BCDB QUALIFYING EXAM **Student X**
May 27, 2009

Question 1

You have isolated a novel protein, AB, based on its ability to bind to actin filaments. Interestingly, when this protein is mixed with actin filaments *in vitro*, filaments are non-covalently cross-linked and bundled together.

a) Actin filaments are polar, and the polarity of actin filaments in bundles can be either in uniform or mixed orientation. Describe one experiment to determine how the polarity of actin filaments is organized in the actin bundles formed in your experiment. (3 points)

b) To make actin filament bundles, AB must bind to at least two filaments to create a cross-link. Therefore, AB can be monomeric (two or more actin-binding sites in a monomer) or dimeric (one actin-binding site in each monomer). Describe one experiment to determine if AB functions as a monomer or a dimer. (3 points)

c) When fibroblasts were stimulated with serum, cells formed actin-rich filopodia. You found that AB is enriched in actin bundles present in the serum-induced filopodia. However, AB is a very stable protein and siRNA cannot be used to knock down the AB protein level. Given this limitation, design an experiment to determine if AB is essential for the formation of serum-induced filopodia. Be sure to include appropriate controls and describe the expected outcome from your experiment. Assume that antibody directed against AB, cDNA clones, and any other reagents for AB are available. Also, assume that you have determined that AB is a monomeric protein and identified two actin-binding sites in the molecule. (4 points)

Question 2

Compound Q44 is a small molecule that is cytotoxic to mammalian cells when added to the culture medium at micromolar levels. However, this same compound appears to have no negative impact on budding yeast (*Saccharomyces cerevisiae*) when added to the culture medium at up to millimolar concentrations.

a) Briefly (a few sentences each) propose THREE COMPLETELY DIFFERENT possible mechanisms that could explain the apparent resistance of budding yeast to compound Q44. (2 points each for a total of 6 points)

b) Select two of the possible mechanisms (clearly state which two you have chosen) and then propose an experiment that would allow you to clearly distinguish between these possible mechanisms. Be sure to state what experimental outcome you would expect for each mechanism and how your experiment will provide clearly distinct outcomes for the potential mechanisms you have chosen to investigate. (4 points)

Assume you have access to a well-equipped lab, a large budget, and any resource you might need, including plenty of Q44 and even ^{14}C-labeled Q44 and fluorescently-labeled Q44.

Question 3

Inflammatory bowel disease is a debilitating condition that affects the colon and small bowel causing dense inflammatory infiltrates in these areas. The patients, who are predominantly young (15-25 years of age), suffer from bloody diarrhea, weight loss and abdominal pain. The etiology or pathogenesis of this entity is not known and hence treatment options are not optimal. Thus there is an unmet need to understand the disease and develop therapeutic options for this condition. On a gene microarray analysis of normal colon versus inflamed colon you discover that the steady-state level of the mRNA transcript for 'curin' is significantly decreased in inflamed colon compared to the normal colon. Upon searching the literature you realize that this protein has not been studied in the intestine. You hypothesize that 'curin' plays an important role in the pathogenesis of inflammatory bowel disease.

a) Briefly describe two methods you could employ to confirm your microarray results, which suggest that curin levels are decreased in the inflamed colon compared to normal. Be sure to state which specific controls would be required to ensure the validity of your assays. (2 points)

b)Assuming that you have confirmed the results of the microarray analysis, design an experiment to assess whether pro-inflammatory cytokines, which are highly upregulated during inflammatory bowel disease, decrease expression of curin in cultured intestinal cells. Be sure to mention specific controls that will be essential to interpret your data. Assume that any reagents required are readily available. (4 points)

c) Given the results of the *in vitro* studies described above, you are now eager to test the hypothesis that curin plays a key role in inflammatory bowel disease *in vivo*. Disease can be induced in mice by treatment with chemical compounds such as dextran-sulfate-sodium (DSS). However, previous work has revealed that curin is required for proper mouse development so a simple knockout mouse will not be informative. Given this information, briefly describe the strategy you would use to create (no need to describe vectors in detail) and use a mouse model to examine the role of curin in inflammatory bowel disease. Be sure to mention any controls required to interpret your experiment. (4 points)

