

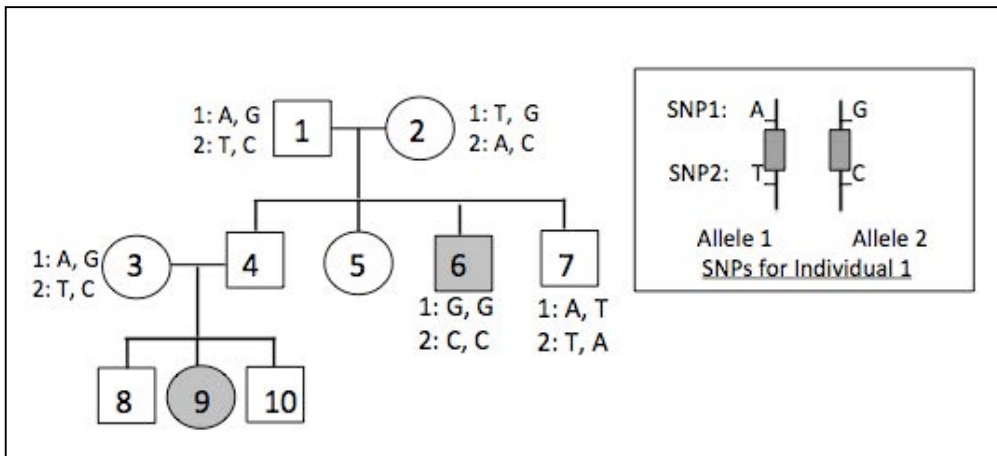
7.016 Problem Set 5- 2018

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Question 1

The following is a pedigree of PKU that is associated with a mutation in the PAH gene.

Note: Filled squares or circles represent PKU patients. Assume that no other mutation arises within the pedigree. Assume complete penetrance (ratio of disease genotype: disease phenotype is 1). The single nucleotide polymorphisms (SNPs); SNP1 and SNP2 that are TIGHTLY LINKED to the PAH gene (shown as shaded rectangle in the schematic below) are given for some of the individuals in the pedigree.



a) What is the mode of inheritance for this form of PKU? _____

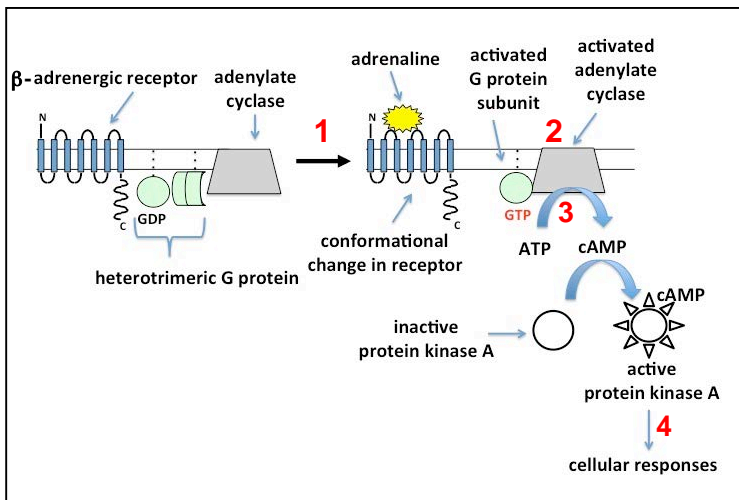
b) What is the probability that **Individual 5** is a carrier of PKU?

c) If Individual 5 is carrier, what are her possible SNP genotypes? **Note:** Assume no recombination.

d) Based on the SNP profile, give all the possible genotypes of **Individual 7** using “A or X^A” for the allele of PAH gene conferring the dominant phenotype and “a or X^a” for the allele conferring the recessive phenotype: _____

Question 2

The diagram below shows a signaling pathway that is activated following the binding of adrenaline to the β -adrenergic receptor. The major steps of this signaling pathway are outlined below and also shown on the diagram.



