added nuclei. If ***all*** the mRNAs in these extracts are degraded by RNAse treatment, no cycling occurs. However, adding back ***solely*** the mRNA for Cyclin B is sufficient to restore cycling.

A. On the graph below, draw the levels of Cyclin E protein in these S-M cycling extracts. (3 points)



B. What is the ***paradox*** between the regulation of S phase and the initiation of DNA replication in the normal cell cycle versus these embryonic cycles? (4 points)

C. In the embryos as well as in these extracts, reformation of the nuclear envelope and nuclear structure is essential for DNA replication to occur, and Cyclin E is observed to be concentrated in the reformed nuclei. ***Explain*** how this resolves the paradox**.** What additional protein that regulates origin firing would you expect to be concentrated in the nuclei? (5 points)

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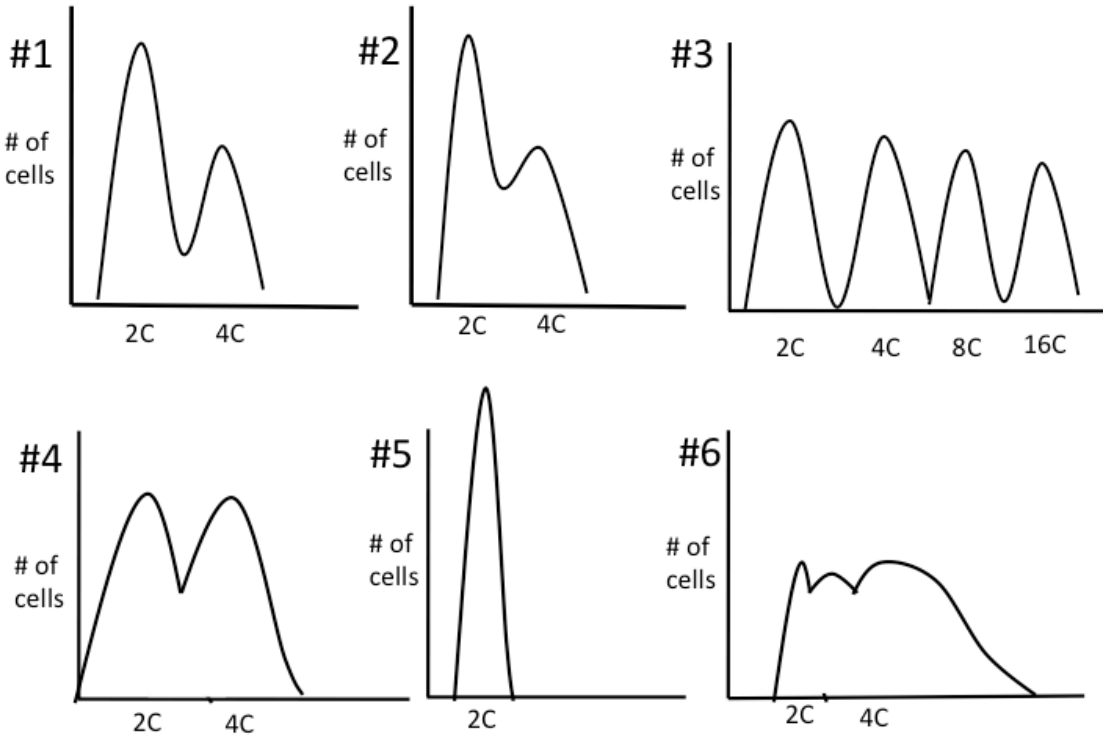
Question 4 (10 points)

For each perturbation below, assume that you can synchronize a mammalian cell culture in G1, replace the endogenous wild type protein with the indicated mutants, and release the cells into the cell cycle. Profile #1 is what would be observed with this synchronization and release, but no perturbation. **For each mutant, indicate the FACs profile you would expect to observe after 48 hours. Briefly explain your reasoning.** (2 points each)



