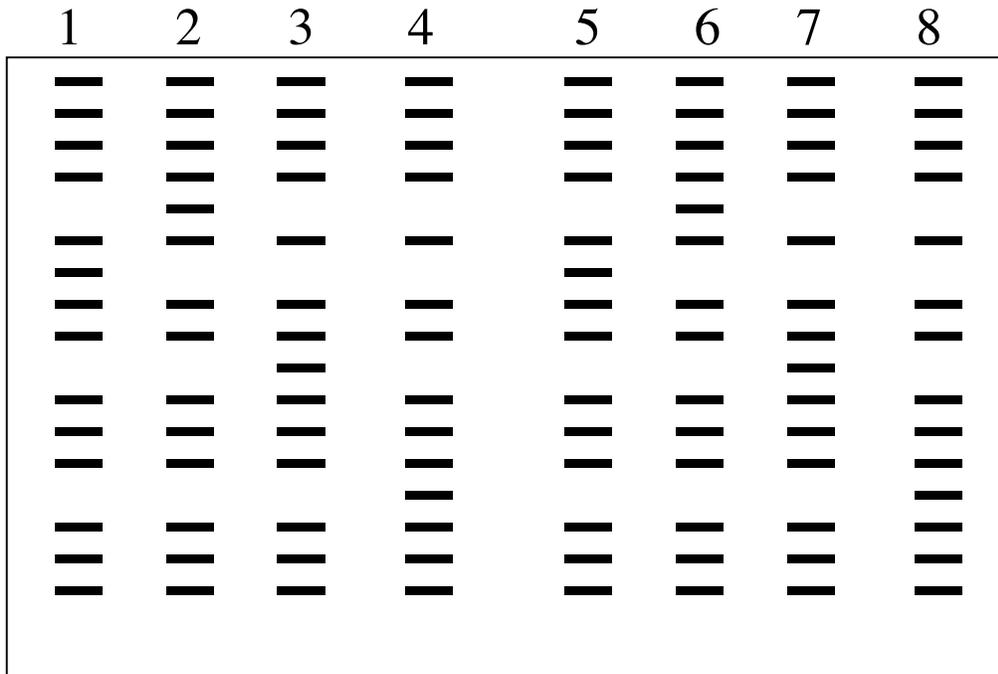


Solutions to 7.012 Problem Set 8

Question 1

You have isolated cells from an alien organism called Sacul Sinned. You want to know if these cells contain proteins that are similar to the cyclins found in eukaryotic cells. To this end, you radioactively label all the proteins in the cell to allow for visualization on a gel. You then synchronize the cells by placing them in low serum for 1 day. Finally, you stimulate the cells with PDGF and isolate protein from the cells at different time points. The proteins are then run out on a gel similar to a DNA gel, such that the proteins are separated by size and you can visualize them through their radioactivity.



a) Circle the protein bands on the gel that would be good candidates for cyclin proteins? Explain your choices.

Any of the bands that appear and then disappear during your time course would be good choices as potential cyclin proteins because cyclin proteins are only present during the part of the cell cycle that they regulate.

b) How many cell cycles did your time points cover if each of the protein bands you selected above are actually cyclin proteins? Explain your answer.

The time points cover 2 cell cycles. Cyclins are proteins that bind to cyclin dependant kinases and activate them. The cyclin proteins are only present during the part of the cell cycle that they regulate. If the bands that you circled are cyclin proteins then the fact that each of them appears twice over your time course suggests that your cells have gone through two cell divisions.

Question 1, continued

c) What does synchronization do to the cells and why is it necessary for this experiment?

Synchronization places all the cells in the same stage of the cell cycle, such that as the cell cycle progresses they enter the other phases at the same time. It is necessary for the above experiment because if the cells were in asynchronous growth, then you would not be able to detect the disappearance and reappearance of individual cyclin proteins because the times at which this happened would be different for each of the cells on your tissue culture plate.

d) Why does low serum or no serum synchronize your cells?

Low or no serum causes all the cells to exit the cell cycle and enter G_0 . The cells are not receiving enough extracellular growth signals in low or no serum to start another cell cycle so they enter quiescence or G_0 . Thus, when serum is added all the cells begin the cell cycle together.

e) Since you used PDGF in your experiment and it stimulates your cells to divide, you assume the alien cells contain a protein similar to the PDGF receptor. How does PDGF binding to the receptor cause the activation of the receptor and subsequent promotion of the cell cycle?

PDGF causes the receptor subunits to dimerize, when they dimerize the cytoplasmic domains cross phosphorylate each other and this activates the receptor. The activation of the receptor and its downstream targets leads to an increase in cyclinD protein levels among other things, which promotes cell cycle progression.

Question 2

Your professor Flow Enirehtac asks you to learn more about the PDGF receptor inside these alien cells. In order to discover the function of various regions in the protein you create cells that no longer express the wild type form of the receptor but various truncated forms.

