

Question 1

a) What role does the acrosome have in fertilization? Circle all that apply. 3 pts

- i) It responds to egg's chemoattractants.
- ii) It has enzymes that digest the zona pellucida.**
- iii) It has enzymes that digest the nuclear membrane.
- iv) It is the part of the egg that prevents sperm from binding.
- v) None of the above.

b) If calcium is injected into an unfertilized sea urchin egg, what would happen? Circle one. 3 points

- i) Capacitation would occur.
- ii) A slow block to polyspermy would be induced.**
- iii) The egg will become fertilized without sperm.
- iv) The egg will become female.
- v) The sperm will undergo an acrosomal reaction.

c) If we now mix sea urchin sperm with these eggs (in b), will fertilization occur? Circle one. 3 points

- i) No, capacitation has already occurred.
- ii) No, the sperm can no longer attach to the egg.**
- iii) Yes, the egg is now in "ready state".
- iv) No, the egg is already fertilized.
- v) None of the above.

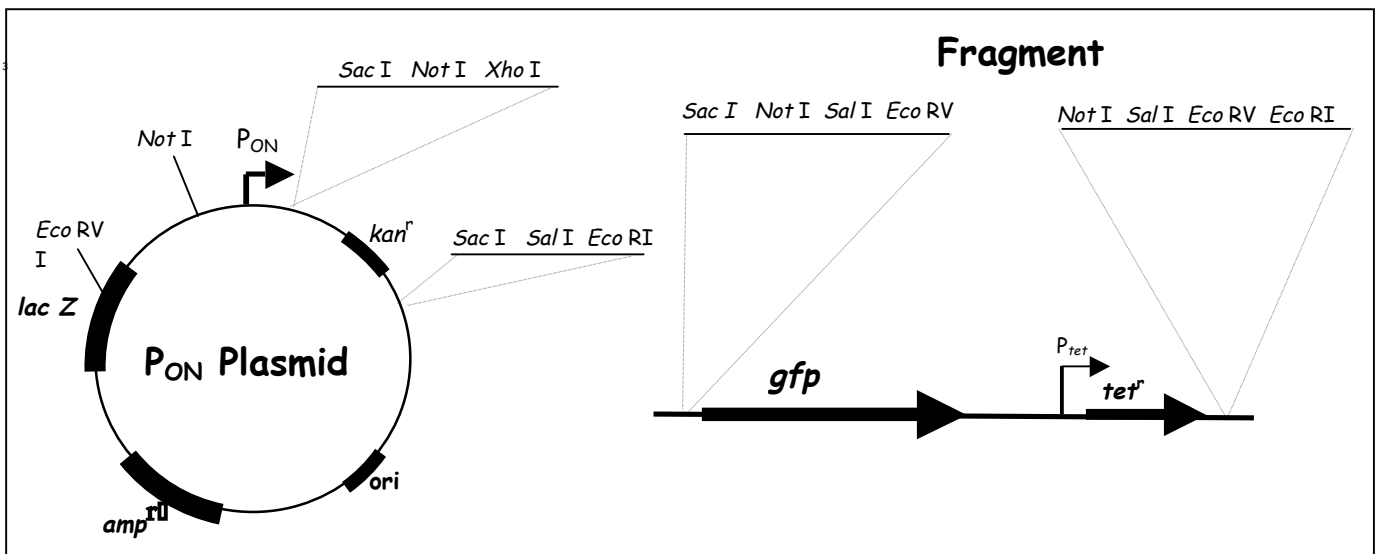
d) Could a human sperm fertilize a hamster egg? Explain your answer briefly. 5 points

No, the egg is protected by a thick zona pellucida that can be penetrated only if the ZP3 ligands within can be bound by the sperm with the appropriate receptor. These interactions are species specific. This triggers the acrosomal reaction which spills out hydrolytic enzymes to make a passage of the sperm through the Zona Pellucida.

Question 2

You wish to make your pet glow in the dark so your first step is to clone *gfp* gene, (encoding green fluorescent protein), downstream of the "ON" promoter which is always active, expressing downstream genes. You have a vector, the P_{ON} plasmid, and you've isolated a DNA fragment containing a promoter-less *gfp* gene as well as the *tet^r* gene conferring resistance to tetracycline.

