Name:_____

TA Name and Recitation Time:
_____

7.06 Cell Biology
QUIZ #4

This is an **open book** exam, and you are allowed access to books and notes, but not computers or any other types of electronic devices.

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

Please write only on the **FRONT SIDE** of each sheet.

And be sure to put your **name on each page** in case they become separated.

There are **10 pages** to the quiz, make sure you have a complete copy!

Remember that we will Xerox all of the quizzes.

Good Luck!

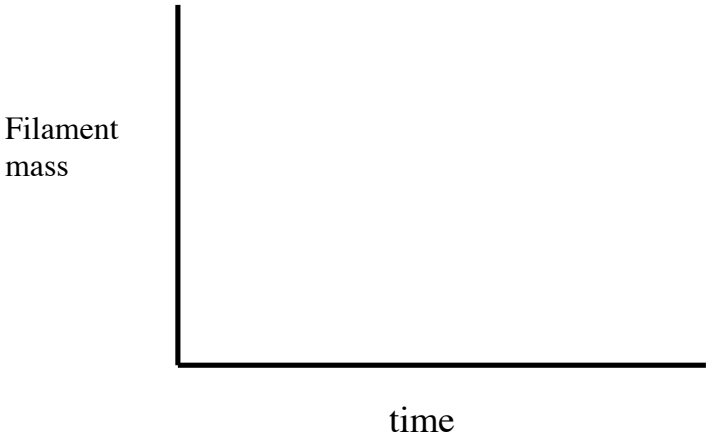
Question 1.	25 pts _____
Question 2.	20 pts _____
Question 3.	30 pts _____
Question 4.	25 pts _____

Name:_____

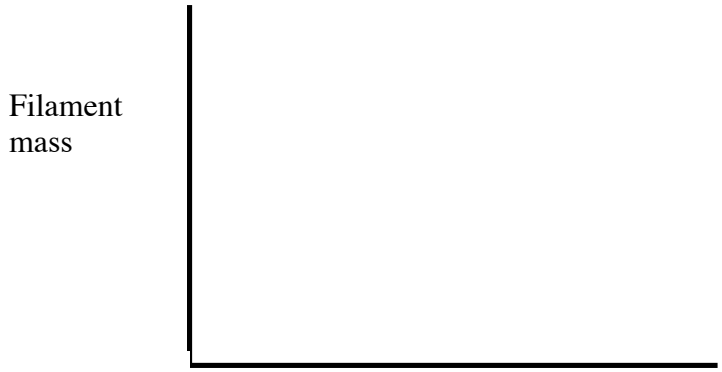
Question 1. (25 points)

You are interested in studying actin dynamics. Your advisor gives you purified G actin-ATP monomers and two proteins she is convinced affect actin dynamics. She also provides you with the Myosin S1 protein fragment.

1a. (2 points) Draw the profile you observe when you add an actin nucleator seed of polymerized actin to a G-actin-ATP solution at a concentration above the critical concentration (Cc) for the - end. Assume the time course goes long enough that equilibrium is reached.



1b. (5 points) Draw the profile you observe when you add actin nucleator seed of polymerized actin to a G-actin-ATP solution at a concentration between the Cc for the + end and the Cc for the – end.



What is the term for what is observed?

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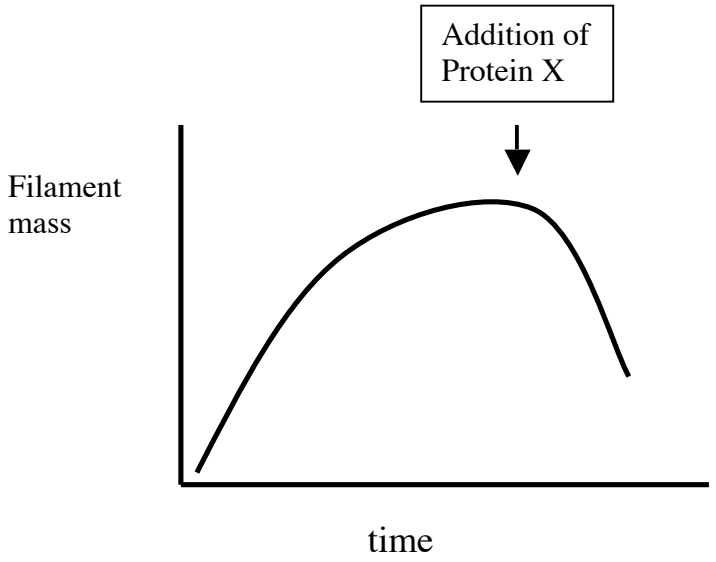
1c. (9 points) When you add Protein X to a reaction with an actin nucleator seed of polymerized actin and G-actin-ATP at a concentration above the critical concentration (Cc) for the – end you observe the following:

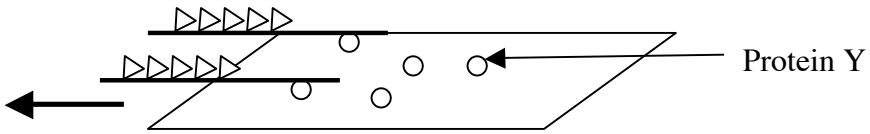


Propose two possible functions for Protein X and design an experiment to distinguish these functions.

1d. (3 points) When you add Protein Y you do not observe an effect on filament mass. You show by co-immunoprecipitation that Protein Y will bind to actin microfilaments but not to monomers. When you attach Protein Y to a microscope slide and then add fluorescently labeled microfilaments you observe they glide across the microscope slide. **Explain this observation and the function of Protein Y.**

Name:_____

1e. (6 points) You coat microfilaments with the myosin S1 fragment and add these coated microfilaments to the slide with Protein Y stuck on a slide. You observe the coated microfilaments move, and they move in the direction with the barbed ends leading.



Microfilament movement

Your advisor is ecstatic with this observation, telling you that you have made a major discovery. **What is novel about this discovery?**

Question 2. (20 points)

You recover a mouse mutant in which the skin is severely blistered. The mutation maps near the gene encoding the alpha6 integrin protein.

2a. (5 points) You make a cell line from the skin epithelial cells of this mutant mouse. **What are two methods you could use with these cells to test whether the mutant phenotype could be the consequence of mutation of this integrin gene? Explain the predicted results for each approach.**

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2b. (5 points) Why would a mutation in integrin cause blistering of the skin? What other proteins would you expect to produce this phenotype when mutated and why?

2c. (5 points) In a wild-type mouse if you irradiate the skin with UV light to produce melanoma skin cancer it is rare for it to metastasize to the lung, but after irradiation of the blistered mutants with UV light the mice readily develop lung cancer from the melanomas. **Explain this observation.**

2d. (5 points) You have a different mouse that has an autoimmune disorder with antibodies against the RGD peptide. **Would you expect it to be more or less susceptible to metastasis than wild type and why?**

Question 3. (30 points)

You are an oncologist asked to treat two patients with early stage prostate cancer. You decide to evaluate the status of candidate oncogenes and tumor suppressor genes and make interesting observations when testing Cyclin D.

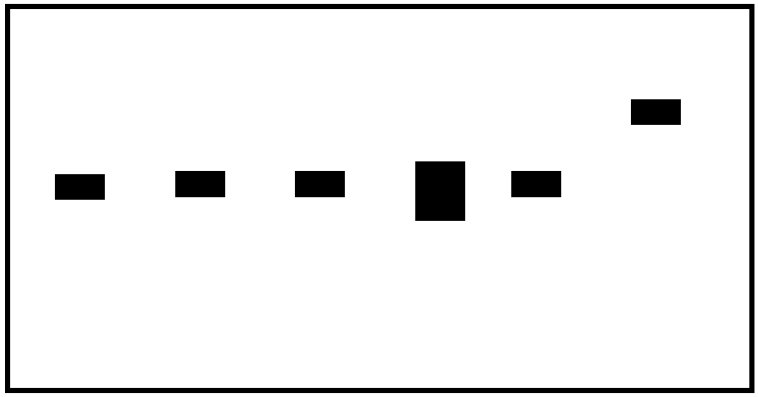
3a. (10 points) Genomic DNA is isolated from blood or prostate. A Southern blot of genomic DNA cut with a restriction enzyme that cuts rarely and hybridized with a probe for Cyclin D shows the following result:

Unaffected male
Blood Prostate

Patient #1
Blood Prostate

Patient #2
Blood Prostate

Name: _____



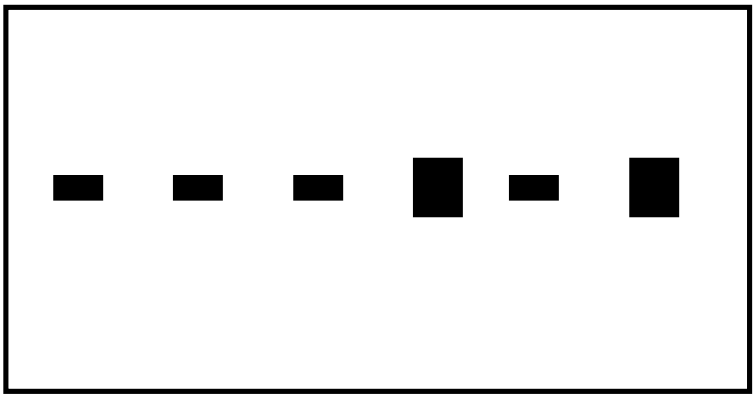
Direction of migration

A Western blot shows the following:

Unaffected male
Blood prostate

Patient #1
Blood Prostate

Patient #2
Blood Prostate



Propose a molecular explanation for the change in the cyclin D gene in each of the patients, explaining how each could account for the prostate cancer. Explain how your model accounts for the Western result.

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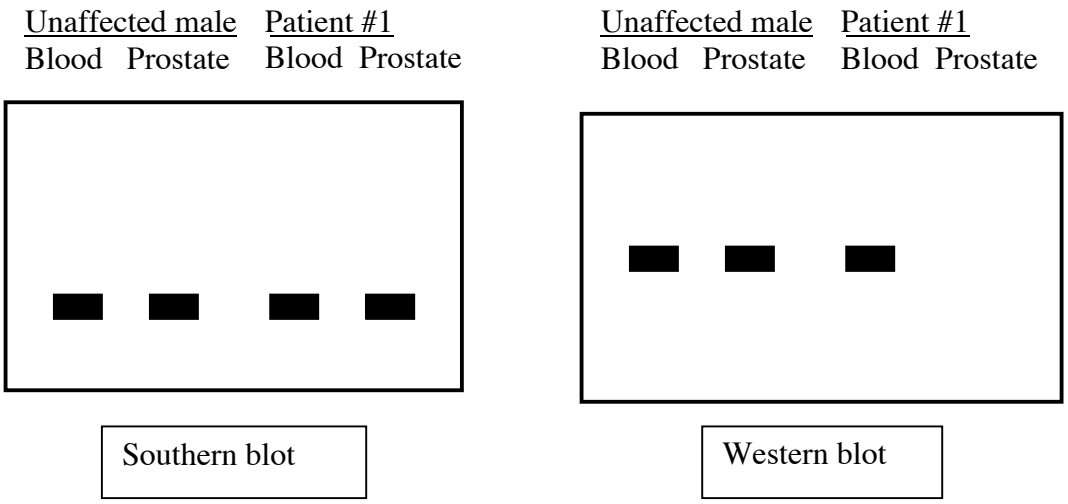
3b. (3 points) Your patients ask whether their sons are likely to be predisposed to prostate cancer. **For each patient do you answer yes or no, and why?**

3c. (8 points) During the course of a year following these patients, the tumor in patient #1 suddenly becomes much larger. You test the p53 gene and protein in this patient by Southern and Western blots.



What type of mutation is consistent with the above data? Propose two reasons why such a change is correlated with the large size of the tumor.

3d. (3 points) Do you think it would be good to treat patient #1 with a drug that blocks blood vessel formation by angiogenesis? Why or why not?