You then use an antibody against the PDGF receptor in order to visualize where in these cells the protein is located. You find that the PDGF receptor protein is found in the cytosol of all the cells.

a) All the truncated forms of the PDGF receptor are missing sequence from one end.

- Which end of the receptor protein is likely to have been deleted?

The N-terminus. This deletion most likely removes the signal sequence which directs this protein to the endoplasmic reticulum.

- Why would this deletion change the localization of the PDGF receptor?

Without this sequence the protein is produced in the cytosol.

b) You also have cells that have a 30 amino acid deletion at the other end of the PDGF receptor. Would you expect this type of receptor to function in the presence of PDGF? Why or why not? *You may expect that this receptor would not function as it is missing 30 amino acids of the region that is phosphorylated.*

c) You look closely at many other cell lines that fail to respond to PDGF.

i) Type 1 cells have a single gene mutation and fail to respond to all growth factors. Antibody staining of these cells shows that many different membrane proteins are now found free in the cytoplasm. Which component(s) of the protein secretion pathway might be missing in Type 1 cells.

The signal sequence or SRP

ii) What other kinds of proteins might be affected in Type 1 cells?

Secreted proteins.

iii) Type 2 cells do not respond to PDGF because PDGF receptor proteins are found outside the cell. What kind of mutation would lead to this result?

A mutation in the transmembrane domain.

Question 3

In order to study genes involved in the progression of cancer you do gene expression analysis. To do this you compare the mRNA levels of normal cells versus tumor cells. What you find from this experiment is that some genes are upregulated in the tumor cells i.e. they have an increase in the mRNA levels of a certain gene and that some genes are downregulated in the tumor cells meaning that they have a decrease in the mRNA levels of certain genes. You also have some genes whose mRNA levels stay the same.

a) What is the most likely function of the genes that are upregulated in the tumor cells?
These genes are most likely involved in the promotion of the cell cycle.

b) What is the most likely function of the genes that are downregulated in the tumor cells?
These genes are most likely involved in the inhibition of the cell cycle.

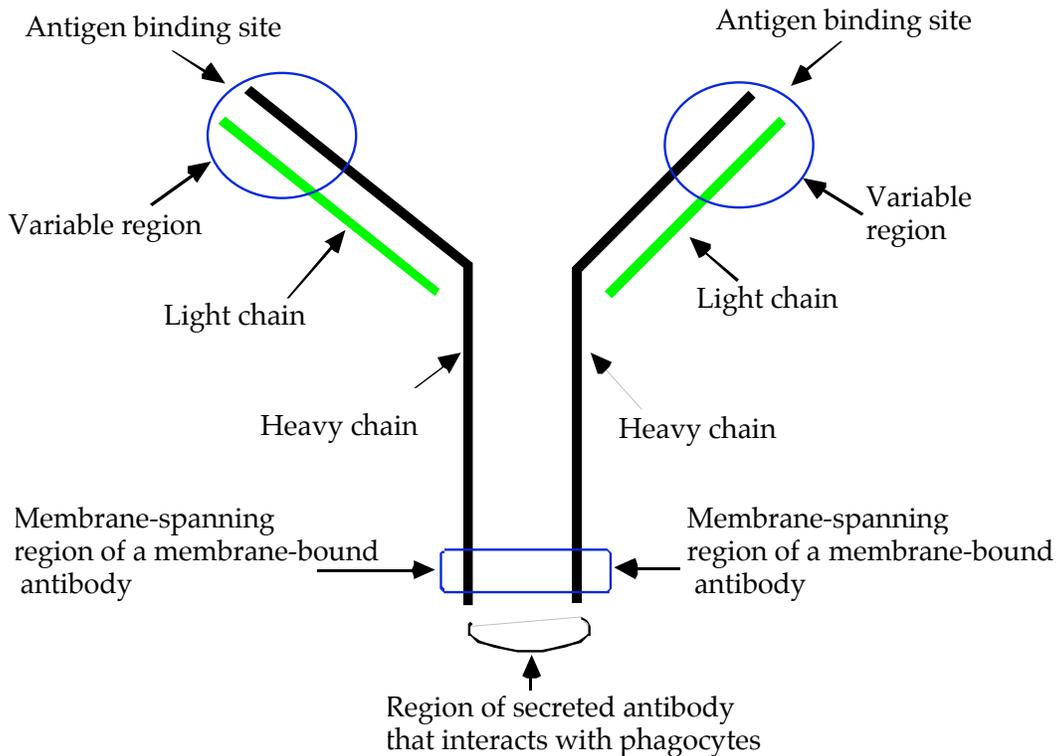
c) What is the most likely function of the genes that are the same in tumor and normal cells?
Genes that stay the same may not be involved directly with the regulation of cellular proliferation.

d) You find out later that one of the genes whose mRNA levels stayed the same actually plays a critical role in the progression of cancer. How can this be?
Either this gene obtained a mutation which changes how the protein acts (a mutation that causes the signaling molecule to be constantly on) or how it is regulated at the translational level such that it has increased protein levels but not mRNA levels.