Step 1: When the β -adrenergic G protein--coupled receptor is not bound to adrenaline, the G protein attached to the receptor has a GDP nucleotide bound to it. Binding of adrenaline hormone (which travels through the blood stream) changes the conformation of the receptor. This conformation change in the receptor affects the conformation of the G protein bound to it and causes the GDP to fall off and be replaced with a GTP molecule.

Step 2: Active GTP--bound G protein detaches from the receptor and it activates adenylate cyclase. The G protein can be inactivated by the hydrolysis of bound GTP to GDP.

Step 3: Activated adenylate cyclase mediates the formation of cAMP from ATP.

Step 4: The cAMP produced, diffuses through the cell to bind and activates Protein kinase A (PKA). PKA affects many proteins in the cell by attaching a phosphate group to them. The phosphorylated proteins induce cellular changes that mediate the increased heart rate and accounts for the flight-or-fight response.

a) To further understand the above signaling pathway, you examine it in the following cells that are cultured in the following conditions (#1-#3)

- #1: Cells are treated with **cholera toxin** that locks the activated G proteins in their GTP bound form.
- #2: Cells are treated with **pertussis toxin** that locks the G proteins in their GDP bound form.
- #3: Cells have a receptor that activates the G proteins independent of the ligand.

In which of the conditions would you see a **constitutive activation** of adenylate cyclase independent of adrenaline hormone? **Explain** why you selected this condition.

b) Why is the effect of adrenaline limited to a specific cell type in an individual?

c) Would you regard the adrenaline hormone mediated signaling pathway as **endocrine/ apocrine/ autocrine/ juxtacrine**? **Explain** why you selected this option as opposed to the others.

Question 2 continued

d) Identify the first step in the signaling pathway where the signal starts to get amplified: **Step 1/ Step 2/ Step 3/ Step 4.**

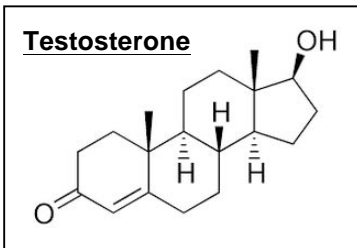
e) Consider the following **homozygous mutations** in different components of this pathway.

- **M1:** The β -adrenergic receptor constitutively stays in its ligand bound form
- **M2:** Adenylate cyclase can no longer bind to ATP
- **M3:** PKA continues to stay in its active form
- **M4:** The cell produces a variant of β -adrenergic receptor that lacks 4 of its 7 transmembrane domains

Complete the table for the cells that are incubated with adrenaline and have the following mutations. Predict the change in heart rate by comparing the mutations with control, wild type, adrenaline treated cells.

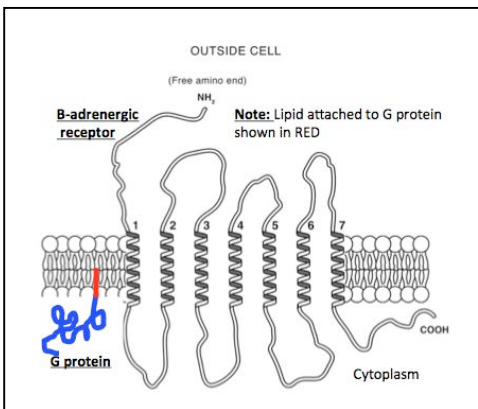
| Mutants | cAMP concentration (High/ Low)? | PKA activated (Yes/ No)? | Does the heart rate <i>increase OR decrease</i> in the presence of adrenaline? |
|---------|---------------------------------|--------------------------|--|
| M1 & M3 | | | |
| M2 & M4 | | | |
| M2 & M3 | | | |

f) Unlike adrenaline hormone, ligands such as testosterone (shown below) can diffuse into the target cell and mediate cell signaling by binding to the intracellular receptors. What property of these ligands allows them to diffuse?