Question 4

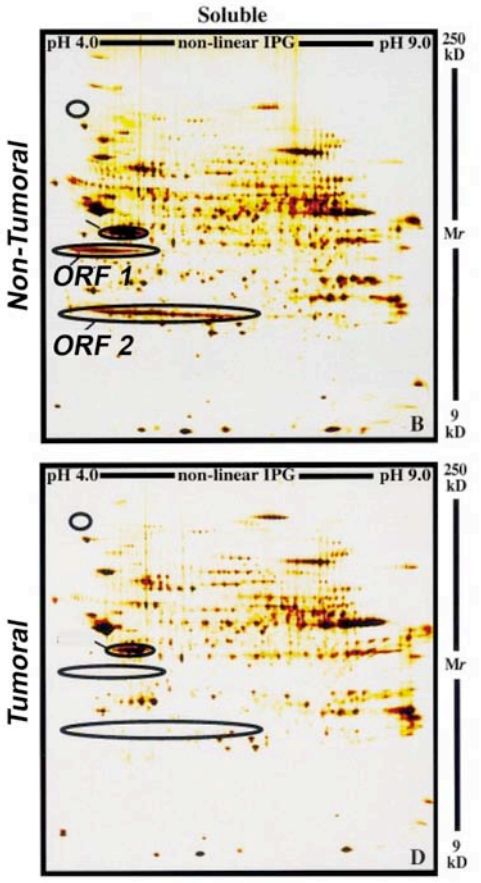


Figure 1. Secreted Proteome of Tumoral and Non-tumoral Human A3341 cells

Human A3341 cultured cells have two clonal cell lines derived from the same parental cell. These two cell clones differ in their capacity to form tumors in nude mice. The non-tumoral variant forms encapsulated cell masses that rapidly regress when injected subcutaneously into mice. In contrast, the tumoral cell variant forms invasive carcinomas that metastasize to bone and lung. Growth factor receptors, such as EGF receptor, are present at similar levels in these two cell variants. Moreover, genome-wide analysis of mRNA transcript levels using microarrays indicates that both cells cultured *in vitro* have similar expression patterns.

Two-dimensional gel electrophoresis (2DIGE) and proteomic analysis of proteins secreted by these two cell variants shows a dramatic difference in the levels of ORF1 and 2. These two protein spots are completely and selectively absent in tumoral A3341 cells (lower panel). Partial primary sequence information reveals that these two spots likely correspond to the same protein. Importantly, the levels of mRNA transcript(s) that code for the protein spots identified as ORF1 and ORF2 (see Figure 1) are identical between these two cells types. Based on this information:

a) Formulate one hypothesis to answer each one of the following questions.

i) How could the loss of a secreted protein lead to a tumoral phenotype? (1.5 points)

ii) How is it that ORF1 and ORF2 are absent from the tumoral cell secreted proteome despite normal levels of the mRNA transcript encoding these two protein spots? (1.5 points)

b) Considering that the primary sequence of ORF1 and ORF2 polypeptides is derived from the same gene and that these polypeptides are secreted:

i) What type of mechanisms or post-translational events could explain the differences in the pattern of 2DIGE migration between ORF1 and ORF2? Provide two distinct hypotheses. (1.5 points)

ii) How can you explain that the isolelectric point of either ORF1 or ORF2 is so broad and spans several pH units? Provide two hypotheses (1.5 points)

c) You hypothesize that the loss of ORF1 and ORF2 is causative of the transformed phenotype observed in human A3341 cells. What experimental strategy would you propose to test this hypothesis? Please be detailed in the formulation of your strategy and include appropriate controls. (4 points)

Question 5

You have been contacted by the producers of the TV show "24". Jack Bauer has been infected by a bioweapon that is destroying the glial cells in his central nervous system leading to seizures. His only hope of survival is to undergo an experimental therapy utilizing stem cells. However the writers of this TV show know nothing about stem cell therapy and need help with the scientific details of the plot. Your ultimate goal is to give Jack Bauer new glia so that he can continue to battle terrorists next season. An exciting potential therapy is nuclear reprogramming of Jack Bauer's own fibroblasts to create pluripotent stem cells. These stem cells could then be induced to differentiate into glial progenitor cells for transplantation. There are two distinct approaches that can be used to achieve such nuclear reprogramming.

a) Briefly describe a strategy to generate glial progenitor cells that involves nuclear reprogramming of Jack's own cells via nuclear transfer. Be sure to state which cells you would employ as a starting point and why. (4 points)

