7.014 Problem Set 6*

*Please note that due to the Patriots Day holiday, the deadline for this problem set has been extended. Copy your problem set as a study aid before you turn it in as problem sets will not be returned before the quiz.

Answers to this problem set are to be turned in at the box outside 68-120 by 4:00 pm, Friday, April 19. Anyone wishing to turn in the problem set early can do so. Problem sets will not be accepted late. Solutions will be posted at (http://web.mit.edu/7.01x/www/).

Question 1

In *E. coli*, the fictitious AB operon is induced by the presence of Compound W. A diagram of the operon, its regulatory proteins and regulatory sites is shown below:

- **P<sub>X</sub>** promoter for the regulatory protein
- **X** gene for the regulatory protein of the AB operon
- **P** promoter for the AB genes
- **S** sequence shown to be important for regulation by W
- **A** structural gene for enzyme A
- **B** structural gene for enzyme B

The following table shows the genotypes of different *E. coli* strains with a wild-type AB operon and various mutant AB operons, and the number of molecules of proteins A and B per cell in the absence or presence of Compound W (–W or +W, respectively). The symbol "+" indicates that the gene or control element is functional (wt) and "−" indicates that the gene or control element is non-functional. Assume the genes not listed are wild type.

<table>
<thead>
<tr>
<th>Strain</th>
<th>X</th>
<th>P</th>
<th>S</th>
<th>A</th>
<th>−W</th>
<th>+W</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>m1</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>200</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>m2</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>m3</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>200</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>m4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

a) For each strain on the table above, label the expression as either inducible, uninducible or constitutive.

b) Based on the data shown above, does the regulatory protein X act as a repressor or an activator of the AB operon? Explain your reasoning.

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7.014 Problem Set 6
Spring 2002
c) You make partial diploids of various *E. coli* mutant strains using a single-copy plasmid introduced into the *E. coli* cell. In cells of the following genotypes, predict the number of molecules of enzyme A per cell (0, 200, 400) produced in the absence or presence of Compound W (–W or +W, respectively). Put the total number of molecules of enzyme A on the lines provided.

<table>
<thead>
<tr>
<th>Genotype</th>
<th># of molecules of enzymes A per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>X+ P+ S+ A+</td>
<td>0</td>
</tr>
<tr>
<td>X+ P+ S+ A+</td>
<td>0</td>
</tr>
<tr>
<td>X– P+ S+ A+</td>
<td></td>
</tr>
<tr>
<td>X+ P+ S– A+</td>
<td></td>
</tr>
<tr>
<td>X+ P+ S+ A–</td>
<td></td>
</tr>
<tr>
<td>X– P+ S+ A+</td>
<td></td>
</tr>
<tr>
<td>X+ P+ S– A+</td>
<td></td>
</tr>
<tr>
<td>X+ P+ S+ A+</td>
<td></td>
</tr>
</tbody>
</table>
d) You examine a different operon, the H operon, containing genes 1 and 2 that encode enzymes 1 and 2. In this operon compound H acts as an inducer. You look at the levels of DNA, mRNA and protein in a typical *E. coli* cell. The results you find are shown below:

<table>
<thead>
<tr>
<th></th>
<th>–H</th>
<th>+H</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>mRNA</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>protein</td>
<td>0</td>
<td>200</td>
</tr>
</tbody>
</table>

At which step of gene expression could this regulation occur? Explain your reasoning.

e) In the lac operon, which is regulated at the transcriptional level, repressor protein is present in *E. coli* at approximately 4 molecules per cell. In the H operon, the repressor protein is present at approximately 200 molecules per cell. Based on your answer to part d, why would you expect there to be more molecules of the repressor protein for the H operon per cell than molecules of the lac repressor per cell?
Question 2

a) Shown below is a schematic of the production of a heavy chain polypeptide for an antibody. At the top is the chromosomal arrangement found in an immature B cell, at the bottom is shown the heavy chain polypeptide.

i) Label the process indicated by each arrow. Choose the one best option for each from:
   - protein processing
   - transcription
   - translation
   - transduction
   - DNA ligation
   - DNA rearrangement
   - RNA splicing
   - RNA ligation

ii) Indicate on the diagram below where you would expect to find each of the following components:
   - Promoter
   - Transcription terminator
   - start codon
   - stop codon

iii) Indicate on the diagram below the variable and the constant region of the heavy chain polypeptide.

V segments  D segments  J segments  constant segment

= intron regions
Question 3

a) Draw a schematic of a secreted antibody molecule and label the following structures on your diagram:  i) the light chains, ii) the heavy chains, iii) the antigen binding sites, iv) the variable regions,  v) If this were a secreted antibody, indicate the region of the antibody that would interact with macrophages.

b)

i) List two ways that combinatorial joining generates antibody diversity.

ii) What are the interactions between the light and heavy chains that make them associate with each other? What kind of bonding is this?
Question 4

a) Name the cell type of the immune system that would:
   i) bind an antigen floating around in the blood.
   ii) recognize an antigenic peptide on a MHC Class II protein displayed by a macrophage.
   iii) recognize an antigenic peptide on a MHC Class I protein displayed by an infected skin cell.
   iv) nonspecifically engulf and digest a variety of pathogens (disease-causing organisms).
   v) secrete large amounts of antibody in response to an infection.
   vi) recognize a virally infected body cell and destroy it.
   vii) the cell type that provides long-lived immunity to measles in children immunized against measles by injection of viral proteins.

b) Will an individual who does not express MHC Class II proteins on the surfaces of B cells and macrophages produce a humoral immune response? Explain your answer.

c) Below is a drawing of a putative human virus pathogen.

![Virus Pathogen Diagram](image)

i) Which viral protein(s) is likely to raise an antibody response in a natural infection? Why?

ii) Which viral protein(s) is likely to raise cell-mediate response (killer T cell lines) in a natural infection? Explain your answer.