Question 3

The following is the β -adrenergic receptor and a **small G protein (shown in blue)** oriented in the membrane. Answer parts (a)-(d) by using the options: *Cytoplasm/ nucleus/ rough endoplasmic reticulum (RER)/ Smooth endoplasmic reticulum (SER)/ vesicles/ cell membrane.*



a) Where in the cell does the translation of the following proteins begin?

- **β -adrenergic receptor?** _____
- **G protein?** _____

b) Where in the cell is the translation of the following proteins completed?

- **β -adrenergic receptor:** _____
- **G protein:** _____

Question 3 continued

c) Where in the cell is the newly translated G protein post-translationally modified by getting covalently coupled to the lipid (shown in red in the schematic for this question)? _____

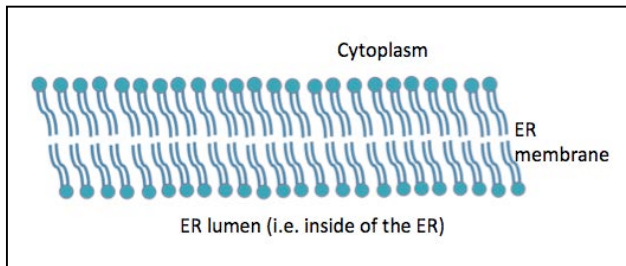
d) Which intracellular structure is involved in transporting these two newly translated proteins to the cell membrane? _____

e) Which of the two proteins have a **signal sequence** and what is its **significance**?

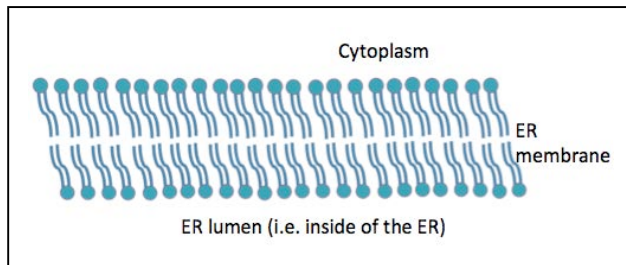
f) On the diagram of **β -adrenergic receptor** on the previous page, circle the part of **β -adrenergic receptor** that is not present in secreted proteins.

g) Based on the information in the schematic above, can you label the $-\text{NH}_2$ and $-\text{COOH}$ ends of the...

i. **β -adrenergic receptor** as it is being translated? If yes, draw this as it is being translated. If no, please write "No".



ii. **G protein**? If yes, draw this as it is being translated. If no, please write "No".

**Question 4**

a) Specific ion channels and pumps regulate the resting membrane potential and action potential of a neuron. You treat a neuron *in vitro* (in a plate) with a reversible ATP synthase inhibitor. Would this inhibitor inhibit a **pump** or **channel**? **Explain.**

b) Multiple sclerosis (MS) is an autoimmune disorder in which the immune system attacks and destroys the myelin sheath of a neuron. What happens to the speed of propagation of action potentials in a neuron when the myelin sheath is destroyed: **slows down/ speeds up/ shows no change**? **Explain** why you selected this answer.

Question 4 continued

c) GABA is a neurotransmitter in the central nervous system (CNS). It acts by binding to GABA-A receptors that are ligand gated chloride channels (Cl⁻) and GABA-B receptors that activate potassium channels (K⁺) via G proteins.

In what direction would Cl⁻ ions flow when the GABA-A receptor is activated: **into OR out** of the target neuron on which this neurotransmitter acts? How does this flow affect the likelihood of an action potential in the post-synaptic neuron? **Explain.**

d) You culture a GABA producing neuron in the presence of the following neurotoxins in two separate cell culture plates (Plate A & Plate B). You then apply excitatory stimuli to neurons in the plates.

