BCDB QUALIFYING EXAM – PART I **Student X**
June 1, 2010
Question 1

Cancer cell metabolism is crucial for tumorigenesis and tumor growth. The Warburg effect describes a pro-oncogenic metabolism switch such that cancer cells take up more glucose than normal tissue and favor incomplete oxidation of glucose even in the presence of oxygen. Using a proteomic approach to identify phosphoproteins, you have identified a metabolic enzyme, pyruvate dehydrogenase (PDH), which is tyrosine phosphorylated in cancer cells that are transformed with an oncogenic receptor tyrosine kinase, fibroblast growth factor receptor 1 (FGFR1). PDH is located on mitochondria and converts pyruvate to acetyl- CoA, the initial substrate for the Krebs/TCA cycle. PDH activity should be inhibited in cancer cells in order to switch the cancer cell metabolism from oxidative phosphorylation to glycolysis. However, tyrosine phosphorylation of PDH has never been reported or connected with human cancers.

You have two cell lines in hand. One is a human leukemia cell line KG-1 that expresses a constitutively active, ligand-independent fusion tyrosine kinase termed FOP2-FGFR1 and the other one is a human lung cancer cell line H1299 that expresses full length FGFR1. You also have a PDH enzyme activity assay, a FGFR1 kinase activity assay in hand with purified, active recombinant FGFR1 fragment, and cDNA constructs of wildtype and control kinase dead human FGFR1 as well as human and mouse PDH constructs in hand.

a) Design one experimental approach to test the hypothesis that FGFR1 directly phosphorylates PDH and affects PDH enzyme activity. Be sure to include appropriate controls necessary to make a firm conclusion. **(4 points)**

b) Assume you find that oncogenic FGFR1 regulates PDH enzyme activity by phosphorylation at one tyrosine phosphorylation site. Based on the biology described for the cancer cells, do you think phosphorylation at this position is likely to activate or inhibit PDH? Provide a rationale for your conclusion. **(2 points)**

c) You now want to test the hypothesis that phosphorylation at this site provides a metabolic advantage for cancer cells to proliferate and form tumors both in cultured cells and in animals. Describe one approach to test this hypothesis in the cultured cell model and one approach to test this hypothesis in an animal model. Be sure to include all necessary controls and clearly describe possible experimental outcomes and interpretations. Please keep in mind that PDH is globally expressed in diverse tissues. **(4 points)**

BCDB QUALIFYING EXAM – PART I **Student X**
June 1, 2010
Question 2

Your favorite enzyme is encoded by a gene in mouse (*YFG1*) that is expressed in all tissues, but that you suspect may be imprinted. You have access to the relevant mouse gene sequence, and you also know that the coding region of *YFG1* is polymorphic, with at least 1 silent coding sequence variation between C57BL/6J mice and DBA/2J mice.

a) Using the information given above, design a straightforward experiment to test whether *YFG1* is imprinted in mouse. Explain your overall strategy and also any important controls. **(4 points)**

b) What will your data look like if *YFG1* is maternally imprinted? paternally imprinted? Explain. **(3 points)**

c) What will your data look like if *YFG1* is <u>not</u> imprinted? Explain. **(3 points)**

BCDB QUALIFYING EXAM – PART I **Student X**
June 1, 2010
Question 3

Working in budding yeast (*S. cerevisae*), you have identified two temperature sensitive cell cycle mutants that you call *cdcX* and *cdcY*. To determine the phase of the cell cycle at which each mutant arrests when shifted to the non-permissive temperature, you use the drug hydroxyurea (HU) which creates a block to DNA synthesis by inhibiting the enzyme ribonucleotide reductase (Rnr) and thus reducing the amount of dNTP precursors available for DNA replication. Adding high levels of HU will block cells at any point in S-phase.

