

2006 7.012 Problem Set 6 KEY

** Due before 5 PM on **WEDNESDAY**, November 22, 2006. **

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

PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

1. You create an artificial cell in a test tube. This artificial cell consists of a “cytoplasm” (i.e. the inside of the cell), a lipid bilayer, and the outside. The “cytoplasm” contains 150 mM NaCl, whereas the outside solution contains 15 mM NaCl. The only kind of proteins present in the membrane are Cl⁻ ion channels, but they are closed.

(a) Are any ions flowing across the cell membrane? If so, which ions are flowing and are they flowing inward or outward?

None are flowing. The only ion channels present in the membrane are Cl⁻ channels, but they are closed. Ions cannot pass through the lipid bilayer membrane without an open channel because they are charged and the inside of the bilayer is nonpolar.

(b) Is there a membrane potential across the cell membrane? If so, is the membrane potential positive on the inside or negative on the inside?

There is no membrane potential across the cell membrane. The charge on each side of the membrane is balanced. On the inside of the membrane, 150mM Na⁺ is balanced by 150mM Cl⁻. On the outside of the membrane 15mM Na⁺ is balanced by 15mM Cl⁻.

(c) Are there concentration gradients across the cell membrane? If so, for which ions are there concentration gradients, and are those ions at higher concentrations or lower concentrations inside the cell?

There are concentration gradients for both Na⁺ and Cl⁻. Both Na⁺ and Cl⁻ are at higher concentrations inside the cell.

Now assume that, all of the sudden, the Cl⁻ channels in the cell membrane open.

(d) Are any ions flowing across the cell membrane? If so, which ions are flowing and are they flowing inward or outward?

Cl⁻ ions are flowing outward. The Cl⁻ channels are now open so Cl⁻ will move down its concentration gradient.

(e) Is there now a membrane potential across the cell membrane? If so, is the membrane potential positive on the inside or negative on the inside?

There is a membrane potential that is positive on the inside. Since Cl⁻ ions have moved to the outside of the cell membrane, the Na⁺ ions inside the cell are no longer balanced by Cl⁻ ions, causing a positive membrane potential inside the cell.

(f) Are there concentration gradients across the cell membrane? If so, for which ions are there concentration gradients, and are those ions at higher concentrations or lower concentrations inside the cell?

There are concentration gradients for both Na⁺ and Cl⁻. Both Na⁺ and Cl⁻ are at higher concentrations inside the cell. Even though the Cl⁻ channels are open, very few ions (~ one millionth of them) leave the cell before the concentration gradient and electric potential are in equilibrium, so the concentration gradient of Cl⁻ remains virtually unchanged.

You now create a different artificial cell in a different test tube. This artificial cell consists of a “cytoplasm” (i.e. the inside of the cell), a lipid bilayer, and the outside. The “cytoplasm” contains 150 mM NaCl, whereas the outside solution contains 15 mM NaCl. The only kind of proteins present in the membrane are H⁺/Na⁺ pumps, which simultaneously pump one H⁺ outside the cell for every one Na⁺ it pumps inside the cell, but these pumps are inactive. (Assume there is an excess of ATP both inside and outside of the cell that allows the pump to function. Also assume the NaCl is dissolved in water, which is where the H⁺ ions are coming from.)

Now assume that, all of the sudden, the pumps in the cell membrane become active.

(g) Are any ions flowing across the cell membrane? If so, which ions are flowing and are they flowing inward or outward?

Yes, H⁺ is moving outward and Na⁺ is moving inward. This is because the pump is actively transporting these ions in these directions.