The vector carries the *lacZ* gene encoding β -Galactosidase which converts the substrate, X-gal, into a blue colored product. It also carries two antibiotic resistance genes: *kan^r* and *amp^r* which allow for growth on kanamycin and ampicillin, respectively. The *lacZ*, *kan^r*, and *amp^r* genes have their own promoters (not shown). Assume all restriction sites are unique unless shown otherwise. Assume that **only** similarly cut DNA ends can ligate with each other. You may use more than one enzyme for your cloning. Assume all digestions are complete (no partial digestions).



a) In order to clone *gfp* in the correct orientation downstream of P_{ON} ...

i) what enzyme(s) would you cut the vector with? 6 points per side (*Eco RI*-*Eco RI* -3 pts) (*Sac I*-*Sac I*-3 points)

- Eco RI***
- Eco RV*
- Not I*
- Sac I***
- Sal I*
- Xho I*

ii) what enzyme(s) would cut the fragment with?

- Eco RI***
- Eco RV*
- Not I*
- Sac I***
- Sal I*
- Xho I*

b) To select for the desired construct with insert you should plate the ligation on medium with...Circle all that apply. 3 points

- Ampicillin
- Chloramphenicol
- Kanamycin
- Tetracycline**
- X-Gal

c) Bacteria transformed with original vector would grow on medium with...Circle all that apply. 4 points

- Ampicillin**
- Chloramphenicol
- Kanamycin**
- Tetracycline
- X-Gal

d) A fellow student cloned the *gfp* insert into the vector using *Not I*. However he found that there was no expression of *gfp*. Why? 3 points

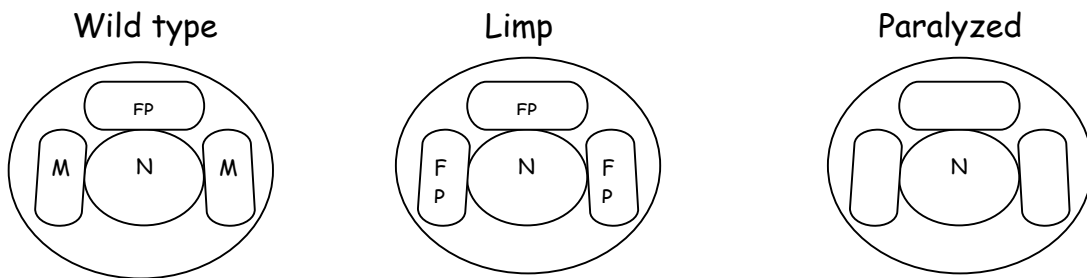
The promoter P_{ON} would be removed.

Question 3

You want to identify genes that control muscle development. Zebrafish are used to study vertebrate development, because the embryos are transparent, abundant and easily harvested. You have two temperature sensitive mutant fish that have mutations in unknown genes causing interesting phenotypes you name "Limp" and "Paralyzed". At 23°, these mutant fish eggs develop normally but if they are shifted to the restrictive temperature, 30°, within 5 hours after fertilization, the embryos exhibit the limp or paralyzed phenotypes.

You grow the wildtype and mutant embryos at 23°C, and then shift them to 30° at 4 hours after fertilization. At hour 10, you make sections through the trunk of the embryos and identify different cell types that should be present at this stage in development with specific dyes.

FP	stained by FloorPlate dye
M	stained by Muscle dye
N	stained by Notochord dye



a) The Limp and Paralyzed mutants could be defective in.... (Circle all that apply.) 5 points

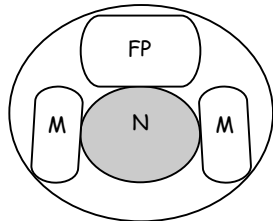
- i) the production of a protein.
- ii) the splicing of an mRNA.
- iii) responding to cell nonautonomous signals.
- iv) post translational modification of a protein.
- v) the transcription of a gene.

b) You grow the wildtype and the mutant fish embryos at 23°C and then shift them to 30° at 6 hours after fertilization. At hour 10, you stain as before. You observe that the mutant strains stain exactly like the wildtype. Why is this? Circle all that could be true. 3 points

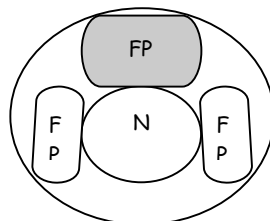
- i) Transcription factors had already induced necessary genes.
- ii) Enough cell specific protein had been made at 23°C.
- iii) Temperature sensitive proteins are no longer required by hour 6.
- iv) Temperature sensitive proteins are required until hour 10.
- v) None of the above.

