

Name: \_\_\_\_\_KEY\_\_\_\_\_

## 7.012 Exam Three -- 2006 KEY

Exam starts at 10:05 am and ends at 10:55 am.

There are 9 pages including this cover page.

Please write your name on each page.

Only writing on the **FRONT** of every page will be graded.  
(You may use the backs, but only as scratch paper.)

**Question 1**            **28 pts**\_\_\_\_\_

**Question 2**            **18 pts**\_\_\_\_\_

**Question 3**            **33 pts**\_\_\_\_\_

**Question 4**            **21 pts**\_\_\_\_\_

**TOTAL**                **100 pts**\_\_\_\_\_

**1. (28 pts)** Below is a list of some potential functions of some new anti-viral drugs that you are considering the possibility of creating and giving to humans:

- Drug #1: blocks RNA-dependent RNA polymerases
- Drug #2: blocks DNA-dependent RNA polymerases
- Drug #3: blocks RNA-dependent DNA polymerases
- Drug #4: blocks DNA-dependent DNA polymerases
- Drug #5: blocks integrases
- Drug #6: blocks ribosomes

**(a, 5 pts)** Which of these six drugs has been designed to specifically block only retroviruses? List all that apply.

**Drug #3.** Reverse transcriptase is an enzyme only produced by retroviruses. Reverse transcriptase is an RNA-dependent DNA polymerase. Retroviruses do not encode any of the other enzymes listed above as being drug targets, except integrase. Retroviruses do encode integrases, but so do dsDNA viruses, so drug #5 would not ONLY target retroviruses.

**(b, 5 pts)** Which of these six drugs would affect the human host? List all that apply.

**Drugs #2, #4, and #6.** Human have RNA polymerase that makes RNA from DNA (i.e. does the process of transcription), DNA polymerase that makes DNA from DNA (does DNA replication) and ribosomes that make protein from RNA (does the process of translation). The other enzymes are all viral-specific enzymes. Humans do not make RNA from RNA or DNA from RNA, so drugs #1 and #3 would not harm humans. Humans also do not express integrase, so drug #5 would not affect humans.

**(c, 5 pts)** Why does a (+) strand ssRNA virus NOT have to carry its viral polymerase protein in its capsid... whereas a (-) strand ssRNA virus MUST carry its viral polymerase protein in its capsid?

A (+) strand ssRNA virus injects the strand of RNA that is able to be translated. This RNA contains a region that is translated to make RNA-dependent RNA polymerase (the viral polymerase that does replication of an ssRNA's genome). Thus this virus does not need to bring in RNA-dependent RNA polymerase protein, because host ribosomes can just make RNA-dependent RNA polymerase protein from a section of the (+) strand of the RNA.

A (-) strand ssRNA virus injects the strand of RNA that is complementary to the strand that is able to be translated. In order to translate protein from the viral RNA, the (-) strand must be converted to a (+) strand. This conversion is done by RNA-dependent RNA polymerase. If the RNA cannot be translated, and no protein were brought in along with the RNA, then the (-) strand could never be converted to the (+) strand, and then RNA-dependent RNA polymerase could never be translated, so the virus could never make more copies of its genome.

**(d, 5 pts)** Rabies infections are often treated with an anti-rabies monoclonal antibody generated from a horse B cell. A patient is infected with the rabies virus and is given this horse antibody treatment. Five years later, the patient again becomes infected with rabies. The patient is treated with another dose of horse antibody treatment, and has a severe reaction to the treatment. Why did this happen?

**The horse antibody is a protein that a human would recognize as foreign. Thus, if you inject a horse antibody into a human, that human will mount an immune response against the horse antibody. The next time you expose the human to that horse antibody, the human will have memory B cells that will allow the human to mount a stronger, faster immune response against the horse antibody. This can lead to the human having a very strong allergic reaction to the horse antibody the second time the human is exposed to it.**

**(e, 4 pts)** In an attempt to avoid this severe reaction, the patient is instead treated with an anti-rabies antibody extracted from human blood. From whom could this antibody have been extracted? Name one reason why such an antibody could exist in the blood of the human from whom it was extracted.

**The human must have either been infected with rabies before, or must have been vaccinated with a rabies vaccine. These are the only two ways that a human would have anti-rabies antibodies floating around in their blood.**

**(f, 4 pts)** Do you think that the human antibody treatment described in part (e) most likely consists of monoclonal or polyclonal antibodies?