3e. (3 points). One of your colleagues has identified a chemical that causes extensive DNA damage that leads to death of proliferating cells. **Would this be helpful as a chemotherapy agent for patient #1? If yes, explain why. If no, explain what properties of a chemotherapy agent are needed.**

3f. (3 points) Your colleague also has a chemical that will induce apoptosis even in cells lacking p53 and that can be specifically delivered to tumors. **Would this be a positive therapy for patient #1? Why or why not?**

Question 4. (25 points)

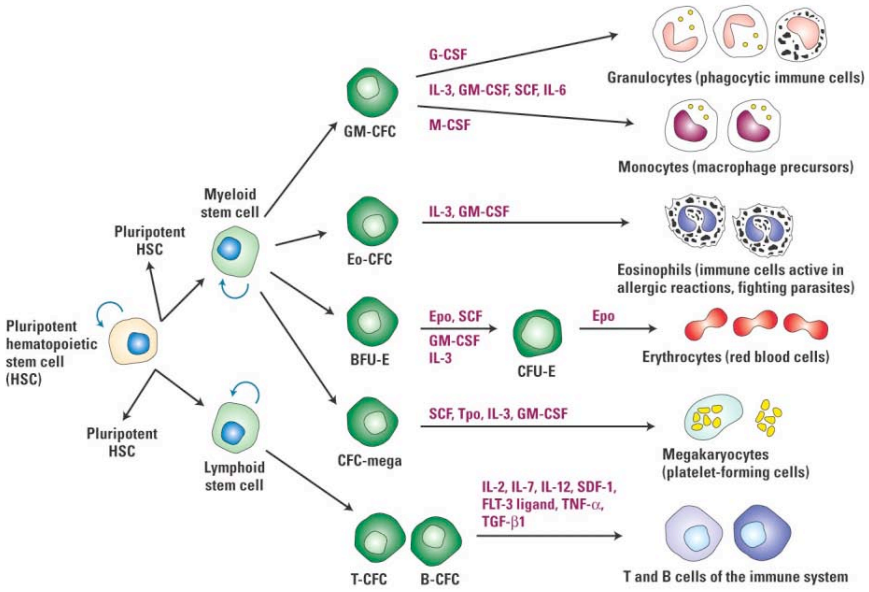
Recent research suggests that some cancers may be caused by unregulated division within populations of stem cells. There is evidence for breast cancer, lung cancer, and leukemia stem cells. You decide to use a mouse model to test whether leukemia is caused by transformed stem cells in the hematopoietic lineage.

4a. (2 points) You have a mouse strain with the bcr-abl translocation that has chronic Chronic myelogenous leukemia (CML). Occasionally one of these mice develops a more severe acute CML. **What is the likely explanation for the change from chronic to acute CML?**

4b. (4 points) You take a mouse with acute CML, irradiate it to eliminate the bone marrow cells, and inject bone marrow from a wild-type mouse. The irradiated mouse recovers and no longer shows signs of leukemia. **Explain why the mouse recovered**

with the donor bone marrow and why its leukemia is in remission. Does this experiment show that the leukemia is caused by stem cells?

4c. (8 points) You isolate myeloid and lymphoid stem cells using antibodies to known cell surface markers and FACS to purify cells.



You grow the lymphoid stem cells in culture media that causes them to maintain a pattern of asymmetric divisions. You plate 200 lymphoid stem cells.

After four division cycles, how many lymphoid stem cells do you have?

How many T cell or B CFC cell progenitors do you have?
(assume they divide at the same rate as the stem cells)

4d. (7 points) You make your bcr-abl mutant mouse transgenic such that GFP is expressed in each of its cells under the alpha-tubulin promoter. You purify lymphoid

stem cells from this mutant mouse and myeloid stem cells from an unlabeled, wild-type mouse. When you inject these two populations of stem cells into an irradiated wild-type mouse you observe that it gets leukemia, and the transformed B cells express GFP. **Explain your conclusions from this experiment.**

4e. (4 points) Your oncologist friend who is treating a patient with human CML wants to give the patient high doses of irradiation and then a bone marrow transplant. To avoid immune rejection he has decided to collect the patient's own bone marrow prior to irradiation and then use this for the transplant after irradiation. **Based on your experiments what advice do you give him about his proposed therapy and what is your reasoning?**