Name _____

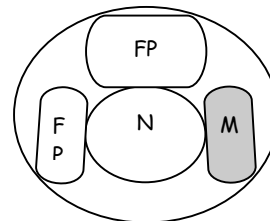
You take Limp mutant fish embryos and transplant in wild type donor cells that have been marked with a Fluorescent dye (gray). You then section the trunk and again stain as above.



marked cells derived from WT notochord precursor cells



marked cells derived from WT floorplate precursor cells



marked cells derived from WT muscle precursor cells

c) These results suggest that the Limp mutation is ... (Not Graded)

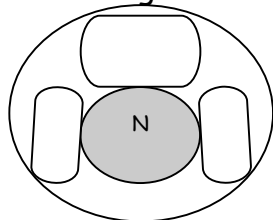
Cell autonomous

Cell nonautonomous

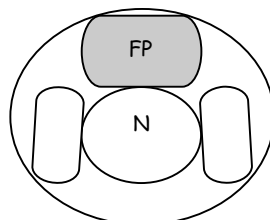
d) Briefly explain your answer in e). (Not Graded because of printing error.) 2 free points.

Wild type Notocord can send a signal to cells with receptors to become muscle.

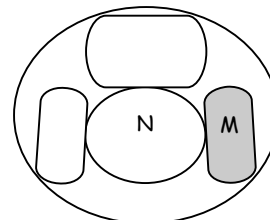
You repeat the above experiments with Paralyzed mutant fish embryos and transplant in wild type donor cells that have been marked with a Fluorescent dye (gray). You then section the trunk and again stain as above.



marked cells derived from WT notochord precursor cells



marked cells derived from WT floorplate precursor cells



marked cells derived from WT muscle precursor cells

f) These results suggest that the paralyzed mutation is ... 2 points

Cell autonomous

Cell nonautonomous

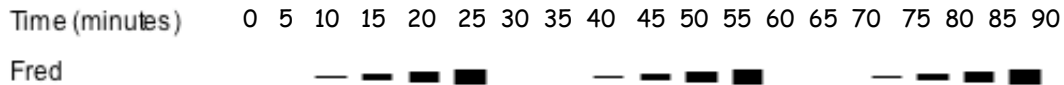
g) Briefly explain your answer in f). 4 points

Unable to receive any signals from the transplants. Possible receptor mutant or something downstream of that.

Question 4

Early frog embryos undergo several rounds of very synchronous cell division. You decide to use them to isolate and study proteins involved in cell cycle regulation. Similar to DNA fragments, proteins can be isolated and viewed on gels.

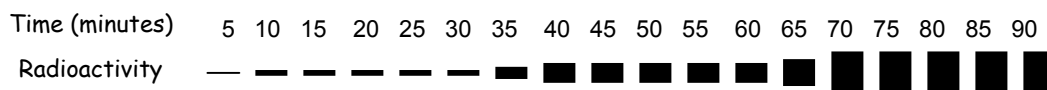
After inducing the frog eggs to begin synchronous cell division, you remove, at regular time points, a portion of the cells to isolate their proteins. After many different time points you run the samples on a gel and observe the following pattern corresponding to one particular protein that you name "Fred".



a) Which of the following hypotheses are consistent with this observation? (Circle all that are consistent with the data.) 5 points

- i) Fred is required in gap phases.
- ii) Fred is required during S phase.
- iii) Fred is required before S phase to enable entry into S.
- iv) Fred is required during M phase.
- v) Destruction of Fred permits cells to exit M phase.

You perform a similar experiment, but this time you add radioactively labeled nucleotides and at each time point measure the amount of radioactivity that has been incorporated into DNA. Your data look like this:



b) Considering this observation and your previous experiment, which of the following hypotheses are consistent with your data? (Circle all that are consistent with the data.) 3 points

- i) Fred is required in gap phases.
- ii) Fred is required during S phase.
- iii) Fred is required before S phase to enable entry into S.
- iv) Fred is required during M phase.
- v) Destruction of Fred permits cells to exit M phase.

c) You perform the following additional experiments.