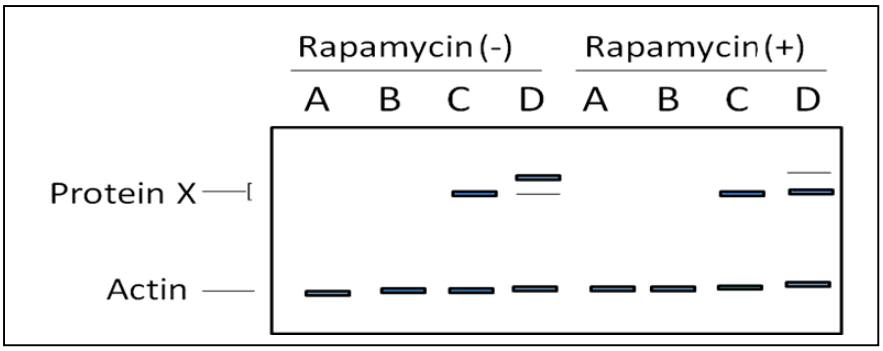
b) What are two problems associated with this method of nuclear reprogramming? (1 point)

c) Devise a strategy to generate glial progenitor cells that involves induced pluripotent stem (iPS) cells. Be sure to include specifics of how you would achieve this strategy. Briefly mention our current general understanding of how this approach leads to the generation of pluripotent stem cells (4 points)

d) What are two problems associated with the generation of iPS cells as a potential tool for stem cell therapy? (1 point)

Question 6

You have obtained three cancer cell lines, A, B, and C, together with the control normal cell line, D, to study expression and phosphorylation-dependent activation of protein X. The result of immunoblotting for protein X is shown below. An mRNA transcript encoding protein X is detected in all cell lines EXCEPT cell line A. Importantly, you observe significantly higher growth rate in the cancer cell lines, which fail to respond to rapamycin-dependent growth inhibition, as compared to the control cells.



Questions: Assuming all reagents needed are available,

a) What type of mutation do you expect to identify in the gene that encodes protein X in each cancer cell line? Design an experiment to test your hypothesis for each cell line. (4 points)

b) Which pathway could regulate phosphorylation of Protein X? Describe one method to determine whether the rapamycin-sensitive migration of protein X observed on the immunoblot is due to phosphorylation of protein X? (3 points)

c) Briefly describe an experiment that you could do to determine whether expression and rapamycin-sensitive phosphorylation of Protein X are functionally required to for rapamycin-dependent suppression of cell growth. Be sure to include any essential controls in your description. (3 points)

Question 7

You have just developed a novel means of permeabilizing synchronized cultured cells at anaphase B, the process of mitotic spindle elongation following anaphase A and resulting in further separation of spindle poles and chromosomes. Additionally, you found that upon adding ATP, GTP and tubulin to the perfusion buffer, anaphase B could be reactivated and continued in the permeabilized cell models. Moreover, you determined that addition of the non-hydrolyzable ATP analog AMP-PNP (5'-adenylyl-beta, gamma-imidodiphosphate), blocked anaphase B.

Importantly, based on additional studies, the mechanism of anaphase B does not involve kinetochore microtubules and the rate of spindle elongation during anaphase B is 10 times faster than tubulin flux or treadmilling. Thus, based on these observations, neither kinetochore microtubules nor tubulin flux plays a direct role in the model.

a) Illustrate, in a pair of simple diagrams, spindle microtubule organization at the beginning and end of anaphase B and label microtubule "plus ends" in your model. (2 points)

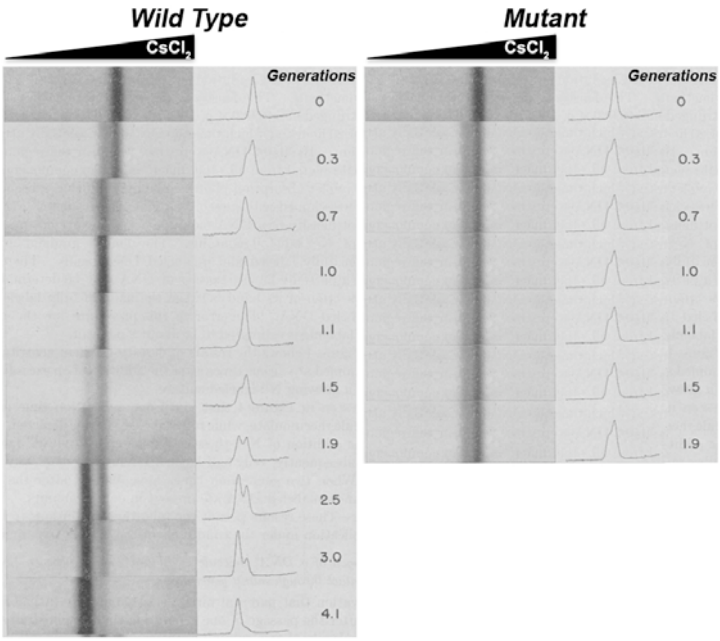
b) Briefly describe a general model for anaphase B based on your model. Describe an experiment to determine where tubulin incorporates in the mitotic spindle during anaphase B. Illustrate as required. (2.5 points)

c) Postulate two roles for ATP in the mechanism of anaphase B. (2 points)

d) Postulate two roles for GTP in the process of anaphase B. (2 points)

e) Postulate and discuss how AMP PNP could block anaphase B. Illustrate as required. (1.5 points)