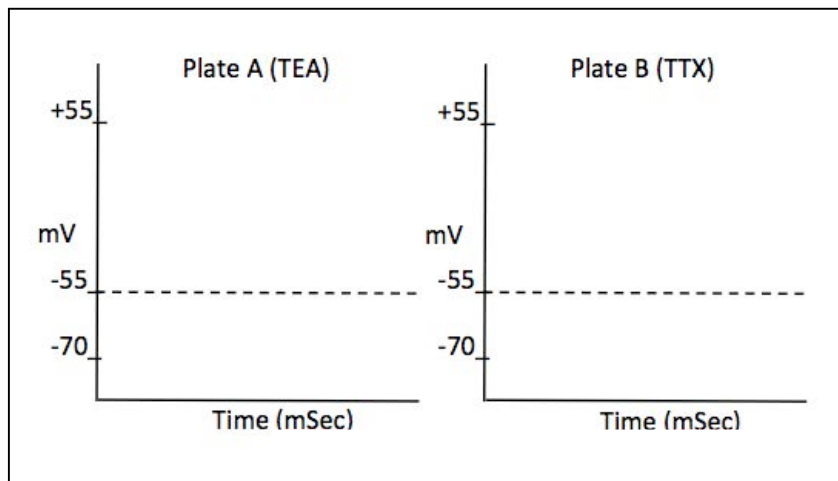


Plate A: Neuron is treated with tetraethylammonium (TEA), which **inhibits voltage gated K⁺ channels.**

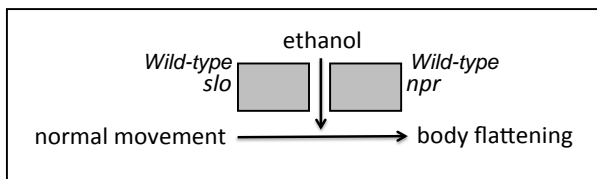
Plate B: Neuron is treated with tetrodotoxin (TTX), which **inhibits voltage gated Na⁺ channels.**

Sketch the alteration in the action potential following the treatment of the GABA producing neuron with each neurotoxin. **Note:** *If there is no action potential,*

please write that on the graph. The resting and threshold membrane potentials are -70mV and -55 mV respectively.

Question 5

C. elegans shows ethanol-responsiveness through body-flattening. Loss-of-function mutants in the *slo* gene are resistant to ethanol and do not flatten their bodies. Null mutants in the *npr* gene show increased sensitivity to ethanol i.e. flatten their body without ethanol.



a) On the shaded boxes in the diagram to the left, indicate whether wild-type alleles of *slo* and *npr* **activate (use an arrow)** or **inhibit (use a T-bar)** the response to ethanol.

The *slo* gene is expressed in the PVM neurons, a specific type of neuronal cell. To test how the slow protein functions in this neuron, you use a PVM neuron specific promoter to express channel rhodopsin.

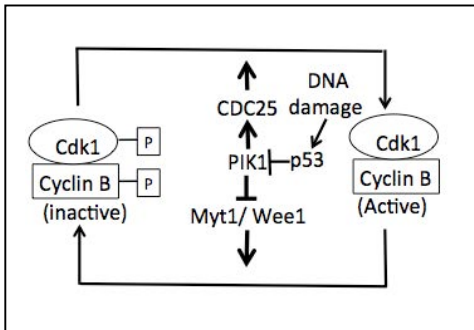
b) What signal/ stimulus is used in optogenetics to open channel rhodopsin?

Question 5 continued

c) You test whether activation of PVM neuron can compensate for *s/o* activity in ethanol responsiveness using channel rhodopsin (CHR) gene. Starting with a *s/o* loss-of-function (LOF) mutant, **explain** how you would perform this test and what result you would expect.

Question 6

The following is the regulatory network that controls the activity of the mitotic regulatory kinase (Cdk-1) that is critical for the G2→M phase transition of a cell during the cell cycle.



- DNA damage or failure to complete DNA replication results in activation of p53.
- The activated p53 regulates Cdk1/ Cyclin B function by inhibiting PIK1.
- PIK1 normally inhibits Myt1/ Wee1 kinase and activates CDC25.
- The Myt1/Wee1 kinase normally inactivates Cdk1 whereas CDC25 activates Cdk1.
- The Cdk1-CyclinB is active when dephosphorylated and inactive when phosphorylated.

a) The mutations in proto-oncogenes that contribute to unlimited cell division are **dominant, gain-of-function (GOF) mutations** as opposed to the mutations in tumor suppressor genes, which are recessive, homozygous, **loss-of-function (LOF) mutations**. Which of the proteins are encoded by....