Expt. 1) You purify large quantities of Fred that you inject into early frog embryos. You observe that this injection:

- blocks cell division.
- results in tightly condensed chromosomes.
- does not interfere with incorporation of radioactive nucleotides into DNA.

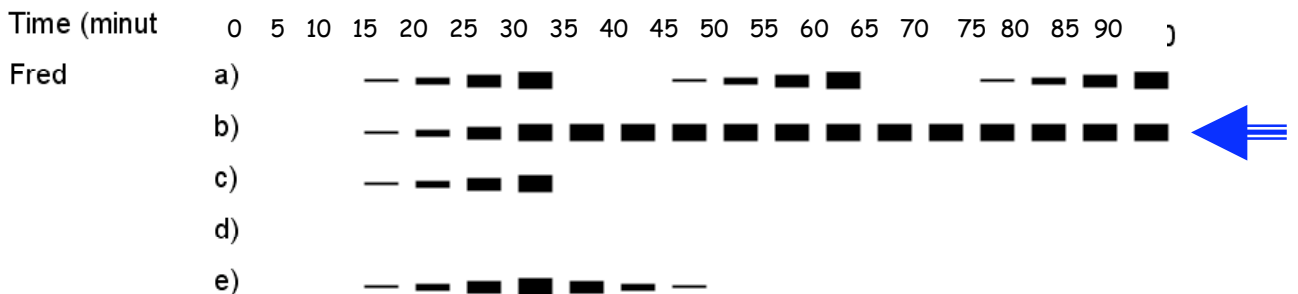
Expt. 2) You inject an inhibitor of Fred into early frog embryos. You observe that this injection:

- blocks cell division.
- results in uncondensed chromosomes.
- does not interfere with incorporation of radioactive nucleotides into DNA.

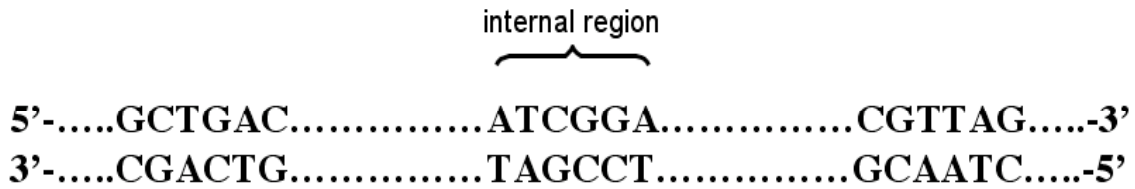
Considering all the results from all of your experiments, which of the following hypotheses are consistent with the data? (Circle all that are consistent with the data.) 2 points

- i) Fred is required in gap phases.
- ii) Fred is required during S phase.
- iii) Fred is required before S phase to enable entry into S.
- iv) Fred is required during M phase.**
- v) Destruction of Fred permits cells to exit M phase.**

d) A colleague suggests that Fred might be degraded when the spindle assembly checkpoint is satisfied. You know that nocodazole, a microtubule depolymerizing agent, disrupts the mitotic spindle and arrests cells in prophase. So you repeat the experiment from part a) using eggs that have been treated with nocodazole. Given this information, what pattern do you predict to be most likely for Fred if your colleague's model is correct? 2 points



You are interested in amplifying the gene encoding Fred from a cDNA library. Parts of the gene are shown below.



e) What reagents would you put into your PCR to amplify the above sequence? (Circle all that apply.) 4 points