Question 8



Meselson and Stahl performed the experiments depicted here to test the hypothesis of the semiconservative nature of DNA replication. Some of these data were published in PNAS 46:671 (1958). The images depict ultraviolet absorption photographs showing DNA bands resulting from density gradient centrifugation of lysates of wild type and a mutant bacteria sampled at various times after the addition of an excess of ^{14}N substrates to a growing ^{15}N-labeled culture. Each photograph was taken after 20 hours of centrifugation. The density of the CsCl solution increases to the right. Regions of equal density occupy the same horizontal position on each photograph. The time of sampling is measured from the time of the addition of ^{14}N in units of the generation time. Microdensitometer tracings of the DNA bands are shown in the adjacent photographs.

Note the contrast in DNA sedimentation of the wild type and mutant bacterial strains. Mutant cells do not growth at the same rate as wild type cells and they begin to die before completion of the second generation or doubling.

a) Describe the phenotype of the mutant bacterial strain. Formulate two hypotheses that could account for the phenotype. (4 points)

b) Choose one of your hypotheses and design an experimental strategy to test your hypothesis. (4 points)

c) The experiments depicted use total bacterial DNA. If you were to isolate both chromosomal DNA and a smaller plasmid DNA from the wild type bacterial strain, how would the sedimentation of the chromosomal DNA compare to migration of the plasmid DNA in this type CsCl gradient analysis? Provide a rationale for your answer. (2 points)

Question 9

Glutathione (GSH) is an important reductant and anti-oxidant required for multiple processes in all eukaryotic cells. It is maintained in a reduced state by the enzyme glutathione reductase which catalyzes the following reaction.

NADPH + GSSG <----------> NADP+ + 2 GSH

Studies on purified liver glutathione reductase (MW=55 kDa) yielded the following data:

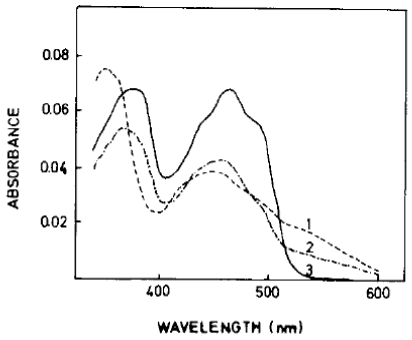
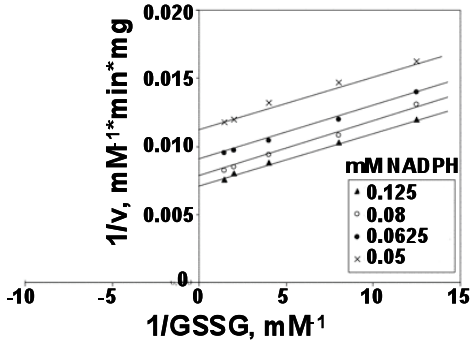


FIG. 3. Absorption spectrum of reduced and spontaneously reoxidized glutathione reductase. The amount of enzyme-bound flavin was 3.0 nmol, and the original spectrum was identical to *Spectrum 3*. Reduction was effected by addition of 10 nmol of NADPH, after which *Spectrum 1* was immediately recorded. Recording of *Spectra 2* and *3* were started 5 and 10 min, respectively, after recording of *Spectrum 1*.
(Carlberg I, Mannervik B., J Biol Chem. 1975 Jul 25;250(14):5475-80.)