You begin your experiments with the *cdcX* mutant. The *cdcX* mutant is first grown at the non-permissive temperature of 37°C for 2 hr (the approximate length of the yeast cell cycle) to allow expression of the phenotype. The *cdcX* cells are then shifted to the permissive temperature of 20°C in the presence of HU and grown for another 2 hr. You score the total number of cells present at T_0 (which is following the two hour shift to 37°C) and end of this protocol as well as in control *cdcX* cultures maintained at 20°C in the absence of HU for 4 hr (see table below). You then reverse the order of the experiment, culturing *cdcX* cells at 20°C in HU for 2 hr, then at T_0 moving them to 37°C in the absence of HU for 2 hr.

cdcX cell counts (as cells/µl)

treatment	**T_0**	**End of protocol**
Control (4 hr, 20°)	113	458
37° (2hr) → 20° + HU (2hr) T_0	111	227
20° + HU (2hr) → 37° (2hr) T_0	115	116

a) At the non-permissive temperature, where in the canonical cell cycle (ie G_1-S-G_2-M) do *cdcX* cells arrest? Explain your conclusion based on the data above and name one gene that when mutated could give this phenotype. **(4 points)**

b) You next repeat the identical experiments with the *cdcY* strain and obtain the following results:

cdcY cell counts (cells/µl)

treatment	**T_0**	**End of protocol**
Control (4 hr, 20°)	118	472
37° (2hr) → 20° + HU (2hr) T_0	114	117
20° + HU (2hr) → 37° (2hr) T_0	117	116

At the non-permissive temperature, where do *cdcY* cells arrest? Explain your conclusion based on the data shown in the Table above. **(4 points)**

Continued on next page

c) You cross your two mutant strains to obtain a haploid double *cdcXcdcY* temperature sensitive mutant. Starting with a non-synchronous culture growing at 20°C, you shift the cells to 37°C, wait 2 hr and score cell number as before. Choose which of the results below (A, B, or C) best fit what you would expect to observe? Explain your conclusion. **(2 points)**

***cdcXcdcY* cell counts** (cells/μl)

	T_0	After 2 hr
A	100	101
B	100	203
C	100	122

BCDB QUALIFYING EXAM – PART I **Student X**
June 1, 2010
Question 4

It has been a long day in the lab but finally you develop your western blot and study it intently to see what your efforts have yielded. As you suspected from your immunofluorescence experiments, your novel protein, NVL, coimmunoprecipitates with tubulin. As you ponder the possibilities, you decide that you want to determine whether this protein might be involved in regulating tubulin assembly.

a) Describe one approach to test the hypothesis that NVL is required for efficient assembly of microtubules in cultured cells. This experiment should be performed in live mammalian cells. Be sure to include proper controls, techniques you would use to obtain results, interpretations, and the limitations of your approach. **(3 points)**

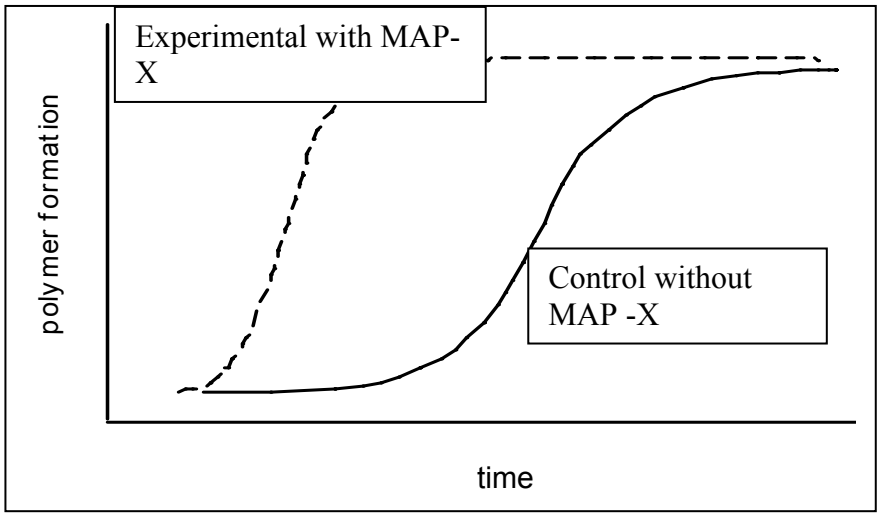
b) You think NVL may be regulated by phosphorylation. How would you definitively determine whether NVL is phosphorylated? Keep in mind that NVL is the same molecular size as alpha tubulin. Also, for this part of this question, assume that you do NOT have any idea of where NVL might be phosphorylated. **(3 points)**

