

2007 7.013 Problem Set 4

Due before 5 PM on WEDNESDAY, April 4, 2007.

Turn answers in to the box outside of 68-120.

PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

Question 1.

Working in the African Congo, you discover a new species of vertebrate in an isolated pond. The animal has no fur and seems equally comfortable on land or in the water. Wondering what type of species this might be, you decide to examine its DNA. First, you obtain a small tissue sample from the organism and extract its DNA. Next you clone a fragment of this DNA into a plasmid vector and sequence it.

To identify your DNA sequence, you decide to do a series of analyses, using the BLAST tools available through the National Center for Biotechnology Information (NCBI). BLAST (Basic Local Alignment Search Tool) is a computational database search method for examining the hundreds of millions of known protein or DNA sequences in the world and rapidly identifying those relatively few sequences that are homologous to a given input sequence (the “query sequence”). BLAST essentially compares the query sequence to every other known sequence and identifies those that are most similar. Using such techniques, researchers can often infer the function of a given protein or DNA sequence simply based on the function of its closest known homologues.

Your sequence (that is, the sequence that you have obtained from this “Mystery animal”) is available in the “DNA Sequence Text File” on the class website. Use the instructions in the file “Supporting File for Question 1 from Problem Set 4” to carryout a BLAST analysis of this sequence

BLASTx is a tool that will first predict what kind of protein might be encoded by a segment of DNA (by electronically “translating” the sequence), and then it will compare the resulting protein sequence to all known protein sequences. To identify what kind of protein might be encoded by your sequence, carry out a BLASTx analysis.

- 1a.** You find that your DNA sequence encodes part of a protein that is homologous to proteins found in many different organisms. Based on the reported names or functions of the homologous proteins, what is the likely name or function of the protein encoded by your sequence.

Protein name or suspected function: Polo-like Kinase 3

- 1b.** From which organism comes the protein sequence that has the greatest homology to your sequence (i.e. what species provides the top hit in the BLASTx results table)?

Species or common name: Pan Troglodytes or Chimpanzee

Puzzled by this outcome, you decide to examine the DNA sequence directly, rather than looking at the protein it encodes. To do this, you carry out a BLASTn of your sequence.

- 1c. From which organism comes the DNA sequence that has the greatest homology to your sequence (i.e. what species has the top hit in the BLASTn results table)?

Species or common name: *Xenopus laevis* or Frog

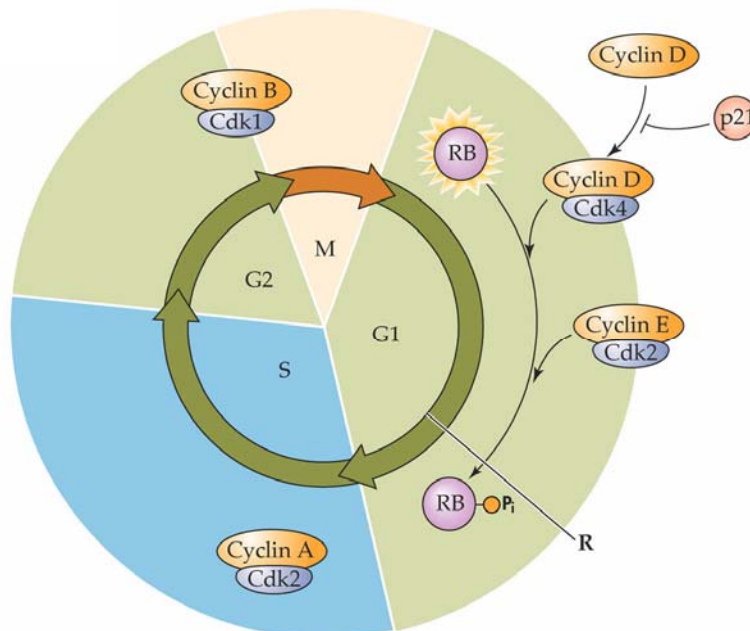
- 1d. What principle of molecular biology or evolution allows the protein and DNA sequences to reveal homology to sequences from two such very different organisms? Explain your answer in 15 words or less.

- “Multiple codons may code for the same amino acid”
 - “Degeneracy of the genetic code”
 - “Redundancy of codon usage”
- or
- “purifying selection has conserved the amino acid sequence but not the DNA sequence”

Question 2.

What stage of the cell cycle best describes the following cells?

- 2a. A cell with condensed chromosomes aligned across the midpoint M Phase or mitosis
- 2b. A cell with very active DNA polymerases S-Phase
- 2c. A brain cell that has not undergone cell division in 19 years G0
- 2d. A cell with microtubules radiating from a centriole All Phases



LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 9.4 Cyclin-Dependent Kinases and Cyclins Trigger Transitions in the Cell Cycle © 2004 Sinauer Associates, Inc. and W. H. Freeman & Co.