A. Sld2 with serine phosphorylated by Cyclin E/CDK2 changed to aspartic acid

B. CAK loss of function

C. SCF loss of function

D. A triple mutant of loss of function Cyclin A, Cyclin B, and CDK1

E. SMC2 loss of function

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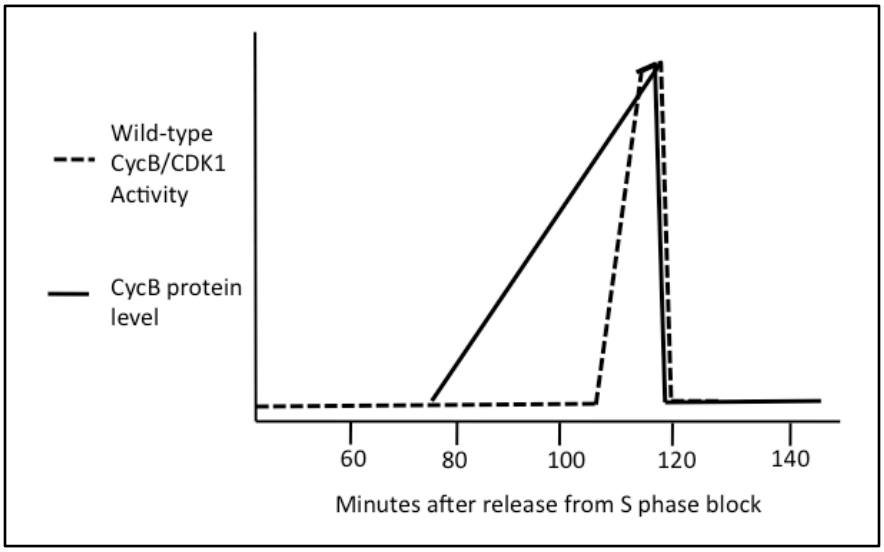
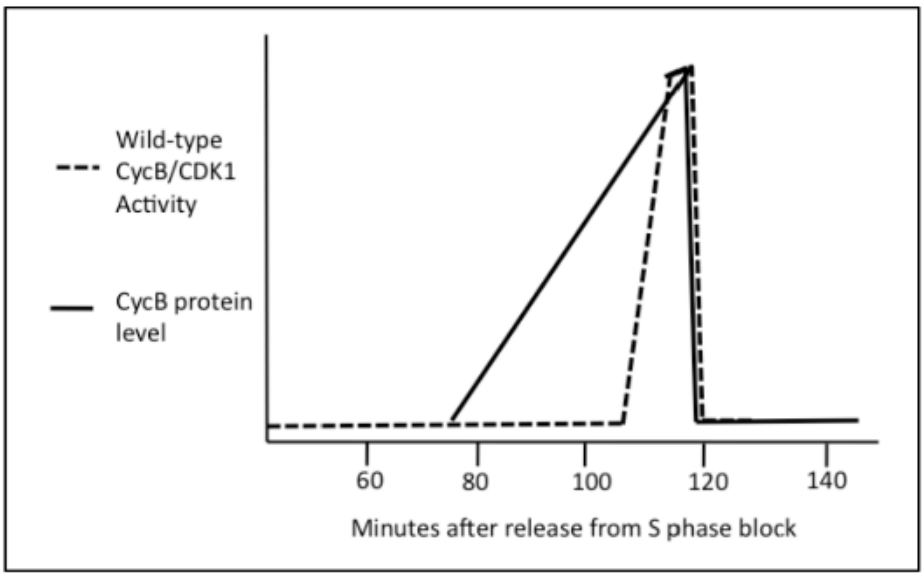
Question 5 (12 points)
Fission yeast has a cell division cycle of about 3 hours, and this yeast does most of its growth in G2. You have a conditional mutant that arrests in S phase at the non-permissive temperature and you use this mutant to synchronize the culture. You then shift the culture to the permissive temperature and measure Cyclin B protein levels and Cyclin B/CDK1 kinase activity.



A. Why aren't the two curves the same? (3 points)

B. On the graph below, ***draw*** what the CyclinB/CDK1 kinase activity will look like in a *wee1* loss of function mutant. (3 points)