e) You clone the gene from (d) and you discover that it is p21^{Ras}. You then take normal fibroblasts and overexpress this allele of the Ras gene in them. To your surprise the cell do not proliferate, but stop dividing. Give an explanation of for this outcome.
Overexpression of Ras in normal fibroblast causes cells to stop dividing and enter a state called senescence. This result can be explained in two ways. Over expression of Ras in an otherwise normal cell causes molecules involved in growth inhibition to be activated. This suppresses the effects of Ras. Also, cells with tumorigenic ability are not produced by a mutation in one gene. It takes several deregulated genes to give a cell that will now grow uncontrollably. Only if you overexpressed Ras in fibroblast that are missing Rb and p53 function would you get cells that grow uncontrollably. You need to get rid of the brake while pressing on the gas pedal.

Question 4

Shown below is a schematic representation of an antibody made in B cells.



a) Label the following structures (i) – (v) on the antibody 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.

vi) If this were a membrane-bound antibody, indicate the membrane-spanning region of the antibody.

b)

i) List **two** ways that antibody diversity is created.

1) Association of different light chains and different heavy chains

2) Recombination of different V (D) and J segments on the immunoglobulin genes.

3) Imprecise joining of nucleotides at the joining sites.

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

Disulfide bridges = covalent bonds

Question 4, continued

c) Name the cell type of the immune system that would:

i) bind an antigen floating around in the blood. *B cell*

ii) recognize an antigenic peptide on a MHC Class II protein displayed by a macrophage. *Helper T cell*

iii) recognize an antigenic peptide on a MHC Class I protein displayed by an infected skin cell. *Killer T cell*

iv) nonspecifically engulf and digest a variety of pathogens (disease-causing organisms). *Macrophage*

v) secrete large amounts of antibody in response to an infection. *Plasma B cell*

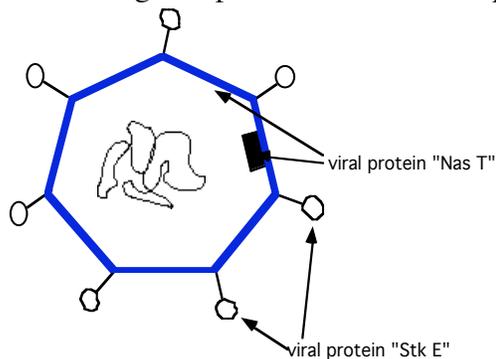
vi) recognize a viral infected body cell and destroy it. *Cytotoxic (killer) T cell*

vii) Provide long-lived immunity to measles in children immunized against the measles virus by injection of viral proteins. *Memory cell.*

d) 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.

Without MHC class II protein expression, antigenic peptides cannot be presented to a helper T cell. As a result, the helper T cells are never activated and neither are the B cells.

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



i) Which viral protein(s) is likely to raise an antibody response in a natural infection? Why?
The Stk E protein is found on the surface of virus and is therefore likely to encounter B cells.

ii) Which viral protein(s) is likely to raise cell-mediated response (killer T cell lines) in a natural infection? Explain your answer.
Both Stk E and Nas T proteins would be present in the cytoplasm of an infected cell so both could give rise to lines of Killer T cells.

Question 4, continued

f) State whether the following are true or false. If the statement is false, explain why it is false.

i) Memory B cells generated in a primary immune response give rise to antibody-secreting cells during a secondary immune response.

True.

ii) Only the variable regions of antibodies make actual contact with the epitopes (antigenic determinants) of the appropriate antigen.

True.

iii) Helper T cells (T_H) are involved in the activation of both B cells and cytotoxic T cells.

True.

iv) Antibody molecules are bivalent, which means they bind two unrelated antigens simultaneously.

False. Antibody molecules are bivalent because they bind two identical antigens simultaneously.

v) Humoral immunity involves circulating antibodies that defend against free bacteria and viruses. Cell-mediated immunity is carried out by specialized T cells that target infected body cells.

True.

g) When an activated helper T cell (T_H) binds to a B cell presenting the appropriate antigen on the B cell's Class II MHC molecules, the T_H activates the B cell. This stimulates the B cell to proliferate and make more antibodies to fight the invasion.

i) Why must the T_H and B cells specifically bind in order for this activation to occur? Why don't the activated T_H cells simply secrete an activating hormone into the blood without ever binding to a B cell?

The T_H cell recognizes and activates only the B cell that is displaying the appropriate antigen on its surface. If the T_H cell secreted the activating hormone into the blood, other B cells that did not make the specific desired antibody might become activated. This is the specificity aspect of the humoral response.

ii) When the animal is no longer fighting the infection, and the antigen is cleared from the body, many of the B cells die since they are no longer needed. Some however, continue to survive. What function do these cells provide?

These B cells serve as memory cells, which can produce antibody (in a secondary response) if the animal is infected with the same antigen at a later point in its lifetime.