**Polyclonal. If a human is exposed to a virus, that human will produce antibodies to many different epitopes on the surface of that virus. Thus there will be a population of different antibodies, all against that virus, floating around in the human's blood. A polyclonal antibody collection is a group of many different kinds of antibodies, all of which react to different parts of the surface of an antigen.**

**2. (18 pts)** Say you are studying early development of zebrafish. You find that the gene *Breatheasy* is required for gill formation in zebrafish.

**(a, 3 pts)** What kind of experiment could you have done to infer this?

**You can mutate the Breatheasy gene such that it is no longer functional.**

**(b, 3 pts)** What would have been the result of this experiment that led you to this conclusion?

**If you inactivate function of the Breatheasy gene, then the fish should not have gills anymore.**

You find that the *Breatheasy* gene is only transcribed in gill cells.

**(c, 3 pts)** What kind of experiment could you have done to infer this?

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**You can do this experiment in one of two ways:**

- Isolate gills away from the rest of the fish. Isolate mRNAs from gills, load that onto one lane of a gel. Isolate mRNAs from the rest of the fish, load that onto another lane of a gel. Design a probe complementary to the Breatheasy mRNA and use that to detect whether Breatheasy mRNA is present in each lane of the gel.**
- Do an in situ hybridization. Design a probe complementary to the Breatheasy mRNA. Incubate a fish with this probe, and visualize to which tissues the probe binds.**

**Note that you can not use a GFP fusion protein to make this conclusion, because that shows you where Breatheasy protein is translated, not where the Breatheasy gene was transcribed. Even if you only saw the Breatheasy-GFP in the gills, that doesn't tell you that the gene is only transcribed in gill cells; it could be transcribed everywhere but only TRANSLATED in gill cells.**

**(d, 3 pts)** What would have been the result of this experiment that led you to this conclusion?

**The mRNA will only be present in the gill cells, so you will only see the signal in the gill cells of the fish. If you chose to run a gel, this would mean that you would only get a band in the lane in which you loaded mRNA from gill cells.**

You find that expression of the *Breatheasy* gene is sufficient to induce the organization and formation of extra gills.

**(e, 3 pts)** What kind of experiment could you have done to infer this?

**You should induce the expression of the Breatheasy gene in other tissues in the organism, by putting the Breatheasy gene under a promoter that is not gill-specific. This way, Breatheasy mRNA and protein would be made in other tissues.**

**Note that putting the Breatheasy gene in other cells in the fish will not work, because those other cells already have the Breatheasy gene in them; the problem is not that the gene isn't there, but that it's not being expressed into mRNA and protein.**

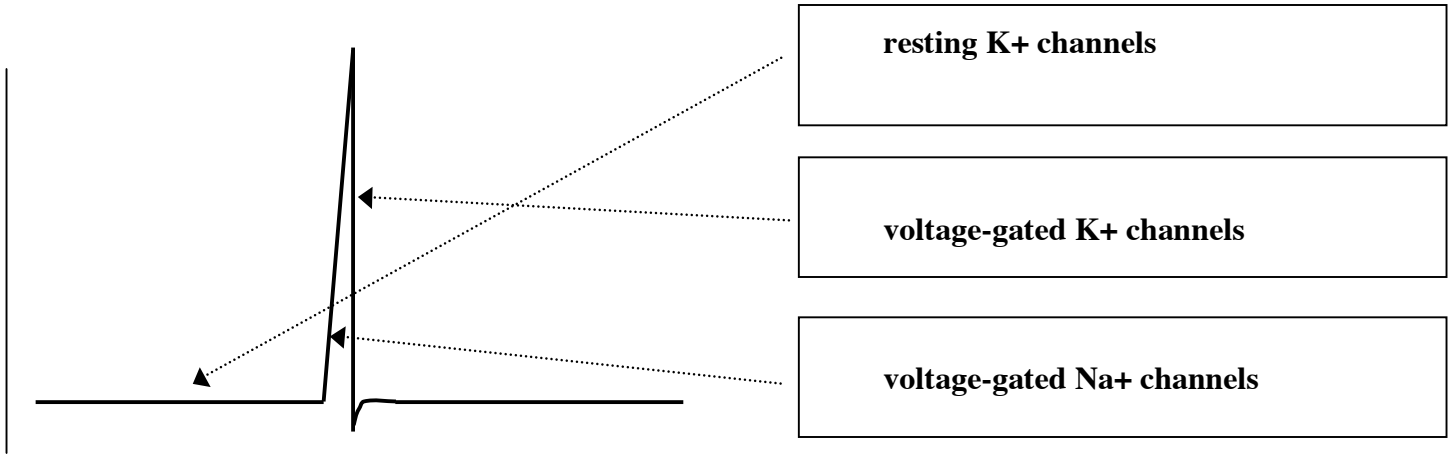
**Also note that transplanting a cell expressing Breatheasy to another part of the organism does not prove that the Breatheasy gene is what is responsible for inducing the extra gills. Cells express 1000s of mRNAs and proteins, and you must do an experiment here that shows that the one gene responsible for inducing extra gill formation is Breatheasy specifically.**