c) It turns out that the localization of NVL varies in mammalian cells depending on growth conditions. It is located in the cytoplasm in growing cells but enters the nucleus when cells are serum starved and enter G_0. You wonder if phosphorylation could regulate NVL subcellular localization. Propose a model to explain the mechanism underlying how the subcellular localization of NVL might be regulated by phosphorylation **(1.5 points)** and give an example of a cellular protein regulated in this manner **(0.5 points)**.

d) Assuming that NVL has one site of phosphorylation, that you know the phosphorylation site and that you have the necessary reagents, describe briefly how you would test whether phosphorylation of NVL alters its subcellular localization in mammalian cells. Briefly discuss controls, including testing for functional protein if you use a mutated protein. **(2 points)**

BCDB QUALIFYING EXAM – PART I **Student X**
June 1, 2010
Question 5

A novel microtubule associated protein, protein MAP- X, has been identified, the protein purified and the gene cloned. Moreover, MAP- X is over-expressed in malignant cancer cells. It is postulated that MAP - X controls microtubule assembly. Illustrated in the Figure below are the results of the time course of microtubule assembly from tubulin dimers *in vitro* either in the presence or absence of MAP - X. Polymer formation was measured in the spectrophotometer using a turbidimetric assay that measures light scattering. (Assembly was performed at 37°C in a PIPES buffer, pH 6.9, containing 1 mM GTP, 2 mM $MgCl_2$, and 0.5 mM EGTA.)



a) Label the phases of microtubule assembly in both graphs, and propose a mechanism for control of microtubule assembly by MAP-X. **(4 points)**

b) Design an experiment to localize MAP- X in the cell and discuss the limitations of the technique you propose. **(3 points)**

c) Based on the data in the Figure above and your answer in part [a], propose where MAP-X is localized in non-malignant cells. Explain your answer. **(3 points)**

BCDB QUALIFYING EXAM – PART I **Student X**
June 1, 2010
Question 6

High throughput technologies have been underway for several years now and include both yeast two-hybrid screening and tandem affinity protein (TAP) or co-IPs for each predicted ORF in an organism's proteome. These strategies have been successful in identifying a large number of predicted protein-protein interactions. However, an examination of the datasets from these strategies reveals them to be unsuccessful in identifying any of the mediators or effectors of regulatory GTPases; including the guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs), and downstream mediators of GTPase signaling (effectors).

(a) Explain the most likely reason for the failure of these approaches to correctly identify GTPase partners. Include in your answer a brief description of the two approaches described above. **(4 points)**

b) Propose a strategy to overcome this limitation that may allow you to use these two methods to identify binding or interaction partners of GTPases. **(2 points)**

c) In general, what is a primary limitation on these two techniques in their ability to identify all biologically relevant binding partners for any given protein (not limited to GTPases). **(2 points)**

d) Although both these methods have been employed to analyze some of the same targets in proteomic studies designed to identify all interacting partners, they often do not identify the same exact spectrum of partners. Drawing on your knowledge of the two methods, propose a reason for the differences in partners identified by the two methods. **(2 points)**