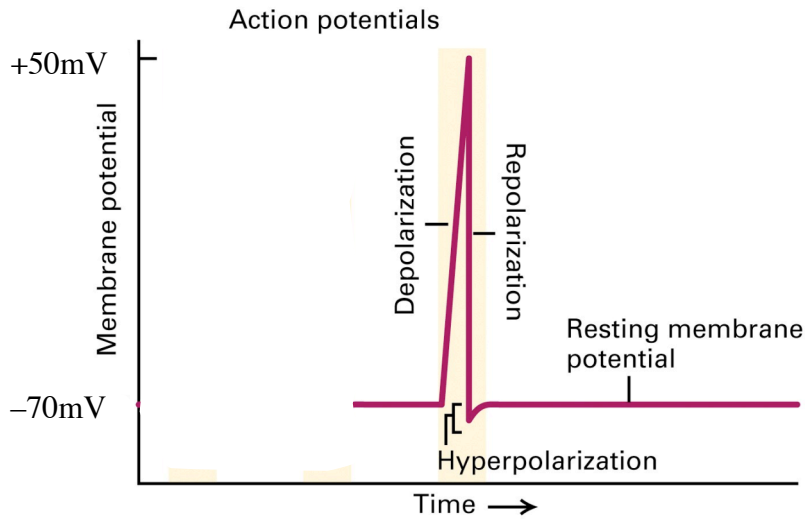
(h) Is there a membrane potential across the cell membrane? If so, is the membrane potential positive on the inside or negative on the inside?

No. For each Na⁺ moved out of the cell, an H⁺ is moved into the cell. This leads to a conservation of charges inside and outside the cell.

(i) Are there concentration gradients across the cell membrane? If so, for which ions are there concentration gradients, and are those ions at higher concentrations or lower concentrations inside the cell?

Yes, both Na⁺ and Cl⁻ have concentration gradients that are higher inside the cell. H⁺ has a concentration gradient that is higher outside the cell.

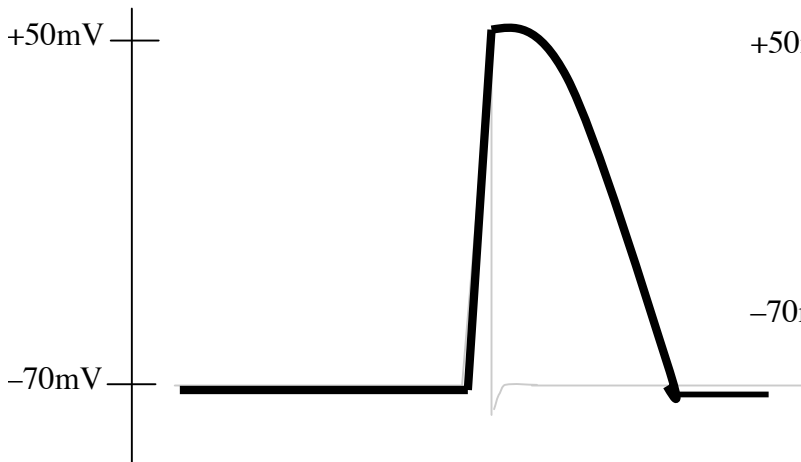
2. A normal action potential can be graphed as follows:



Draw what an action potential would look like if you **inhibited** the following voltage-gated channels in the following graphs. A normal action potential is drawn into each graph lightly, for comparison.

(a) ... voltage-gated K⁺ channels

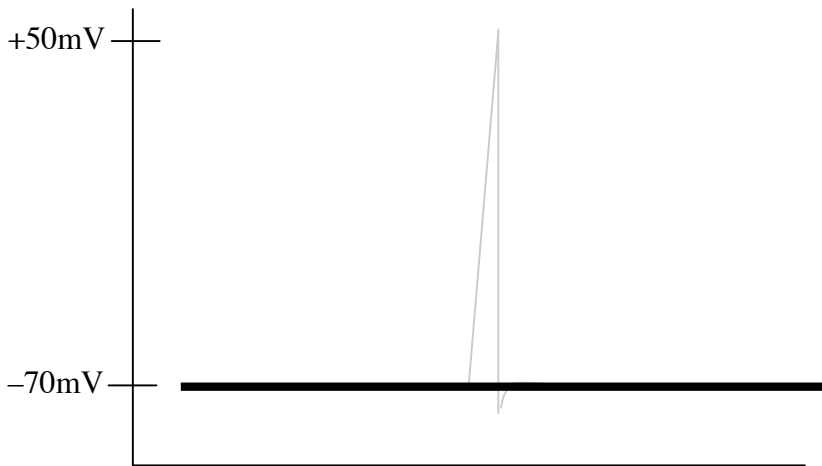
(b) ... voltage-gated Ca⁺⁺ channels
Voltage-gated Ca⁺⁺ channels are not involved in the actions potentials we have talked about.