Above is a diagram of the cell cycle, displaying the interactions of several regulators. In your laboratory, you have identified several homozygous mutant cell lines, each carrying a temperature sensitive mutation in one of the above indicated factors. In each case, the protein in question becomes inactivated when the cells are grown at temperatures above 30°C. In cells carrying temperature sensitive mutations for each of the following factors, at what point in the cell cycle would you expect to see cells arrest (halting further progression through the cell cycle) once you shifted their incubation temperature to 37°C?

- 2e. Cyclin A S
- 2f. Cyclin B G2
- 2g. Cyclin E G1
- 2h. RB No arrest

Question 3.

PtsG is a membrane protein with an extracellular domain and an intracellular domain, and it is involved in bacterial uptake of the six-carbon sugar glucose. When there is no glucose in the culture medium, enzymes within the cell phosphorylate part of the PtsG.

3a. Which portion of PtsG is most likely phosphorylated by these enzymes?

- The extracellular domain
- The membrane-spanning domain
- The intracellular domain
- The tryptophan residue

Phosphorylated PtsG translocates glucose into the cell. As it does so, it transfers its own phosphate group to the glucose molecule, leaving the protein unphosphorylated and producing glucose-phosphate, a form of the sugar that is activated for further metabolism.

3b. Which term best describes PtsG?

- Protein kinase
- Receptor
- Second messenger
- Responder

Mlc is a regulator that can bind to the promoters of genes that are required for the cell to further metabolize glucose. When Mlc is bound to the promoter, RNA polymerase can no longer recognize the promoter.

3c. Which word best describes Mlc?

- Translational activator
- Translational attenuator
- ~~Transcriptional Activator~~
- Transcriptional repressor
- Translation elongation factor

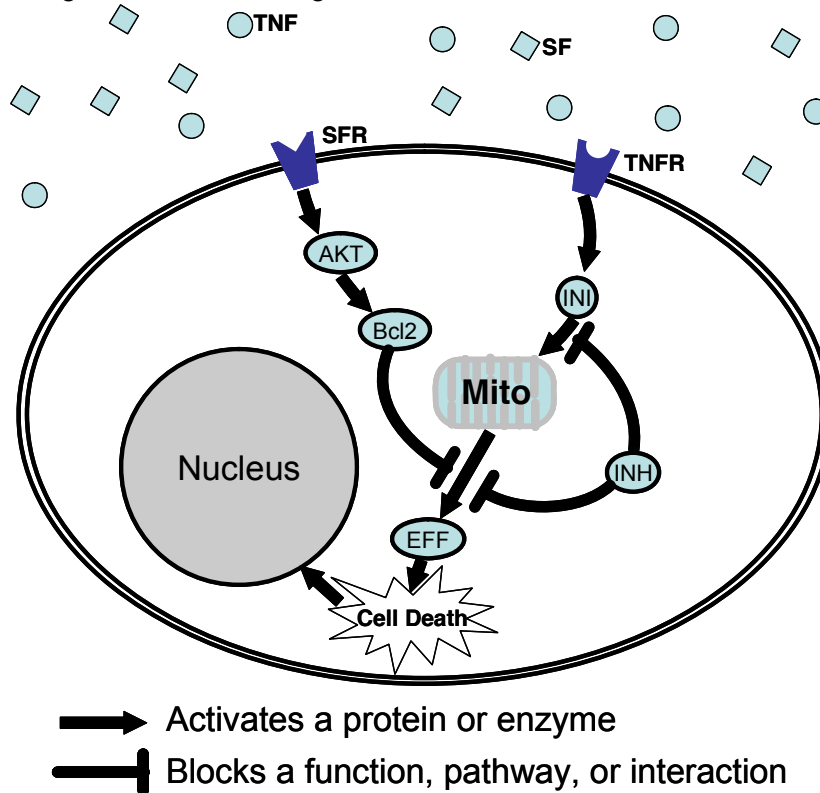
3d. Direct interactions between the proteins Mlc and PtsG are central to the regulation of glucose metabolism in bacterial cells. Given the above properties of Mlc and PtsG, is it more likely that Mlc binds the phosphorylated PtsG or the unphosphorylated PtsG?

Briefly explain your answer.

The cell wants to prevent transcription when there is no glucose in medium. Mlc prevents transcription when there is no glucose in medium by binding DNA. PtsG is phosphorylated when there is no glucose in medium. Mlc prevents transcription when PtsG is phosphorylated. Mlc binds DNA when PtsG is phosphorylated. So, **Mlc more likely binds PtsG when PtsG is unphosphorylated.**

Question 4.

Consider the following simplified diagram of a signal transduction pathway that regulates programmed cell death in mammalian cells. Assume that this diagram represents the behavior of a single cell within an organism.



Key: TNF (small circles) and SF (small diamond shapes) are circulating in the plasma that bathes the surface of the cell; TNFR = TNF receptor; SFR = SF receptor; As indicated, arrowheads indicate an activating relationship between one protein and another, while the lines with perpendicular bars at their ends indicate a disruptive or inhibitory relationship between one protein and another protein or pathway.