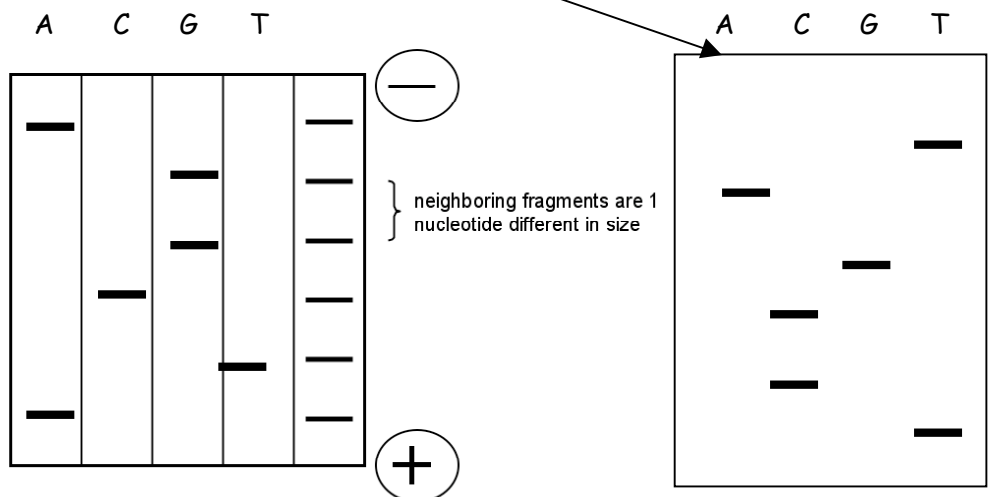
- | | |
|--|--|
| a) primer: 5'-GCTGAC-3'
b) primer: 5'-CGACTG-3'
c) primer: 5'-CGTTAG-3'
d) primer: 5'-GCAATC-3'
e) primer: 5'-GTCAGC-3'
f) primer: 5'-CAGTCG-3'
g) primer: 5'-GATTGC-3'
h) primer: 5'-CTAACG-3' | i) DNA polymerase
j) RNA polymerase
k) DNA ligase
l) primase
m) <i>Eco</i> RI
n) dNTPs
o) ddNTPs
p) ribosomes |
|--|--|

You decide to sequence your PCR fragment to make sure you amplified the correct sequence.

f) What would you put into your sequencing reaction? (Circle all that apply.) 4 points

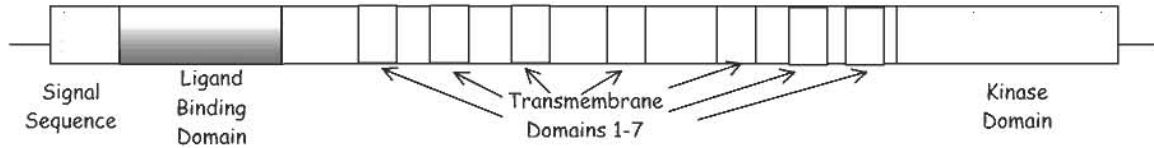
- | | |
|--|--|
| a) primer: 5'-GCTGAC-3'
b) primer: 5'-CGACTG-3'
c) primer: 5'-CGTTAG-3'
d) primer: 5'-GCAATC-3'
e) primer: 5'-GTCAGC-3'
f) primer: 5'-CAGTCG-3'
g) primer: 5'-GATTGC-3'
h) primer: 5'-CTAACG-3' | i) DNA polymerase
j) RNA polymerase
k) DNA ligase
l) primase
m) <i>Eco</i> RI
n) dNTPs
o) ddNTPs
p) ribosomes |
|--|--|
- OR

g) What would your sequencing gel look like corresponding to the part of the sequence labeled "internal region" in the figure above? 4 points



Question 5

Consider the following 477 amino acid protein ADRB1, the human beta-adrenergic receptor. ADRB1 is a transmembrane protein kinase that activates a G-protein when bound to adrenaline. (Length of individual domains is not drawn to scale.)

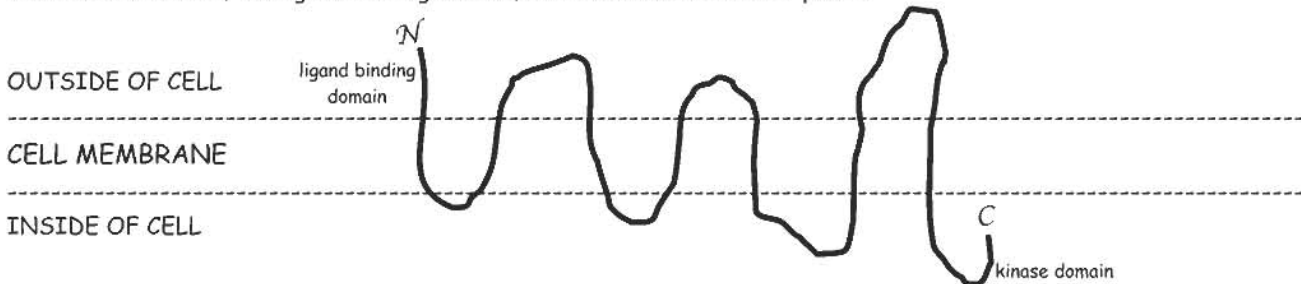


a) The predominant amino acids found in the transmembrane domains of ADRB1 are shown below. What property do they share? One word answer in box. 2 points