For part (a), voltage-gated K⁺ channels are normally involved in repolarization, so repolarization will not happen so quickly. It will still happen, though, just more slowly, because the resting K⁺ channels that exist in the membranes of all cells are still open. Thus those channels will allow for eventual repolarization of the cell.

(c) ...voltage-gated Na⁺ channels

If the Na⁺ channels do not open then there will be no depolarization of the cell and no action potential.



(d) After depolarization occurs during a normal action potential, is the concentration of sodium inside the cell high or low with respect to the concentration of sodium outside the cell?

The concentration of sodium will be low inside. The Na⁺ concentration will have barely changed. So few sodium ions actually move into the cell during an action potential that the concentration will change only slightly, but not enough to even out or reverse the gradient.

(e) Do you think that action potentials in a neuron would cease immediately after one exposes the neuron to a drug that inhibits the Na⁺/K⁺ pump?

No. Many action potentials could happen because the concentrations gradients of ions barely change with each action potential. After many hundreds of action potentials there eventually will be a great enough change in the concentration gradients to require the pump to restore them. However a single action potential or a few action potentials does not lead to enough change in the concentration gradients to require the pumps to restore them before the next action potential can be sent.

Loss of function mutations in some genes expressed in neurons cause flaccid paralysis (i.e. muscles cannot contract at all), while loss of function mutations in other genes cause rigid paralysis (i.e. muscles are permanently contracted).

(f) Into which one of the two categories of mutants would the gene encoding the acetylcholine receptor found in muscle cells fall?

Flaccid paralysis. Without the acetylcholine receptor, the muscle cells would never receive the signal of acetylcholine from the neurons. This means that the muscle cells would never become depolarized and therefore would never receive the signal to contract.

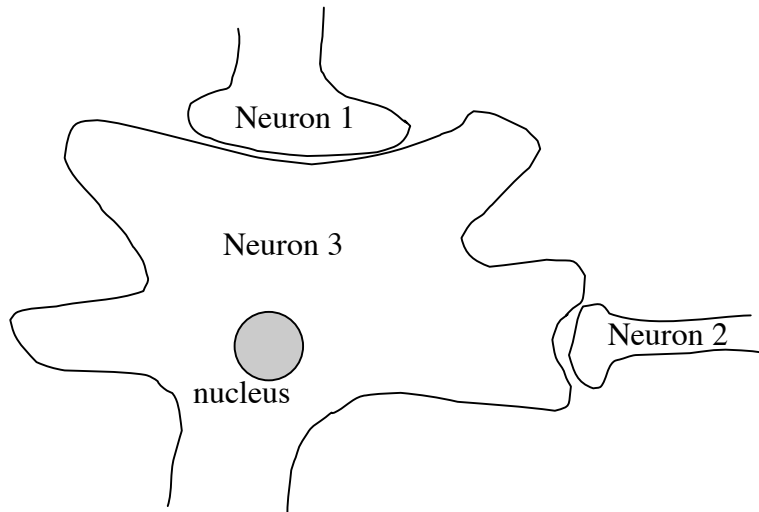
(g) Into which one of the two categories of mutants would the gene encoding acetylcholine esterase fall?

Rigid paralysis. A loss of function mutation in acetylcholine esterase would cause acetylcholine to persist in the synaptic cleft, since it will not be degraded. This would cause acetylcholine to continuously signal the muscle to contract.

(h) Into which one of the two categories of mutants would the gene encoding the voltage-gated Ca^{2+} channel fall?

Flaccid paralysis. If the voltage-gated Ca^{++} channels did not function, then synaptic vesicle fusion and release of neurotransmitters would never occur. Therefore the muscle would never be signaled to contract.

In the schematic below, Neuron 3 receives signals from two different presynaptic cells, Neuron 1 and Neuron 2. The input from Neuron 1 is excitatory, whereas the input from Neuron 2 is inhibitory.