BCDB QUALIFYING EXAM – PART I **Student X**
June 1, 2010
Question 7

The human *MYH9* gene encodes a non-muscle myosin heavy chain that belongs to a class of myosin II (a conventional myosin that forms bipolar filaments). A number of mutations in the *MYH9* gene have been found in patients with defective platelets that fail to change shape upon thrombin stimulation. Your collaborator found two new missense point mutations in *MYH9* and asked you to investigate why these mutations cause disease. Mutation A is mapped to the motor domain and causes disease as either a heterozygous or homozygous mutation. Mutation B maps to the coiled-coil domain and causes disease only when the mutation is homozygous. You have been successful in setting up systems to express and purify any mutants or fragments of myosin. Antibody against *MYH9* myosin heavy chain is available and works for immunofluorescence, Western blot, ELISA, and immunoprecipitation.

a) Briefly describe the function of the motor domain of myosin heavy chain, and design one experiment to test how the mutations described above could affect this function. **(3 points)**

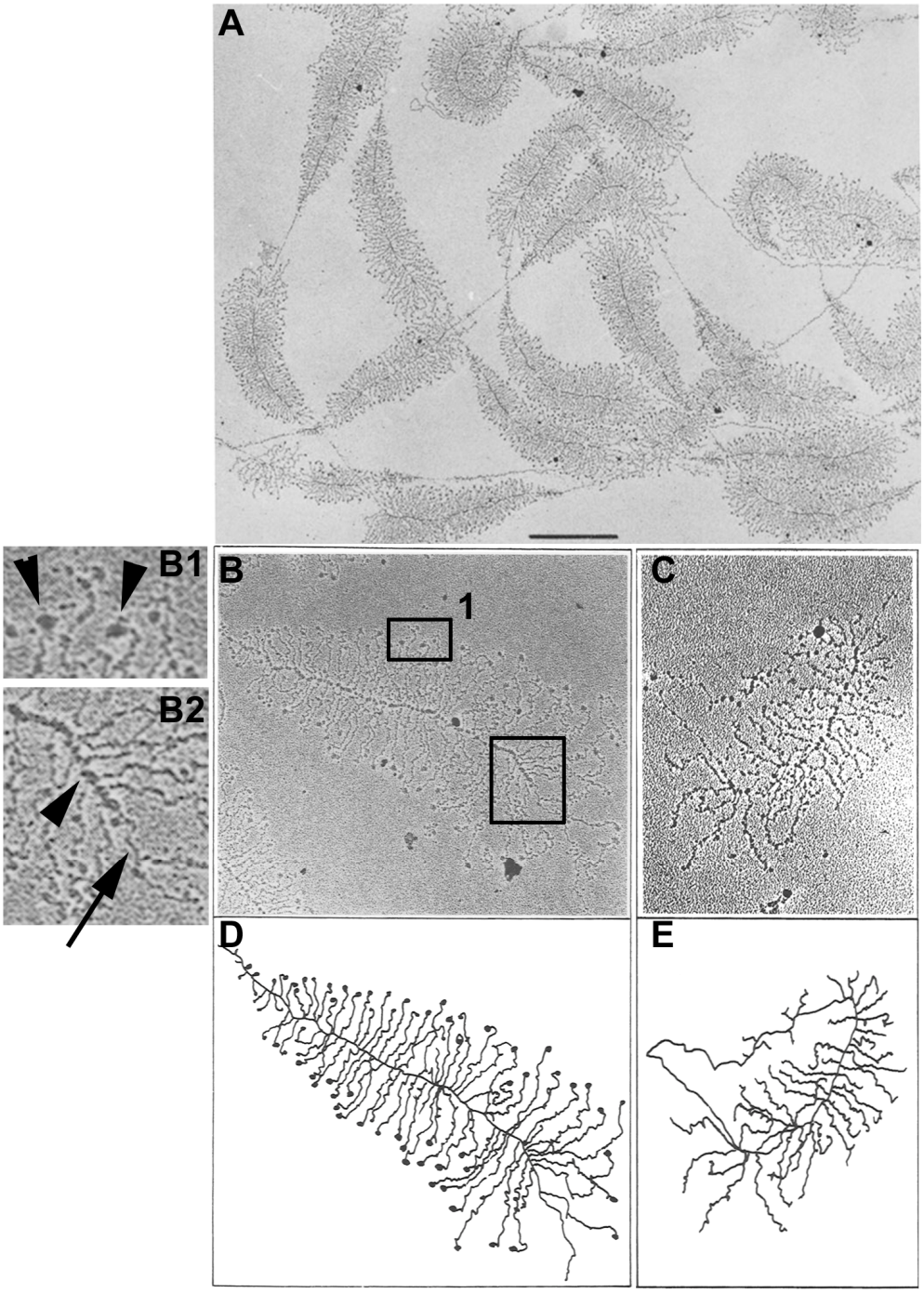
b) Briefly describe the function of the coiled-coil domain of myosin heavy chain, and design one experiment to test how the mutations described above could affect this function. **(3 points)**