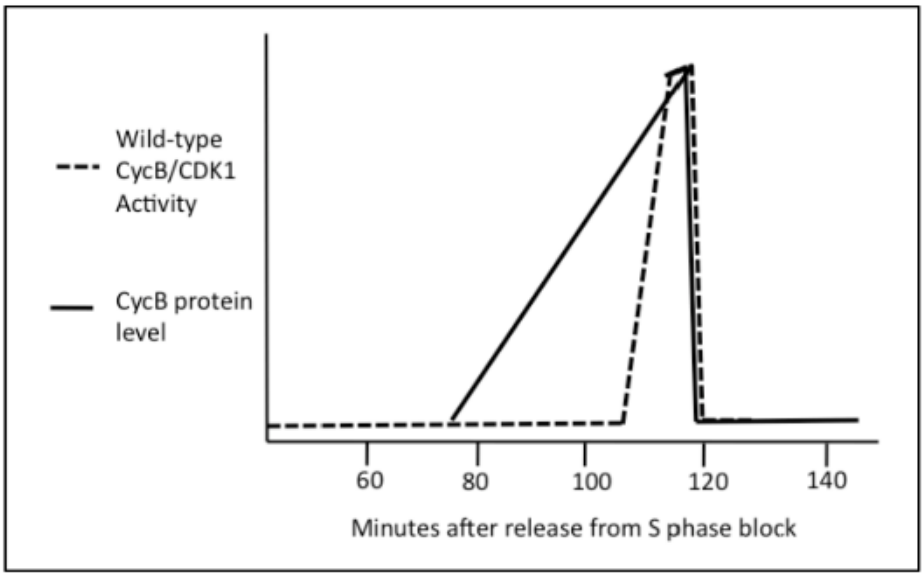
Name _____



C. You have a Wee1 mutant in which Serine 123 (the residue phosphorylated by Cyclin B/CDK1) is changed to aspartic acid. (3 points)

• State your predictions for how this mutation will affect Wee1 activity.

• On the graph below, ***draw*** what the CyclinB/CDK1 kinase activity will look like in this mutant:



D. Cdc25 is subject to multiple regulatory inputs. You have a Cdc25 mutant in which Threonine 48 and 67 (the two residues phosphorylated by Cyclin B/CDK1) are changed to alanine. (3 points)

• Given that Cdc25 is subject to multiple regulatory inputs, state your prediction for how these mutations will affect Cdc25 activity

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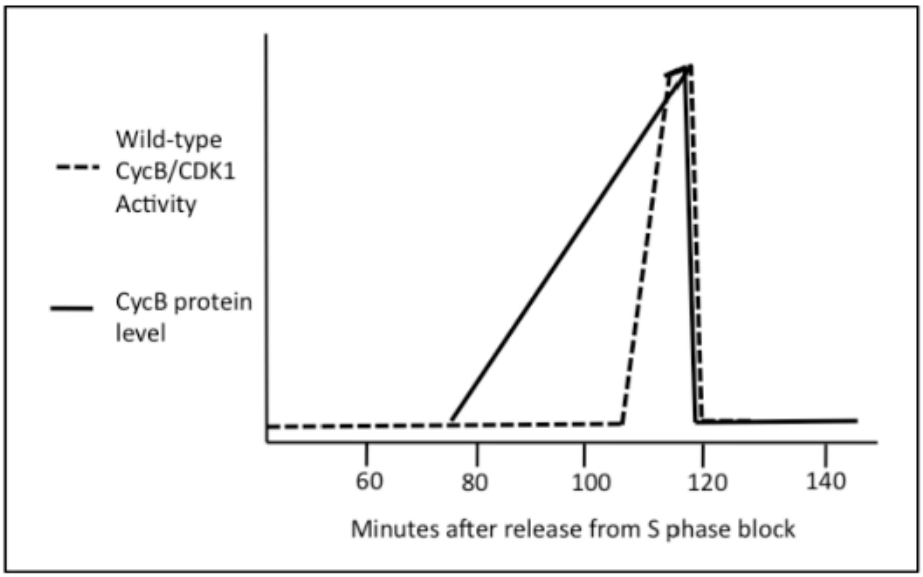
• On the graph below, ***draw*** what the Cyclin B/CDK1 kinase activity will look like in this mutant:



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Question 6 (15 points)
In class, we discussed three different assays that measure aspects of actin assembly:

1. The bulk pyrene actin assembly assay
2. A TIRF-based assay to visualize actin assembly
3. Listeria motility in cell extracts.

For each of the proteins listed below, **indicate "Yes" or "No"** for whether you would be able to visualize their activities in these assays. For each "Yes", additionally **describe the nature of the change**.

A. Pyrene Assay. For the pyrene assay, indicate (Yes/No) for whether the ***addition*** of the protein to an assembly reaction containing ATP, actin, buffer, and ActA would have a detectable effect. For any "Yes", indicate whether polymerization will be "Increased" or "Decreased".