(i) Put an X in each box where the following proteins would be found.

Proteins	Location				
	Terminal of Neuron 1	Axon of Neuron 1	Terminal of Neuron 2	Axon of Neuron 2	Dendrites of Neuron 3
Vesicle docking and fusion proteins (e.g. synapsin)	X		X		
Ligand-gated Cl^- channels					X
Voltage-gated Ca^{++} channels	X		X		

Name: _____ KEY _____

Ligand-gated Na ⁺ channels					X
Voltage-gated K ⁺ channels		X		X	
Voltage-gated Na ⁺ channels		X		X	

Vesicle docking and fusion proteins are involved in allowing the membrane vesicles that contain neurotransmitter to fuse with the cell membrane to release neurotransmitter into the synaptic cleft. These proteins must be found wherever the vesicles are found, which is at the axon terminus of a pre-synaptic cell.

Ligand-gated channels are channels that are opened by the presence of their ligand, or the molecule that binds and interacts with them. The ligand gated channels we have discussed are ion channels that are opened in response to binding neurotransmitter. These channels are found on the dendrites of the post-synaptic cell.

Voltage-gated calcium channels are found at the axon terminus of a pre-synaptic neuron. When an action potential reaches the end of the axon, it stimulates these channels to open. Thus calcium rushes in to the terminus, triggering fusion of vesicles to the membrane.

Voltage-gated K⁺ and Na⁺ channels are involved in the formation of action potentials. Action potentials occur along the axon of the neuron.

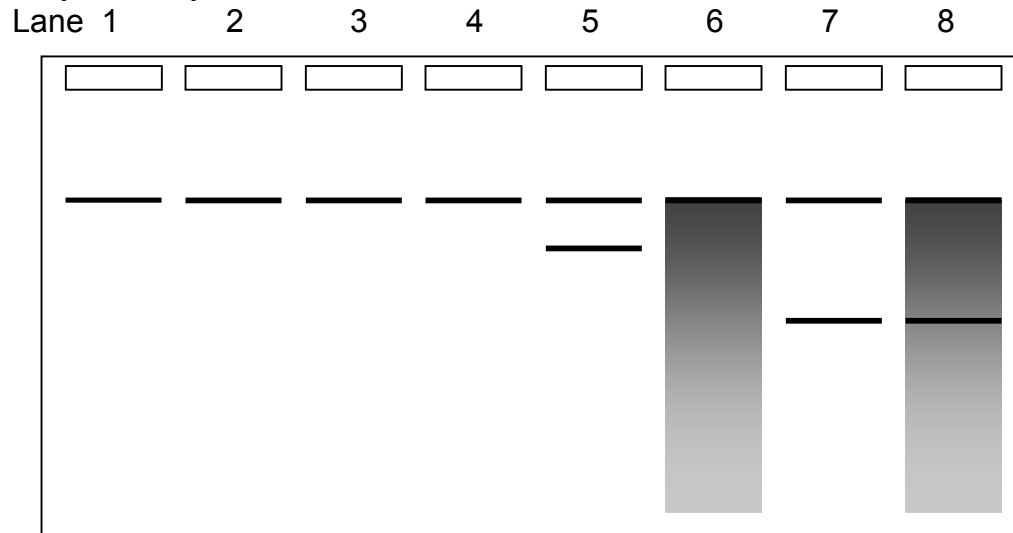
3. For the sake of this problem, assume that, during VDJ recombination, one allele of an antibody locus in every diploid B cell gets rearranged, and the other one doesn't (as is often true for B cells). Say you developed a PCR assay to test whether the two alleles of the antibody locus had been rearranged or not. You design primers to the regions of the genome directly flanking this gene. You do the PCR reaction on DNA extracted from the following cell populations:

- one skin cell (lane 1 of the gel below)
- a large population of skin cells (lane 2 of the gel below)
- one sperm cell (lane 3 of the gel below)
- a large population of sperm cells (lane 4 of the gel below)
- one mature B cell (lane 5 of the gel below)
- a large population of mature B cells (lane 6 of the gel below)

(a) You load each separate reaction into one lane of the gel below. In the gel below, draw all of the bands you would expect to see in lanes 2 - 6 of the gel. Lane 1 has been done for you, and lanes 7 and 8 will be filled in during part **(b)**.