c) Because platelets from the patients are difficult to work with, you decide that a human fibroblast cell line that also expresses *MYH9* might be a good model system. These cells respond to serum stimulation and increase cell motility. You express wild-type, mutant A, or mutant B alleles of *MYH9* in these cells by transfection, with each transgene expressed at the same level as endogenous *MYH9,* and examine serum-stimulated cell motility. Only expression of mutant A significantly slowed down serum-stimulated cell motility.

Describe one model to explain why expression of mutant A *MYH9* but not mutant B *MYH9* impaired cell motility **(1.5 points)** and describe an experiment to test your model. **(2.5 points)**

BCDB QUALIFYING EXAM – PART I **Student X**
June 1, 2010
Question 8

During early growth of the *Xenopus laevis* oocytes the nucleolus is amplified to ~ 1000 nucleoli.



Nucleoli can be easily isolated because of their abundance, compactness, and density. All these nucleoli are transcriptionally active. Isolated and active nucleoli can be unwound by incubation in low salt buffer and splayed into an electron microscopy grid. Negatively stained grids allow the visualization of macromolecular complexes (depicted in panel A-C) composed of nucleic acids and

Continued on next page

proteins. These structures are described as “Christmas trees” with different parts named “trunk” (Fig B2 arrowhead), “branches” emerging from the trunk, and “bulbous terminations” at the end of “branches” (Fig B1 arrowheads). Panels D and E represent diagrams of the trees in electron micrographs B-C. Bar in A corresponds to 1 micrometer.

Based on this information:

a) What is the molecular identity (DNA, RNA/type of RNA(s), and protein/type of protein (s)) of the different parts in a “Christmas tree”? Propose an experimental strategy to test your hypothesis about the molecular nature of the tree components. **(3 points)**

b) Tree trunks are continuous with a filamentous structure connecting several trees (see A). However, tree trunks are four times thicker than the filamentous structure connecting them (compare arrowhead and arrow in B2). What molecular explanation could account for the differential apparent thickness of the trunk when compared to the connecting filamentous structures? **(1 point)**

c) What branches of the tree are <u>older</u>, the long or the short ones? Propose an experimental strategy to test your hypothesis. **(3 points)**

d) Panels C and E depict a “mutant” tree. Describe the phenotype **(1 point)** and propose a mechanism to explain this phenotype **(2 points)**. Note: The closed circles in C and E are NOT the phenotype of interest. These circles merely indicate the use of closed plasmids in the experiment.

