Bi/Ch 113 Final Exam Instructions

This is an open-book, open-notebook exam. Your class notes, the lectures, the two texts (Lodish et al and Alberts et al), and the assigned section/review papers may be consulted. The use of the internet and discussions with other persons are not allowed. Do not exceed 3 hours for the exam.

The best answers are clear and concise, but complete. For each question, the correct answer is the simplest answer consistent with all the data.

There are 4 pages.

There are no extensions.

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Question 1: Cytokine receptor (25 points)

You have been studying the properties of a secreted cytokine (cytokine X) that has potent activity in directing the proliferation of certain hematopoietic cells. You would like to clone the receptor for cytokine X using an expression cloning approach.

1) As a first step towards characterizing the receptor, you need to identify some cell lines that express the receptor and then determine the cell line that expresses the highest number of receptors. Once you find such a cell line, you would like to determine the number of receptors present on each cell and their affinity for the ligand. Explain how you would do all this.

2) Suppose the best cell line makes 1500 receptors. A 15 cm plate contains ~10 million cells. Using the guess that the receptor is a medium-sized protein (~50 kDa), how much receptor does a plate of cells contain (in mass)? Is this enough protein to allow for biochemical purification and peptide sequencing of the receptor?

3) You make a fusion protein consisting of cytokine X fused to the Fc region of human immunoglobulin G1. The Fc region can dimerize. Just like cytokine X, you find that this fusion protein is capable of causing proliferation of hematopoietic cells.

Now you would like to use expression cloning to identify the gene encoding the receptor. Explain how you would do this.

4) For the expression cloning approach of part C to be successful, what assumptions must be true?

5) Using the approach in part C, you indeed identify the cytokine X receptor. It has the typical sequence features of a cytokine receptor. You notice that this receptor is mutated in a human disease involving hematopoietic dysfunction. The mutation introduces a premature stop codon into the C-terminal region of the receptor, causing a short truncation. You analyze the mutant receptor and find that it is capable of binding cytokine X but does not transmit a proliferation signal. Describe two distinct mechanisms that could explain this molecular defect.

Question 2: Apoptosis

1) Apaf1 is the mammalian ortholog of CED-4 in nematodes. Mice lacking Apaf1 die perinatally. They often have craniofacial abnormalities, including ectopic masses in the forebrain. What do you think is the cause of these ectopic masses and how can you test this idea?

2) Two common ways to access apoptosis are: (a) treatment of fibroblasts with etoposide and (b) treatment of lymphocytes with anti-Fas antibody. If wildtype cells versus Apaf1 mutant cells are tested with these assays, what do you expect the result to be?

3) Another common apoptosis inducer is UV irradiation. If wildtype versus Apaf1 mutant cells are irradiated with UV, do you expect to observe a difference in cytochrome *c* release? Explain.

4) If cytochrome *c* and Apaf1 work together to promote apoptosis, why do cytochrome *c* knockout mice have a much more severe phenotype than Apaf1 knockout mice?

5) In *C. elegans*, a loss-of-function CED-9 allele (when homozygous) causes ectopic cell deaths and embryonic lethality. In contrast, a gain-of-function CED-9 allele causes loss of developmentally regulated cell death but the worms appear to develop normally. Provide a rationalization for these phenotypes.

Question 3: Oncogenesis

1) Women inheriting a heterozygous BRCA1 mutation have a lifetime breast cancer risk of 80-90%. The disease is autosomal dominant and often results in bilateral breast tumors. In contrast, sporadic breast cancer can also involve BRCA1 mutations. However, in this case, the disease is usually unilateral. Explain.

2) With the identification of tumor promoting genes, it has been possible to develop drugs that can inhibit specific oncogenes, such as tyrosine kinases. Even though such drugs can be very effective initially, often a small subset of the tumor cells is resistant, and this leads to eventual recurrence of the tumor. Why do many cancers show an ability to overcome targeted drug treatments?

3) The Rous Sarcoma Virus (RSV) is an acute acting retrovirus that causes tumors in chickens. Why does RSV cause rapid, polyclonal tumors in chickens, whereas some other similar oncogenic viruses are much slower acting and cause monoclonal tumors?

4) v-src lacks the last 18 residues of c-Src. Within this deleted region, c-Src contains a tyrosine residue that when phosphorylated causes down-regulation of its tyrosine kinase activity. The domain structure of c-Src consists of an SH3 domain, an SH2 domain, and the tyrosine kinase domain. Given this domain organization, propose a molecular model for how phosphorylation of the tyrosine might reduce c-Src activity.