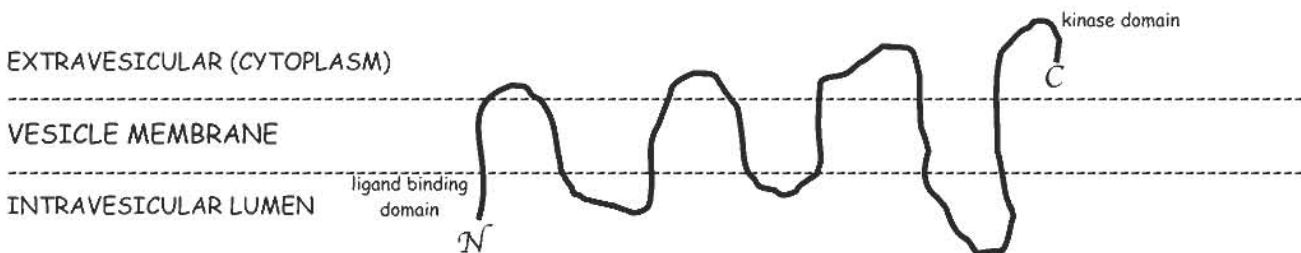
ala leu ile met phe val

HYDROPHOBIC

b) Draw what the protein would look like in the cell membrane, given that the N-terminus is outside of the cell. Label the N and C termini, the ligand binding domain, and the kinase domain. 7 points



c) Draw what the protein would look like in the secretory vesicle membrane. Label the N and C terminal ends of the protein, the ligand binding domain, and the kinase domain. 3 points



d) Circle a proper sequence of steps through the cell for the ADRB1 protein. 3 points

- i) Rough ER -> mitochondria -> Golgi -> secretory vesicle -> plasma membrane
- ii) Ribosome -> Rough ER -> Golgi -> secretory vesicle -> plasma membrane
- iii) Ribosome -> Golgi -> Rough ER -> secretory vesicle -> plasma membrane
- iv) Rough ER -> Golgi -> secretory vesicle->Smooth Endoplasmic Reticulum-> plasma membrane
- v) Ribosome-> secretory vesicle-> Rough ER->Smooth ER-> Golgi-> plasma membrane.