BCDB QUALIFYING EXAM – PART I **Student X**
June 1, 2010
Question 9

a) The specific activity of T4 DNA ligase from Invitrogen = 2000 Weiss units/mg. The molecular weight of T4 DNA ligase = 60 kDa. A molecular biologist typically puts 0.1 Weiss units of T4 DNA ligase into a 20 microliter ligation reaction. What is the molar enzyme concentration of T4 DNA ligase in such a reaction? **(2 points)**

b) A molecular biologist cuts insert (1 kb) and vector (3 kb) DNAs with restriction endonucleases and gel purifies the appropriate resulting fragments. The molecular weight of dsDNA = (# nucleotides x 607.4) + 157.9 Da). How many nanograms of each fragment must she pipette into a 20 microliter ligation reaction in order to get 20 fmoles of each fragment? When first assembled, what is the molar concentration of DNA in this ligation reaction? **(2 points)**

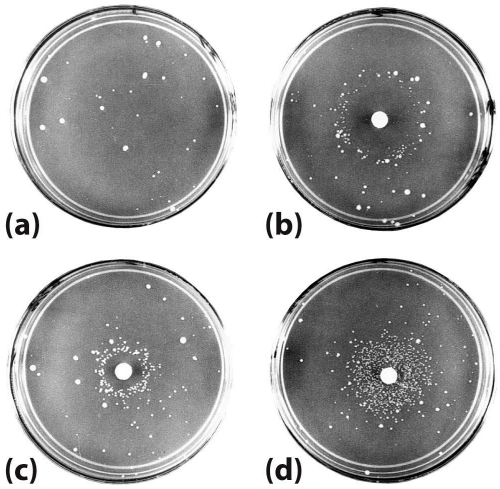
c) All experiments should include controls. What positive and negative control reactions are appropriate for the ligation reaction described above? **(2 points)**

d) The molecular biologist analyzes her ligation reaction products by agarose gel electrophoresis but observes no evidence of ligation of the vector and insert (relative to control reactions). K_M of ligase/sticky end complex = 600 nM. Would you expect the addition of higher concentrations of T4 DNA ligase to improve the yield of ligated product? Why or why not? **(2 points)**

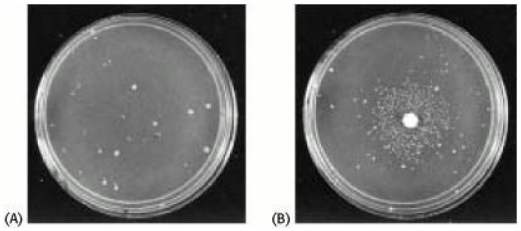
e) If the ligation product yield is low, would you expect the addition of higher starting concentrations of the vector and insert DNA fragments to improve matters? Why or why not? **(2 points)**

BCDB QUALIFYING EXAM – PART I **Student X**
June 1, 2010
Question 10

Exposure of organisms to DNA damaging agents can result in different biological consequences (cytoxicity and mutagenicity) depending on the exposure dose and the ability of the cells to repair the induced damage. Two different isogenic strains of *Salmonella typhimurium* lysine auxotrophs with DNA repair backgrounds X and Y are used in the following experiment where cells are plated onto petri dishes containing lysine-deficient growth media onto which are placed (in the center of the dish to allow outward diffusion of the chemical) filter disks soaked in high, medium, or low concentrations of the toxic and mutagenic DNA damaging agent methylmethane sulfonate (MMS). Following overnight incubation at 37°C, revertant colonies (lysine prototrophs) can be seen on all of the plates, but at different amounts and patterns (shown below).



Mutant strain X. (a) no MMS exposure; (b) high conc. MMS; (c) medium conc. MMS; (d) low conc MMS



Mutant strain Y. (A) (left) no MMS exposure; (B) (right) high conc. MMS

Provide concise interpretations of these experimentmal results with respect to the following:

a) The respective DNA repair proficiencies of strains X and Y. **(2 points)**

b) The relationship(s) between MMS exposure dose and relative numbers of colonies observed for each strain exposed to a particular dose. **(4 points)**

c) The relationship(s) between MMS exposure dose and growth patterns of colonies observed for each strain exposed to a particular dose. **(4 points)**