5) Like other SH2 domains, the SH2 domain of c-Src binds to ligands containing phosphotyrosine in the context of a specific consensus sequence. How might binding of such ligands lead to activation of c-Src activity?

Question 4: Membrane protein

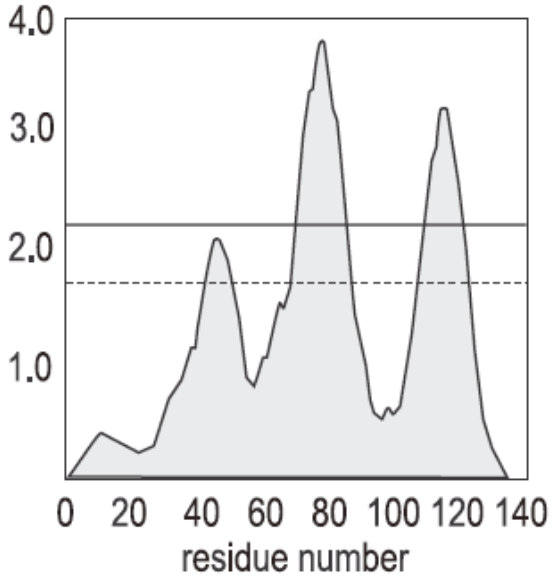
Using yeast genetics, you have just identified and cloned gene X, which you think controls mitochondrial biogenesis. This novel gene has never been studied before, and it is up to you to do some basic characterization. Based on the gene sequence, the encoded protein has 140 residues.

1) One fact you would like to know is where the protein is localized within the cell. Specifically, you would like to determine the subcellular localization of the *endogenous* protein. One method is to use an antibody against the protein to perform immunocytochemistry; another method is to express a GFP fusion of the protein. Discuss the pros and cons of these two methods, given your goal.

2) Suppose your experiment in part 1 shows localization to discrete structures in the cytosol, but you are not sure whether these structures are endoplasmic reticulum, mitochondria, vesicles, etc. How would you resolve this issue?

3) Explain the difference between a polyclonal antibody versus a monoclonal antibody. In addition, what are the pros and cons of using these reagents for immunocytochemistry?

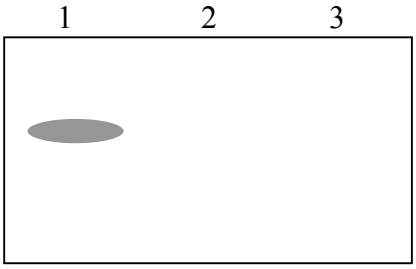
4) Suppose the combined results in part 1 and 2 show that the protein is mitochondrial. You run the protein sequence through a hydropathy program and get the following plot, where more positive values indicate increasing hydrophobicity:



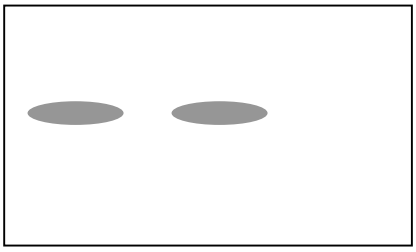
Based on this plot, there are 3 peaks corresponding to 3 hydrophobic regions. The last two peaks are very likely to be true transmembrane domains, whereas the first peak is smaller and marginal.

So you conclude that the protein is likely to be a mitochondrial membrane protein, but you are not sure whether it has 2 or 3 transmembrane domains.

You raise an antibody (called "Antibody A") to the first 20 residues of the protein. You also want an antibody to the carboxyl terminus of the protein, but because there are so few residues beyond third hydrophobic region, you decide instead to make a construct that expresses the protein with a Myc epitope tag fused to the carboxyl terminus. You generate yeast expressing such a construct, purify intact mitochondria by differential centrifugation, treat the mitochondria as indicated below, and examine the protein by Western blotting with Antibody A or anti-Myc antibodies.



Western blot with Antibody A



Western blot with anti-Myc antibody

Lane 1: No protease
Lane 2: Protease added
Lane 3: Protease and Triton X-100 added

Based on these results, how many transmembrane segments are likely to exist, and what is the most likely membrane topology of this protein in mitochondria? Draw a diagram.

5) In an *in vitro* import assay, do you expect CCCP (a mitochondrial uncoupler) to affect the import of protein X into mitochondria?