Lanes 1 through 4 will all have the same size bands because both skin cell alleles and the allele in each sperm cell have not been rearranged. Thus, although skin cells are diploid,

both their alleles will be unarranged, and thus will be the original size. Rearrangement will only happen to one allele in B cells. Thus, for every B cell, you will see one unarranged allele of the original size, and the other allele will be some length that is shorter. It is shorter because most of its V and D and J segments have been cut out. Lane 5 shows one unarranged allele and one rearranged allele in a single B cell. Lane 6 shows a band for the WT allele and then a smear where all of the different B cells have arranged their second allele differently. (The smear occurs because there are so many different bands of a light intensity that they all blend in with each other.)



(b) A patient with multiple myeloma has a clonal over-proliferation of one B cell that had been mature enough (at the time of cancer development) to secrete antibodies. Go back to Lane 7 of the gel and draw in the bands that you would see there if you had done your PCR reaction on a single cancerous B cell from the myeloma of a multiple myeloma patient. Go back to Lane 8 of the gel and draw in the bands that you would see there if you had done your PCR reaction on a population of B cells from that same patient with multiple myeloma.

Lane 7 shows a single B cell myeloma cell with one unarranged allele and a rearranged allele. (The rearranged allele will be a different size than the rearranged allele in the B cell from lane 5 since every different B cell will rearrange its one allele differently.) Lane 8 shows the same smear as lane 6 since this is a population of B cells, but since the myeloma has led to overproliferation of a particular B cell (namely, the one drawn in lane 7) into a clonal population, there is also a strong band for the rearranged allele that was seen in lane 7. One way to think about this is to imagine that a person without cancer has 1 million B cells in the sample you took from them, and each one has a different arrangement. The PCR in that case would look like lane 6. A myeloma patient might have 3 million B cells, one million that a non-cancer-patient has, and then 2 million B cells that are all direct descendants of the B cell that you drew in lane 7. Thus their entire immune system would give the PCR pattern shown in lane 8, where there are the normal 1 million cells that give

the smear pattern, and then an additional 2 million cells that give the same pattern as the one single cancer cell shown in lane 7.

4. As we learned in the cancer unit, *myc* is one example of an oncogene. One way that *myc* can be mutated into its oncogenic cancer-causing form is by a translocation. A translocation is when two different chromosomes (e.g. chromosome #9 and chromosome #22) exchange segments at their ends. There is a specific translocation found in the tumor cells of Burkitt's lymphoma patients that places the coding region of *myc* (found on chromosome #8) under the promoter of an antibody-encoding gene (found on chromosome #14).

For part (a) only, assume that this translocation occurred early on in development (say at the 64-cell stage) in a cancer patient, such that lots of cells in the body contained this same translocation.

(a) Why would this patient get a lymphoma specifically, as opposed to any other type of cancer?

The antibody encoding gene promoter on chromosome #14 is only active in B-cells so there will be no overexpression of *myc* in other cell types in the body. Although many cell types were affected by this translocation since it occurred so early in development, only those that normally activate the antibody gene promoter (i.e. only B cells) will actually be overexpressing the *myc* oncogene.

For parts (b) - (d), assume that the translocation discussed occurred in only one developing B cell in each patient.

(b) Why does the *myc* open reading frame being under control of the antibody promoter lead to cancer?

The antibody promoter will lead to *myc* overexpression in the B cell with the translocation. The *myc* gene activates the cell cycle so its overexpression will lead to loss of cell cycle control and hyperproliferation of the B cell.

There is a specific translocation found in the tumor cells of follicular B-cell lymphoma patients that places the coding region of *Bcl-2* (found on chromosome #18) under the promoter of an antibody-encoding gene (found on chromosome #14). The function of *Bcl-2* is to prevent apoptosis.