BCDB QUALIFYING EXAM – PART I **Student X**
June 1, 2010
Question 11

You are a first year graduate student doing a lab rotation. Your project is to analyze whether a given growth factor can stimulate an increase in the levels of beta-actin mRNA in cells. Draw on your knowledge from the 2010 BCMB Symposium to answer the following questions:

a) What technique which allows morphologic preservation of cells and does not employ cell fractionation can be applied to fixed cultured cells enabling you to visualize and quantify beta-actin mRNA levels in the nucleus and cytoplasm,? Describe the type of probe used, what the probe is labeled with, the type of microscopy performed, and appropriate controls. How does this method allow one to distinguish beta-actin mRNA signals in the nucleus versus cytoplasm? **(2 points)**

b) You perform an experiment to examine the time course of beta-actin mRNA induction in response to treatment with your growth factor. As expected, you observe an increase in beta-actin mRNA levels in the nucleus after five minutes of treatment. After a 30 minute treatment, however, you notice that there is an unusual amount of signal for beta-actin mRNA at each treated fibroblast's leading edge (lamellae). Your advisor tells you that your probe is sticking non-specifically and that you need to refine your technique. However, you think there might be some other explanation. What is your hypothesis? **(1 point)**

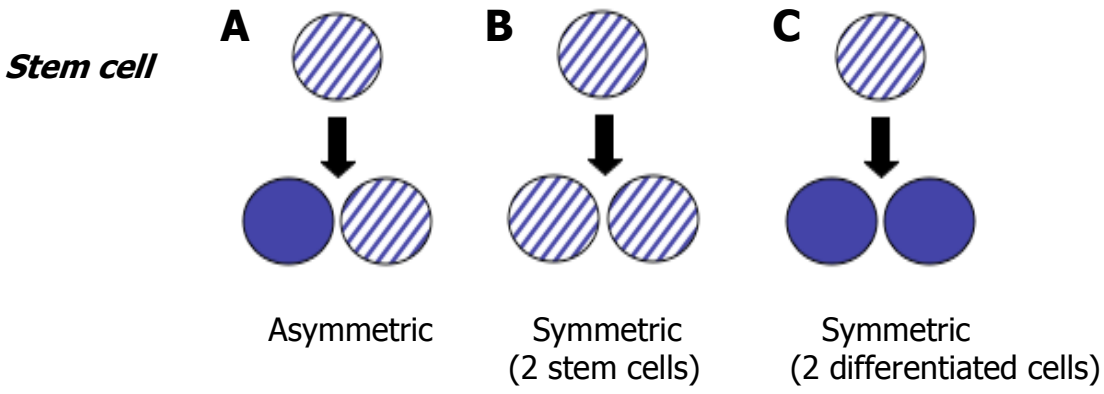
c) You decide to perform some fibroblast microinjection experiments with fluorescently tagged beta-actin mRNA followed by live cell imaging. What would happen if you injected beta-actin mRNA into the nucleus? Would the RNA exhibit any directed movements or non-probabilistic movement **(0.5 points)**? What would happen after the mRNA was transported through a nuclear pore and entered the cytoplasm? Would the RNA exhibit any directed or non-probabilistic movement **(0.5 points)**? Comment on the role of a specific mRNA sequence that is involved. What part of the beta-actin mRNA molecule includes this sequence **(1 point)**? Design an experiment to test your hypothesis using appropriate controls **(2 points)**.

d) You finish your rotation and decide to pursue this research project further for your dissertation, under the direction of your cautious advisor. You use the specific mRNA sequence (discussed above) as an affinity probe to purify and ultimately clone a binding protein from a cell extract. You call this protein, BARBP-1, for beta-actin mRNA binding protein-1 (BARBP-1). Describe a BARBP-1 domain that is relevant for binding of beta-actin mRNA.