	Pyrene Assay	If "Yes", indicate nature of the change
Arp2/3 complex	Yes / No	Increased / Decreased
Capping protein	Yes / No	Increased / Decreased
Fimbrin	Yes / No	Increased / Decreased
Formin	Yes / No	Increased / Decreased
Myosin	Yes / No	Increased / Decreased

B. TIRF Assay. For the TIRF assay, indicate (Yes/No) for whether the addition of the protein to an assembly reaction containing ATP, actin, buffer, and ActA would have a detectable effect. For any "Yes", describe any visual changes to the actin filaments in their length and/or organization

	TIRF Assay	Nature of visual change to actin filaments
Arp2/3 complex	Yes / No	Shorter filaments (due to increased nucleation), more total polymer, highly branched
Capping protein	Yes / No	Shorter filaments (and less polymer)
Fimbrin	Yes / No	Bundled/Crosslinked Filaments
Formin	Yes / No	Longer filaments, more of them (more nucleation)
Myosin	Yes / No	(if yes and considering a tetrameric myosin, okay to say bundled)

C. Listeria Motility Assay. For the Listeria motility assay, indicate (Yes/No) whether depletion (removal) of the protein from cell extract would have a detectable effect. For any "Yes", indicate whether the rate of movement will be "Increased" or "Decreased".

	Listeria Motility	If "Yes", indicate nature of the change
Arp2/3 complex	Yes / No	Increased / Decreased
Capping protein	Yes / No	Increased / Decreased (counterintuitive, but needed to focus polymerization at the surface)
Fimbrin	Yes / No	Increased / Decreased
Formin	Yes / No	Increased / Decreased
Myosin	Yes / No	Increased / Decreased

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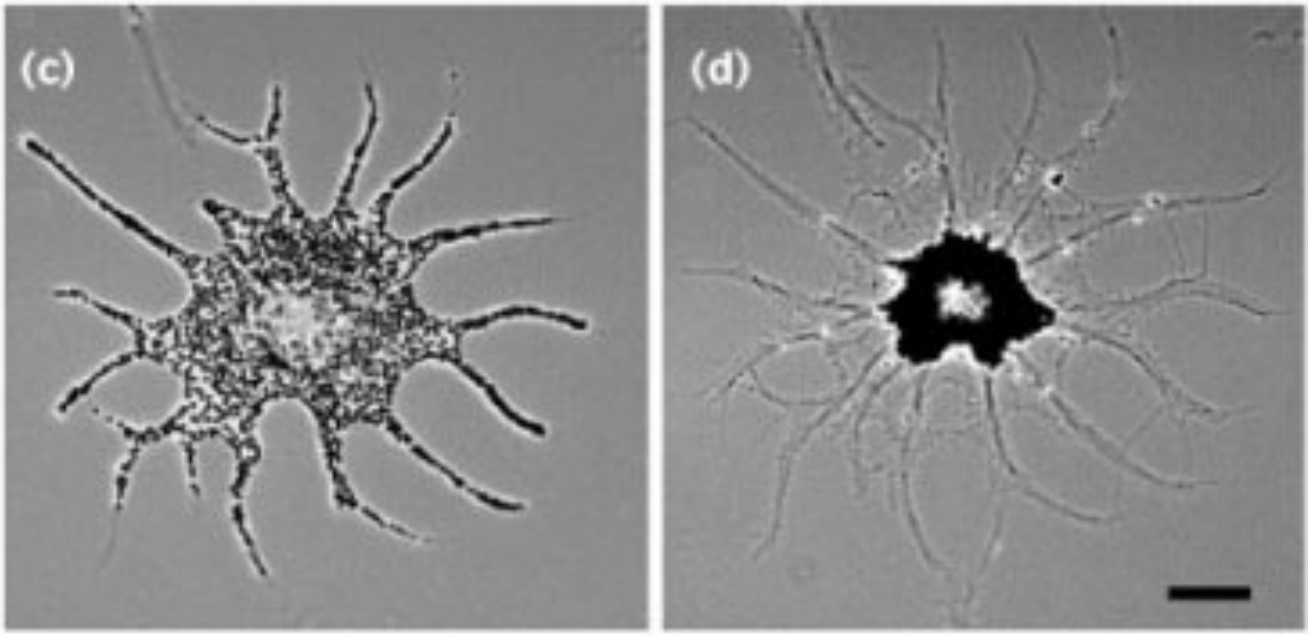
Question 7 (25 points)

Melanophores are cells found in some frogs and fishes that allow the organism to dynamically change its coloration for camouflage. This change requires the movement of pigment-containing vesicles, called melanosomes, around an *interphase* cell. For the cell to become darker, the melanosomes must move over longer distances outward from the center of the cell. To become lighter, the melanosomes move inward to the center of the cell.