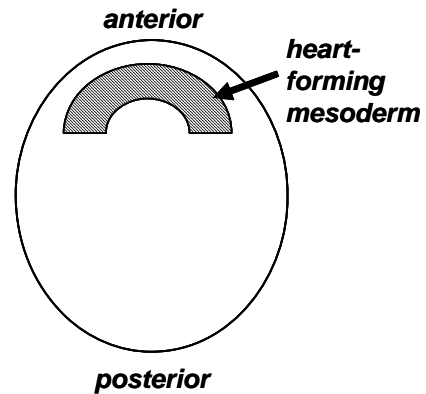
Based on the regulatory network described above, state whether mutations that knockout each of the following proteins would either **increase** apoptosis or **decrease** apoptosis.

4a. TNF decrease4e. SFR increase4b. Bcl2 increase4f. AKT increase4c. INH increase4g. TNFR decrease4d. EFF decrease4h. SF increase

Question 5.

Regeneration of heart tissue is of great medical interest. One idea is that by understanding how an embryo forms a heart, scientists will be able to recapitulate that process to make replacement heart tissue. As a new PhD graduate, you decide to study heart development in the chick embryo, with the ultimate goal of growing replacement heart tissue in the lab.

The first thing you need to do is figure out which part of the chick embryo forms the heart. You place dots of green dye at various places in an early chick embryo (stage 2 or stage 4), and find that a region near the front of the embryo (anterior), always goes on to form the heart. Stage 2 and stage 4 embryos look similar, and do not have a heart, which only appears and starts to beat, 3 days later, at stage 18. The embryo has three layers of cells, but when you cut the embryo open, you find that just one layer of cells, the mesoderm, forms the heart.



Early Chick Embryo

5a. What is the labeling technique described called?
(2 words) **fate mapping or lineage analysis/tracing okay**

You then ask when the future heart cells decide to become such. You do this by isolating a small piece of tissue (called an “explant”), of about 200 cells, from the heart forming anterior mesoderm of both a young stage 2 embryo, and from a slightly older stage 4 embryo (about 6 hours older). You place the tissue in culture medium, and examine it three days later. You obtain the following results.

Stage 2 anterior mesoderm explant 3 day culture no change from original cells

Stage 4 anterior mesoderm explant 3 day culture beating heart

5b. At the time of explantation, are the stage 2 cells determined, differentiated or neither? Explain your answer in 15 words or less.
Neither. The tissue does not become heart. So, it has not decided its fate (been determined), and has not assumed final fate (differentiated).

5.c At the time of explantation, are the stage 4 cells determined, differentiated or neither? Explain your answer in 15 words or less.

Determined (not differentiated). The tissue has not differentiated yet, but has decided to become heart.

5.d Are the beating heart cells determined, differentiated or neither? Explain your answer in 15 words or less.

Differentiated. They have already become heart cells and therefore have assumed final fate. (Would also accept both determined and differentiated).

Intrigued, you ask why the isolated stage 2 cells did not become a heart. You remember that the anterior mesoderm cells actually lie on top of another cellular layer in the embryo, called “endoderm”, and that you did not isolate (explant) these endoderm cells in the previous experiment. You perform the following experiment, and obtain results indicated.

Stage 2 anterior mesoderm explant alone (labeled with green dye)	3 day culture	no change (green)
Stage 2 anterior mesoderm explant (green) <u>plus</u> underlying endoderm (unlabeled)	3 day culture	beating heart (green)
Endoderm alone (unlabeled)	3 day culture	no change

5e. Why does the stage 2 anterior mesoderm explant plus endoderm make a heart, whereas the stage 2 anterior mesoderm explant alone does not? (15 words or less)
Endoderm signaled to the mesoderm causing it to become determined/ instructed it to become heart.

5f. What is the general biological term for the influence of the endoderm? (1 word)
induction

5g. What was the reason to label the anterior mesoderm with green dye in this experiment? (10 words or less)
To distinguish it from endoderm.

Later, in your studies, you are interested to find that a purified protein, BMP4, is able to substitute for the endoderm. Thus

Stage 2 anterior mesoderm explant + BMP4	3 day culture	beating heart
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5h. Based on all the data above, where in the embryo would you expect BMP4 to be expressed if it normally directed heart formation? (5 words or less)
Expressed in endoderm or in other cells nearby prospective heart.

Posterior mesoderm does not form the heart. However, you would like to know how powerful an influence BMP4 can have, and so you test whether BMP4 is able to turn posterior mesoderm into beating heart, and get the following results.

Stage 2 posterior mesoderm explant	3 day culture	no change
Stage 2 posterior mesoderm explant + BMP4	3 day culture	no change

5i. BMP4 is a secreted protein that activates its receptor. The activated receptor phosphorylates a transcription factor, Smad1. Phospho-Smad1 moves to the nucleus to change transcription of target genes. Using this information, suggest why posterior mesoderm does not respond to BMP4 to make a heart (15 words or less).

Posterior mesoderm does not express the BMP4 receptor or Smad1 transcription factor proteins. (other acceptable answers: produces an inhibitor of BMP signaling, or target genes are not in correct chromatin configuration for activation.)