(c) Why does the *Bcl-2* open reading frame being under control of the antibody promoter lead to cancer?

The antibody promoter will lead to overexpression of the *Bcl-2* gene in the B-cell with the translocation. Since *Bcl-2* prevents apoptosis, there will be enhanced survival of this particular B-cell, leading to a larger-than-normal population of B cells. Its proliferation will not be kept in check by the normal apoptosis machinery that is utilized to regulate the number of each B cell population.

(d) Why do you think that it is so common to have translocations involving chromosome #14 in cancers that are specifically cancers of B cells?

Since B-cells are actively rearranging their antibody alleles on chromosome #14 by breaking and rejoining chromosome segments to do VDJ rearrangements, sometimes the machinery involved in this process will accidentally cause a translocation of another chromosome to chromosome #14 (which is NOT supposed to happen) instead of a rearrangement within chromosome #14 itself (which is supposed to happen).

5. When an activated helper T cell (T_H cell) 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.

(a) Why must the T_H and B cells contact each other and specifically bind in order for this activation to occur? (i.e. What would be the problem if the activated T_H cells simply secreted an activating hormone into the blood without ever binding to a B cell?)

The specific binding of a helper T cell to a B cell ensures that only a particular B cell is activated and stimulated to produce more clones of itself to fight an infection. If this process was not specific and helper T cells released activating hormones into the blood then many B cells would be activated to proliferate and would this be a waste of the body's resources for fighting a specific infection, and would result in an abnormally high number of B cells in the bloodstream.

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

These cells serve as memory for the immune system. If the body is ever infected by the same antigen-producing pathogen again, then it can immediately respond to the infection without the delay of finding a new B cell that responds to the antigen. This is how vaccines work. They expose the immune system to specific antigens that allow the immune system to create memory B cells that can respond if the body is ever infected with the pathogen against which the vaccine was formulated.

(c) When a B cell is first generated, the antigen that it recognizes is determined based on whichever amino acid sequence and structure is possessed by the antibody that B cell produces. Many of these newborn B cells are selected to undergo apoptosis. Why might an autoimmune disorder result in a patient who is unable to properly select which B cells are supposed to undergo apoptosis and which B cells are not?

Autoimmune disorders result when B cells produce antibodies that react to antigens from our own bodies. Generally these particular B cells are eliminated from the B cell pool via apoptosis, but if a patient cannot properly select which B cells to eliminate, then the self-reacting B cells will persist and will attack the patient's own body.

6. Fill in the blocks in the following chart.

	macrophages	B cells	Helper T cells
Name a cell surface protein involved in immunology that they produce. Choose a different protein for each column.	<u>MHC class II</u>	<u>Antibody</u> (also called <u>immunoglobulin</u>) (also has <u>MHC class II</u>)	<u>T cell receptor</u> (also has <u>CD4</u>)
Name a protein that physically interacts with the protein listed above.	<u>T cell receptor</u> and <u>the antigen</u> and <u>CD4</u>	<u>The antigen</u> (MHC class II binds T cell receptor, the antigen, and CD4)	<u>MHC class II</u> (and the <u>antigen</u>) and <u>CD4</u> (CD4 binds MHC class II and the T cell receptor)
Do they undergo genome rearrangements?	<u>No</u>	<u>Yes</u> (the antibody gene is rearranged)	<u>Yes</u> (the T cell receptor gene is rearranged)
Do they recognize specific antigens?	<u>No</u> (a macrophage will eat anything)	<u>Yes</u> (only the one recognized by the antibody)	<u>Yes</u> (only the one recognized by the T cell receptor)
Do they display antigens on MHC class II molecules?	<u>Yes</u> (anything they eat will be displayed)	<u>Yes</u> (they will only eat and display the one antigen they recognize)	<u>No</u> (T cells don't eat and display anything)
Do they undergo clonal expansion?	<u>No</u>	<u>Yes</u>	<u>Yes</u>
Do they emit growth signals? If so, which cell types do they affect?	<u>Yes – T cells</u> are affected and subsequently proliferate	<u>No</u>	<u>Yes -- B cells</u> are affected and subsequently proliferate