Fig. 5. Double reciprocal plots of initial velocity of the bovine liver GR against as GSSG varied substrate at different fixed NADPH concentrations. The assays were carried out at 37°C in 100 mM sodium phosphate buffer, pH 7.4 (Ulusu NN, Tandoğan B., Mol Cell Biochem. 2007 Sep;303(1-2):45-51).



a) How were the points (triangles, circles and x's) in Figure 5 derived? Describe the experiments done to generate these data. (2 points)

b) How would you calculate the k_{cat} for this enzyme? Be specific about the additional plots and calculations necessary and give your estimate of k_{cat}. (3 points)

c) How would you calculate the K_m for GSSG from these data? Be specific about the additional plots and calculations necessary and give your estimate of the K_m for GSSG. (3 points)

d) How do figures 3 and 5 help suggest the mechanism of this enzyme? (2 points)

Question 10

You have discovered a previously unidentified orphan nuclear receptor, NR-1, in a parasitic nematode that infects human liver. NR-1 is expressed at the critical transition from the larval to adult stage and preliminary data indicates that the receptor requires the bile acid deoxycholate as a transcriptional co-activator. You hypothesize that NR-1 transcriptional activation is required to allow transition from the larval to the adult stage by binding to the promoter of a key target gene, WM. The sequence:

AGGTCTGCGTAGACTAGGTCAGCATAGACCTTG

is found 300 bp upstream of the transcription initiation site of the WM gene and it is required for proper expression *in vivo*. You already know that forced over expression of the WM gene drives larval worms into becoming adults. You may also assume that NR-1 can be expressed in E. coli and purified in a biologically active form.

a) Which region within the AGGTCTGCGTAGACTAGGTCAGCATAGACCTTG sequence suggests that it is a nuclear receptor response element? Briefly explain why you selected this region. (1 point)

b) How would you test the hypothesis that the binding of NR-1 to the promoter region of the response element you identified in part "a" causes transcriptional activation? (2 points)

c) Describe two methods you would use to quantify NR-1 binding to the sequence in "a" and describe how the experiment would be done to test the binding. In your answer, be sure to include the macromolecules that are required for this experiment. (3 points)

d) How would you test the hypothesis that deoxycholate is an activating ligand for NR1 in cells in culture? (2 points)

e) Assume that deoxycholate is a ligand that activates transcription *in vivo*. How would you quantify this binding *in vitro*? (2 points)

Question 11

Mr. and Mrs. Mickey Mouse decide to start a family. They take all the usual measures and Mrs. Mouse becomes pregnant. However, one of her eggs kicked out the maternal nucleus and got fertilized by two sperm cells. So, this egg is diploid, but has two paternal haploid nuclei and develops as a metastatic choriocarcinoma (a malignant, trophoblastic germ cell tumor). However, the DNA in a choriocarcinoma is normal. Usually, we think of cancer as a genetic disease where mutations accumulate and disease follows.

a) Explain why having two paternal nuclei in an egg could cause such a dramatic problem. (2 points)

b) Devise an experiment where you would test the hypothesis that gene expression in normal mouse embryos differs from that of a choriocarcinoma. (3 points)

c) You end up discovering that six genes show elevated transcription in choriocarcinomas vs normal embryos. Briefly describe an experimental approach where you could learn the reason for this elevated gene expression. (3 points)

d) Mrs. Mouse has a tumor in her brain. Devise an experimental approach to determine if it is derived from the choriocarcinoma in her uterus. (2 points)

Question 12

Dysregulation of tyrosine kinases has been associated with pathogenesis and disease progression of many human cancers including solid tumors and hematopoietic malignancies. You have identified an interesting point mutation in the open reading frame of a protein tyrosine kinase called LEUTK1 (leukemogenic tyrosine kinase 1) in a handful of CML (chronic myelogenic leukemia) patients. LEUTK1 has not been reported previously to be mutated in CML cases. Fortunately you have identified a human leukemia cell line HLCL1 that harbors this LEUTK1 mutation and injection of HLCL1 cells into nude mice through a tail vein induces a "leukemia-like" disease characterized by splenomegaly (big spleen). Also you have a murine hematopoietic cell line BaF3 that is normally dependent on cytokine IL-3 for survival and proliferation, but becomes IL-3-independent in its proliferation and survival upon expression of a leukemogenic tyrosine kinase such as BCR-ABL. Finally, you have a cDNA encoding LEUTK1 in hand.

a) Design one experimental approach to determine whether this LEUTK1 mutation affects LEUTK1 catalytic activity. Be sure to include appropriate controls necessary to make a firm conclusion. (5 points)

b) Assume you find that this mutation activates LEUTK1. Design one experiment to determine whether this mutation is a "driver" to induce hematopoietic transformation in cultured cells and one experiment to determine if it is a driver for transformation in an animal model. Be sure to include all necessary controls and clearly describe possible experimental outcomes and interpretations. (5 points)