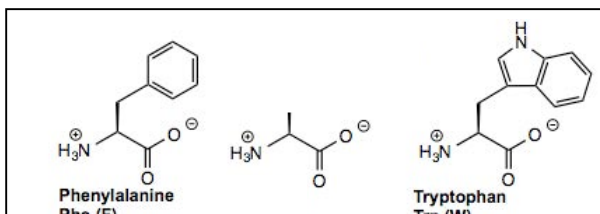
- Proto-oncogenes: Myt1/ PIK1/ p53/ CDC25/ Cdk1/ Cyclin B?**
- Tumor suppressor genes: Myt1/ PIK1/ p53/ CDC25/ Cdk1/ Cyclin B?**

b) In a diploid yeast undergoing the cell cycle, you identify a Cyclin B (or M-Cyclin), which functions at the G2→M transition. A temperature sensitive Cyclin B mutant fails to complete cell cycle at 42°C since it arrests the cell at a particular cell cycle phase. Give the ploidy of the arrested cell: **n/ 2n/ 4n?**

c) Would a **PIK1** temperature sensitive mutant, grown at the non-permissive temperature (42°C), progress from G2 → M phase? **Why or why not?**

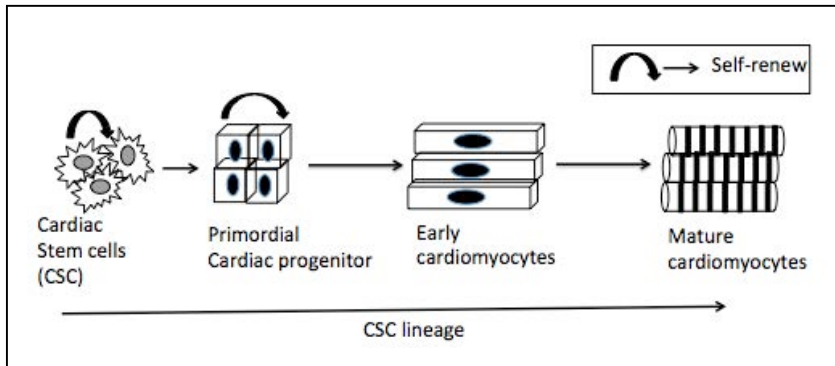
d) Cisplatin is a chemotherapeutic drug that disrupts DNA replication and thus activates the DNA damage checkpoint such as p53. How would the activation of this checkpoint disrupt tumor growth?

e) **Circle** the amino acid that can be phosphorylated by the Cyclin D-CDK complex.



Question 7

The following drawing represents a cardiac stem cell (CSC) lineage commitment and differentiation in mice.



a) List **two** characteristics of CSC that qualifies them as stem cells.

b) Briefly describe **one** experiment that would test whether a “test cell” is likely to be a CSC. **Note:** Your answers may vary.

c) The CSCs have transcription factors such as *Oct4* and *Nanog* and cell surface proteins such as *CD90*. Which of these proteins would be best to use to purify living CSC from a mixed population that has multiple cell types? **Explain** your choice.

d) In adults, CSCs reside in defined regions called “niches” and require the Epidermal growth factors (EGF) and Fibroblast growth factor (FGF) for their proliferation (Cell division) and differentiation. You do the following experiments.

You ablate the CSCs from mutant mouse embryos 1 and 2. You implant the GFP labeled CSCs from normal mouse embryo into mutant embryos 1 and 2. You observe that the GFP labeled CSCs proliferate and differentiate to form the cells of CSC lineage in mutant mouse embryo 1 but not in embryo 2.

Why do the CSCs from a wild-type mouse embryo grow in **embryo 1** but not in **embryo 2**?

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