**(f, 3 pts)** What would have been the result of this experiment that led you to this conclusion?

**Extra gills would form wherever the Breatheasy mRNA and protein were made.**

3. (33 pts) Below is a picture of a normal action potential.

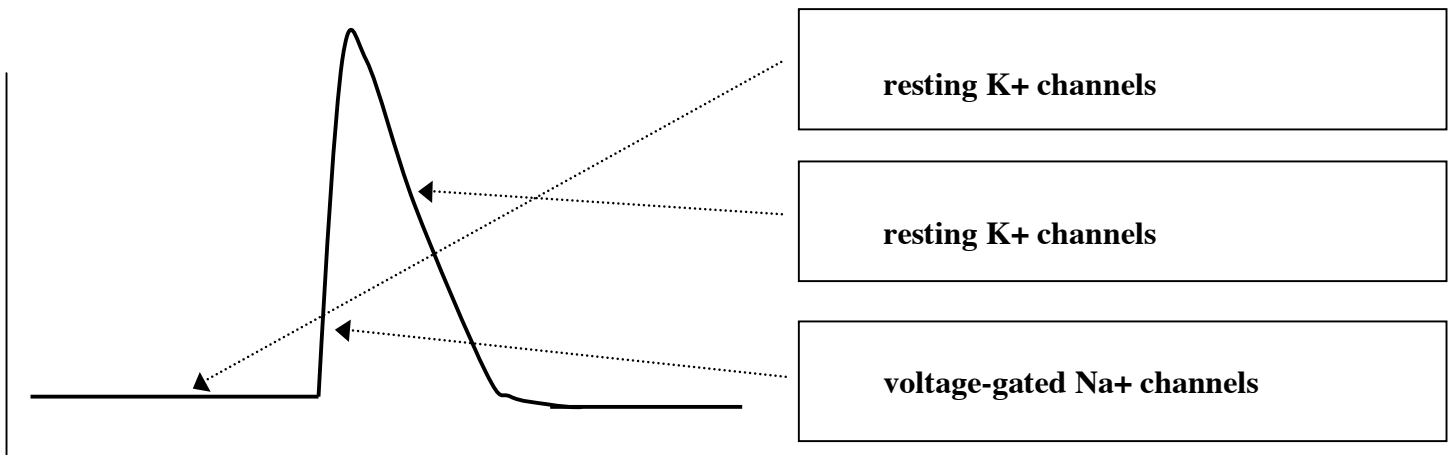
(a, 3 pts) Fill in each blank box in this diagram with the **full name** of the channel that allows for that part of the membrane potential to be achieved.



Note that the question asks for channels (so answers including pumps were not accepted, because pumps and channels are two completely different kinds of proteins). The resting membrane potential of the cell is  $-70\text{mV}$ , and this potential is generated because of the resting  $\text{K}^+$  channel, which allows some  $\text{K}^+$  ions to flow outside of the cell, thereby making the inside of the cell negative. The depolarization part of an action potential is due to the opening of voltage-gated  $\text{Na}^+$  channels, through which  $\text{Na}^+$  flows into the cell, making the inside of the cell positive. The repolarization part of an action potential is due to the opening of voltage-gated  $\text{K}^+$  channels, through which  $\text{K}^+$  flows out of the cell, making the inside of the cell negative.