e) Where would the ADRB1 protein be localized if it were missing ... 2 points

i) its signal sequence? _____CYTOPLASM_____

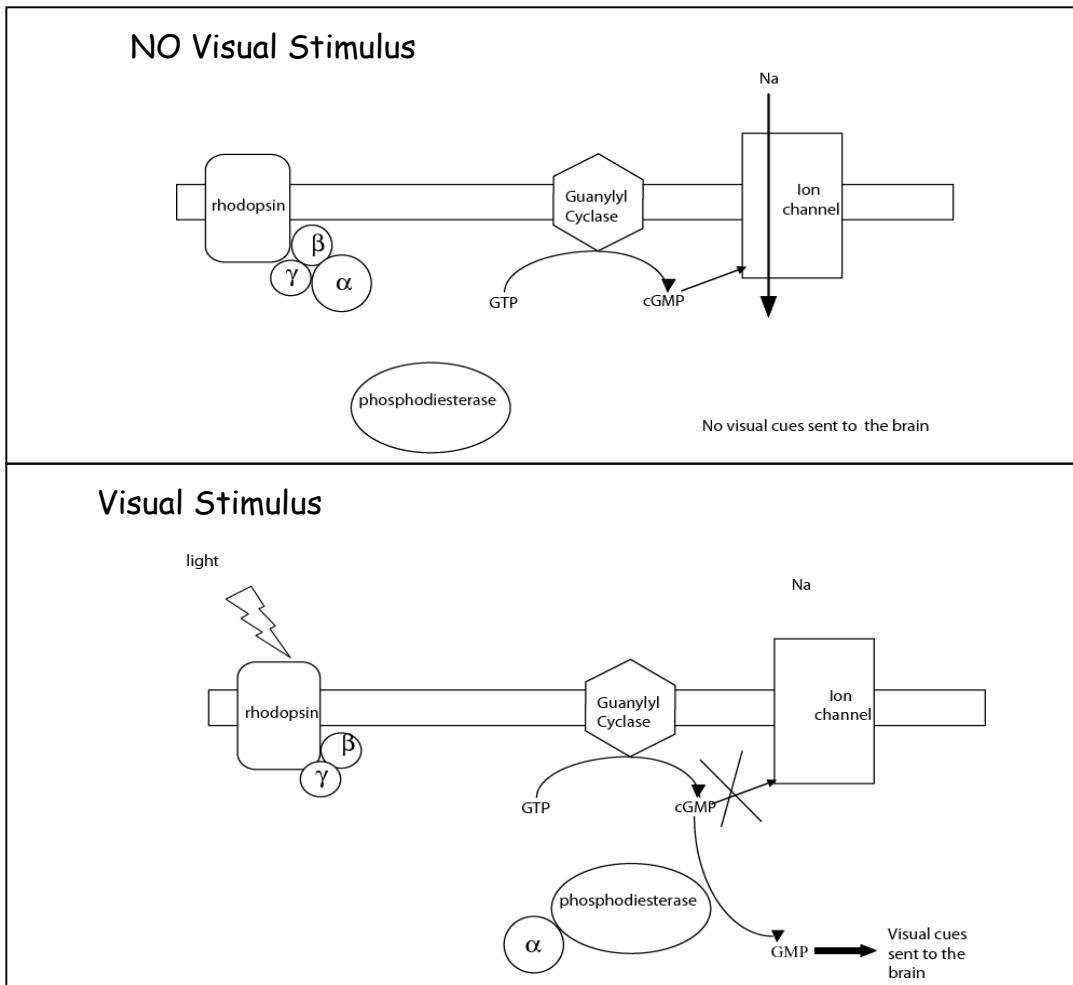
ii) the 1st transmembrane domain? _____PLASMA MEMBRANE_____

Question 6

The mammalian visual system works through the pathway shown below.

In the absence of visual stimulus guanylyl cyclase produces *cGMP*, which opens a sodium channel.

In the presence of visual stimulus, phosphodiesterase is activated by a *G* protein, called transducin. Phosphodiesterase catalyzes the conversion of *cGMP* to *GMP*, enabling the sodium ion channel to close and sending visual cues (signals) to the brain for processing.



a) Which form is the α subunit of transducin in when there is a visual stimulus? 2 points

- i) the *GDP* bound form
- ii) the *GMP* bound form
- iii) the *cGMP* bound form
- iv) the *GTP* bound form**
- v) both *cGMP* and *GDP* bound forms

b) At what step(s) in the pathway does signal amplification occur? 4 points

- i) guanylyl cyclase creating *GDP*
- ii) transducin activating phosphodiesterase**
- iii) phosphodiesterase creating *GMP***
- iv) all of the above
- v) none of the above

The pathway below shows a map kinase cascade that occurs when a growth factor binds to its receptor. Activated SOS activates Ras, which in turn activates Raf, which in turn activates MEK, which in turn activates MAPK, which results in the synthesis of proteins that trigger cell division.

Growth Factor → Growth Factor Receptor → Sos → Ras → Raf → MEK → MAPK → New Protein Synthesis → Cell Division

c) How is the signal amplified in this pathway? Circle all that apply. 2 points

- i) MAPK phosphorylates Sos
- ii) The growth factor receptor degrades upon ligand binding.
- iii) Each kinase phosphorylates multiple molecules of its downstream target in the pathway.**
- iv) The growth factor is degraded upon binding receptor.
- v) None of the above.

d) How does Sos activate Ras (a monomeric G protein)? Circle all that apply. 3 points

- i) promotes exchange of GDP for GTP**
- ii) promotes exchange of GTP for ATP
- iii) promotes hydrolysis of GTP to GMP
- iv) promotes hydrolysis of GTP to GDP
- v) attaches a phosphate group
- vi) None of the above.

e) How would a mutant form of Raf that could not be inactivated affect the pathway? Circle all that apply. 2 points

- i) There would be no cell division; the pathway would be off.
- ii) There would be no cell division; the pathway would be on.
- iii) There would be continuous cell division; the pathway would be off.
- iv) There would be continuous cell division; the pathway would be on.**
- v) This pathway would not be affected.