Melanosomes dispersed throughout the cell **Melanosomes aggregated in the cell center**



A. Previous work on these melanosomes indicated that they ***primarily*** move along microtubule polymers, but not actin (in cells lacking microtubules, melanosomes no longer move). Based on what you know about the properties and organization of these cytoskeletal elements, explain why this would make sense. (3 points)

B. Describe two potential mechanisms by which the melanosomes could move outward towards the periphery of a cell. Be as specific as possible for the mechanisms of force production and the identity of any proteins involved. (2 points)

C. Describe two potential mechanisms by which the melanosomes could move inward towards the center of a cell. Be as specific as possible for the mechanisms of force production and the identity of any proteins involved. (2 points)

D. Propose ***two*** experiments that would each (independently) allow you to quickly and unambigiously distinguish between the models you proposed above for the mechanism of force production for ***inward*** movement from part C (for example, with a perturbation affecting one mechanism, but not the other). (4 points)

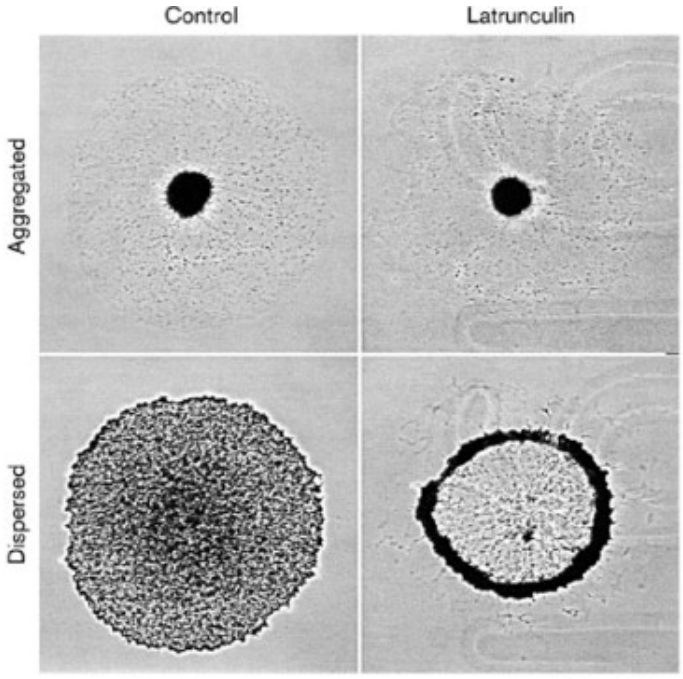
E. To change an organism's color and appearance, the distribution of the melanosomes within the cell must change in response to upstream signals. Assume that the parameters of microtubule dynamics are ***not*** altered by these signals. Propose a model for how these vesicles would switch the directionality of their motility is response to specific signals. (3 points)

F. You are analyzing the requirements for melanosome movement in a cell in which the melanosomes are initially distributed to the cell periphery (edge). At the beginning of the experiment, signaling is altered to cause the melanosomes to move to the center. At the start of the experiment, you also overexpress and activate **Katanin** (assume that this occurs instantaneously). Indicate the most likely effect on melanosome distribution. ***Briefly*** explain your reasoning. (3 points)

G. In the same experiment as Part F, you instead overexpress and activate the tetrameric kinesin-5 (Eg5). Indicate the most likely effect on melanosome distribution. ***Briefly*** explain your reasoning. (3 points)

In cells lacking microtubules, melanosomes no longer move, consistent with a primary role for microtubules in their distribution. However, some researchers have proposed a role for actin in observed final melanosome distribution. For the experiment below, cells were first induced to either

aggregate or disperse their melanosomes (left column). They were next treated with the actin depolymerizing drug Latrunculin A. Note that Latrunculin treatment will decrease cell size slightly.



H. Based on these data, what is the effect of latrunculin treatment on melanosome distribution? (2 points)

I. Provide a model to explain this behavior. (3 points)