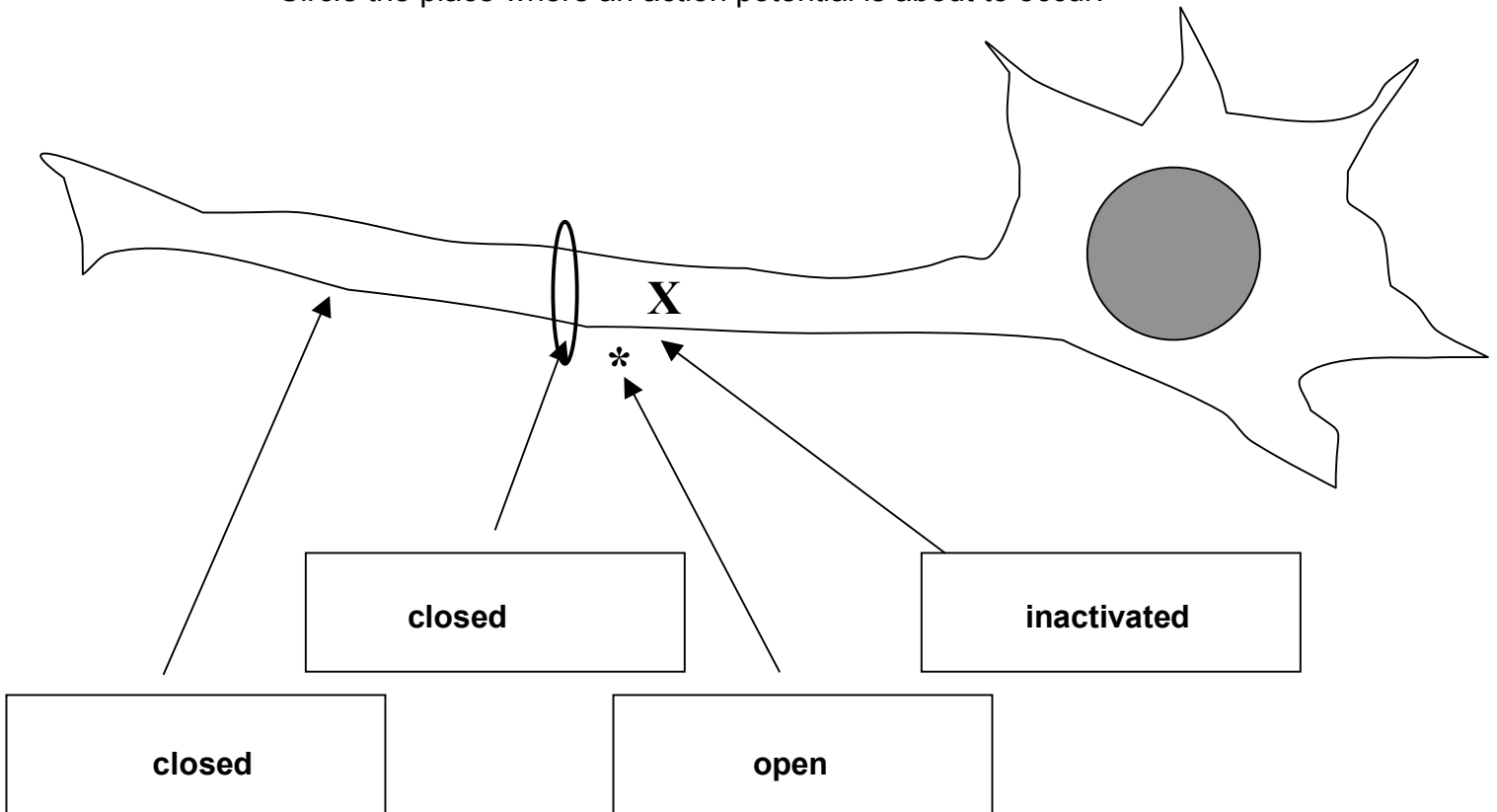
Below is drawn an action potential in a mutant neuron in which a single type of channel has been inactivated.

(b, 3 pts) Fill in each blank box in this diagram with the **full name** of the channel that allows for that part of the membrane potential to be achieved in this mutant neuron.