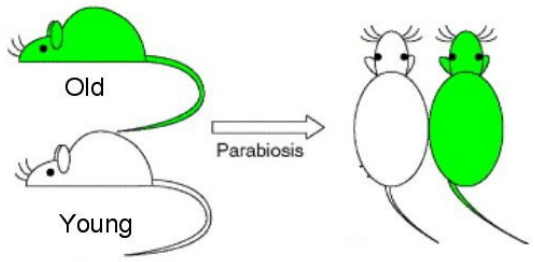
Design an *in vitro* experiment to test your hypothesis that a sequence specific molecular interaction occurs between BARBP-1 and the signal in the beta-actin mRNA **(1.5 points)**.

Design an experiment using cultured fibroblasts to test your hypothesis that BRABP-1 is necessary for proper beta-actin mRNA sorting **(1.5 points)**.

Stem cells can undergo either asymmetric or symmetric cells divisions as shown below:



The stem cell niche is an important regulator of stem cell fate. You are studying a mouse mutant in which muscle stem cells are depleted with age due to enhanced symmetric divisions as in C above. You hypothesize that such impairments in stem cell fate are due to changes in circulating factors. To test this hypothesis, you set up an experimental system in which tissues in old mice can be exposed only to the circulating factors of young animals, and vice versa. As shown below, you establish parabiotic pairings between young and old mice, with parabiotic pairings between two young mice or two old mice serving as controls. In parabiosis, animals develop vascular anastomsoses and thus a single, shared circulatory system.



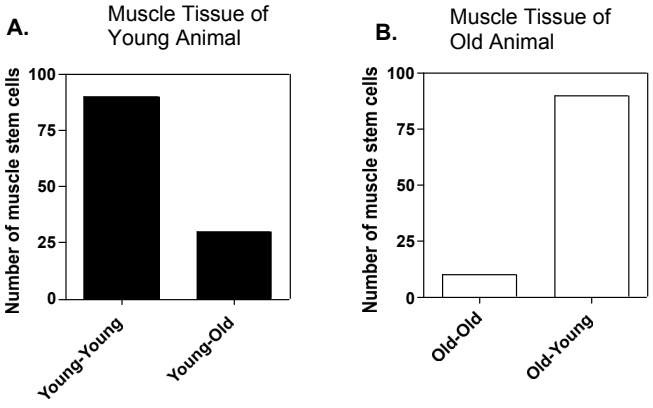
After 2 months of parabiotic pairing, you analyze the number of muscle stem cells in muscle sections and the results are plotted in the graphs below. Consider the data shown in graphs A and B (below) independently from each other in answering the questions below.

a) What conclusion can be drawn about the role of systemic factors in regulating stem cell fate from the data presented for the tissue from the Young Animal in Graph A? Explain your reasoning. **(5 points)**

Continued on next page

Question 12 (Continued)

b) What conclusion can be drawn about the role of systemic factors in regulating stem cell fate from the data presented for the tissue from the Old Animal in Graph B? Explain your reasoning. **(5 points)**



The serine protease trypsin cleaves peptides on the C-terminal side of Arg or Lys residues. This specificity is due to the presence of an aspartic acid residue at position 189 (Asp189) which is located at the bottom of a hydrophobic binding pocket. A modified version of the enzyme, 'trypsin-D189S', was created using site-directed mutagenesis and the catalytic activity of the wild-type and mutant enzymes compared (**Table 1**). Next, the effect of a high salt concentration on the kinetic properties of each enzyme was examined (**Table 2**).

Table 1. Catalytic parameters for trypsin and trypsin-D189S

Enzyme	**K_M (μM)**	**k_{cat} (s^{-1})**
Trypsin	3.7	61
Trypsin-D189S	2100	1.2

Table 2. Effect of sodium acetate (3 M) on enzyme activity, quantified by the parameter k_{cat}/ K_M

Enzyme	**k_{cat} / K_M**	
	No sodium acetate	**+ 3 M sodium acetate**
Trypsin	1.65×10^7	4.70×10^6
Trypsin-D189S	571	1.50×10^5

a) Explain the effect of the D189S mutation. **(*5 points*)**

b) Explain the effect of sodium acetate on the two enzymes. **(*5 points*)**