Note that the question asks for channels (so answers including pumps were not accepted, because pumps and channels are two completely different kinds of proteins). This mutant neuron must have had its voltage-gated K<sup>+</sup> channels inactivated, because they are what is normally responsible for the repolarization, but in this mutant neuron, the repolarization is too slow. The resting membrane potential of the cell is -70mV, and this potential is generated because of the resting K<sup>+</sup> channel, which allows some K<sup>+</sup> ions to flow outside of the cell, thereby making the inside of the cell negative. The depolarization part of an action potential is due to the opening of voltage-gated Na<sup>+</sup> channels, through which Na<sup>+</sup> flows into the cell, making the inside of the cell positive. The repolarization part of an action potential in this mutant neuron CANNOT be due to the opening of voltage-gated K<sup>+</sup> channels, because these channels are inactivated in this mutant. However, K<sup>+</sup> can still flow out, albeit slower, through the resting K<sup>+</sup> channels that are always open in all cells of our body, including our neurons.

**(c, 4 pts)** Voltage-gated Na<sup>+</sup> channels have three states: open, closed, and inactivated. These channels adopt the inactivated state for a brief period of time right after they have just been open. Label the following diagram of a neuron, in which an action potential is occurring at the place marked with a star (\*), in the following ways:  
 -- Mark the place where an action potential just occurred with an "X."  
 -- Circle the place where an action potential is about to occur.



An action potential occurs because of the opening of voltage gated Na<sup>+</sup> channels, so the channels must be open at the point where the star is found. Action potentials travel from

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the dendrites to the axon terminus, so the action potentials in this diagram would flow from right to left. This means that an action potential has just fired at the place marked by the X, and the action potential is about to travel with the place that is circled.

**(d, 4 pts)** At each arrow in the diagram above, fill in the box to indicate whether the voltage gated Na<sup>+</sup> channels found there would be open, closed, or inactivated.

An action potential occurs because of the opening of voltage gated Na<sup>+</sup> channels, so the channels must be open at the point where the star is found. Action potentials travel from the dendrites to the axon terminus, so the action potentials in this diagram would flow from right to left. This means that an action potential has just fired at the place marked by the X, and the action potential is about to travel with the place that is circled. This means that the voltage-gated Na<sup>+</sup> channels must be inactivated (i.e. they were just open a moment ago) at the place marked with an X, and closed (but about to be open) at the place that is circled, and every other place to the left of the star.

**(e, 5 pts)** What is the reason why a normal action potential only propagates unidirectionally down an axon?

Because the voltage-gated Na<sup>+</sup> channels become inactivated right after they have been opened. This means that, at the place marked with the star, Na<sup>+</sup> is flowing in, and making that part of the membrane more positive. These positive charges can diffuse somewhat to the left and right. However the voltage gated Na<sup>+</sup> channels to the right are inactive, so even though that spot on the membrane might reach threshold, those channels cannot be opened. Only the channels to the left of the starred location can be opened, so action potentials will only travel right to left in this diagram.

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**(f, 5 pts)** What would happen if you inserted an electrode into the middle of an axon that is at rest, and thereby induced a change in the membrane potential at that location to make it  $-40\text{mV}$ ? Would an action potential result? If yes, how would this action potential be similar and different from a normal action potential? If no, why not?

**Yes. An action potential would result, and it would have all of the same properties as a normal action potential, except that it would travel bidirectionally instead of unidirectionally. This is because an action potential wouldn't have just fired anywhere along the neuron, so none of the voltage gated  $\text{Na}^+$  channels would be inactive, so when you make a single point along the neuron more positive (to  $-40\text{mV}$ ), these positive charges would diffuse in both directions and activate voltage gated  $\text{Na}^+$  channels on either side of the original site of depolarization.**

**(g, 5 pts)** What would happen if you inserted an electrode into the middle of an axon that is at rest, and thereby induced a change in the membrane potential at that location to make it  $-60\text{mV}$ ? Would an action potential result? If yes, how would this action potential be similar and different from a normal action potential? If no, why not?

**No. An action potential would not result, because the threshold for opening voltage-gated  $\text{Na}^+$  channels is  $-50\text{mV}$ , and you have not allowed this neuron to reach threshold, so the voltage-gated  $\text{Na}^+$  channels would never open.**

**(h, 4 pts)** All synapses need a way to stop signaling from occurring continuously after an initial signal has been sent. How is this signal termination accomplished at nerve-muscle synapses?

**Signal termination is accomplished by the enzyme acetylcholine esterase, which cleaves and thereby inactivates the neurotransmitter acetylcholine.**

**4. (21 pts)** The following is a description of the pathway in which Ras acts. A growth factor binds to the growth factor receptor, causing it to form dimers. This activates the enzymatic activity of the receptor, leading to activation of a protein that helps Ras bind to GTP instead of GDP. Active Ras activates a protein called MAPK. Active MAPK phosphorylates a transcriptional activator. When the transcriptional activator is phosphorylated, it undergoes a conformational change that allows it to be imported into the nucleus, where it turns on expression of its target genes.

**(a, 3 pts)** What is the highest level of protein structure for the receptor protein that is altered during the action of this pathway? (Your choices are: primary, secondary, tertiary, or quaternary.)

**Quaternary. The receptor forms dimers, meaning that two protein subunits of receptor come together. Quaternary structure refers to the structure of proteins that are made up of more than one chain of amino acids, and is a level of structure that only applies to a protein that forms multi-subunit complexes.**

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**(b, 5 pts)** Are the target genes of this pathway expressed under the following conditions, in the following cells? Fill in each block of this table with the word “yes” or the word “no.”

GROWTH CONDITIONS

<i>Mutant property of cell</i>	<i><b>NO</b> growth factor present in the environment</i>	<i>Growth factors are present in the environment</i>
None (cell is wild-type)	<b>NO</b>	<b>YES</b>
Ras cannot hydrolyze GTP	<b>YES</b>	<b>YES</b>
Receptor cannot form dimers	<b>NO</b>	<b>NO</b>
Receptor protein lacks transmembrane domain	<b>NO</b>	<b>NO</b>
Activator protein has an aspartate at the position where it is normally phosphorylated	<b>YES</b>	<b>YES</b>

-- The first line of the chart is the wild-type, regulated situation, where the target genes can be turned on and off based on the usual environmental conditions.

-- If Ras cannot hydrolyze GTP into GDP, then Ras will always be bound to GTP, and thus will always be active. If Ras is always active, target genes will always be expressed.

-- If the receptor cannot form dimers, then Ras will never be activated, and target genes will never be expressed.

-- If the receptor protein lacks its transmembrane domain, then the receptor protein will not be found on the cell surface in the cell membrane. If this is true, then the receptor will not be able to bind to growth factor and thus the pathway will never be activated.

-- If the activator protein is mutated in such a way as the last line of the chart, then the activator protein will always think it is phosphorylated (because aspartate is negatively charged, like a phosphate group), and it will always go into the nucleus and activate expression of target genes.

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**(c, 5 pts)** At what level are these genes regulated in this pathway? Your choices are: transcriptionally, post-transcriptionally, translationally, post-translationally.

-- the growth factor receptor

**Post-translationally. The growth factor receptor protein is always present, but is regulated based on whether it adopts its monomer form or dimer form.**

-- Ras

**Post-translationally. The Ras protein is always present, but is regulated based on whether it is bound to GTP or to GDP.**

-- MAPK

**Post-translationally. The MAPK protein is always present, but is regulated based on whether it is in its active form or inactive form.**

-- The transcriptional activator

**Post-translationally. The transcriptional activator protein is always present, but is regulated based on whether it is in the conformation that goes into the nucleus or stays in the cytoplasm.**

-- The target genes of this pathway

**Transcriptionally. These genes are regulated at the level of whether they are transcribed or not, because they are regulated by a transcriptional activator protein.**

**(d, 4 pts)** Is the growth factor receptor gene a tumor suppressor gene or an oncogene?

**An oncogene. The growth factor receptor's function is to promote the cell cycle and encourage cell growth and division, and is thus an oncogene.**

**(e, 4 pts)** Which kind of mutation in the MAPK gene would promote the development of cancer, a loss-of-function mutation or a gain-of-function mutation?

**A gain-of-function mutation. MAPK's function is to promote the cell cycle and encourage cell growth and division, and is thus an oncogene. Gain-of-function mutations in oncogenes cause them to be hyperactive, and thus they always promote the cell cycle, and this leads to cancer.**