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7.013 Problem Set 7 - 2013

Question 1

a) Neuronal path finding is crucial for structured cellular organization and development of neural circuits. The elongation or retraction of the growth cone is dependent on the guidance cues. You are looking at the response of the growth cone to the following guidance cues. ***Note:*** *In this example you may assume that both these guidance cues serve as* ***attractants****.*

- ***Guidance cue 1: Fibronectin*** *protein that is a part of ECM.*
- ***Guidance cue 2: Ephrins****, which diffuse along their concentration gradient.*

i. Classify the two guidance cues as **short-** or **long-** range signals.

Fibronectin: **Ephrins:**

ii. You do a stripe assay to determine if fibronectin serves as attractive guidance cues. Briefly **explain** how this assay works.

iii. The stripe assay is an *in vitro* assay, that is, performed outside the body. You next need to determine whether fibronectin and ephrins are the normal guidance cue for these neurons in the brain. Give an experiment that can help you answer this question.

b) A key question in neurobiology is how neurons find their targets to generate circuits. Retinal neurons transmit information about the light that the eye perceives to the brain in the form of electrical signals. The cell bodies of these neurons are in the eye. During development they connect to a region of the brain known as the optic tectum. The goal of recent research has been to figure out how they do this. The following inhibitors disrupt innervation of the tectum by retinal neurons. Complete the table for each of the following treatments.

Treatments	What would be the effect of this treatment on axon elongation?
Nocodazole (disrupts microtubules)	
A collagen specific antibody that inhibits/ disrupts extracellular matrix (ECM)	

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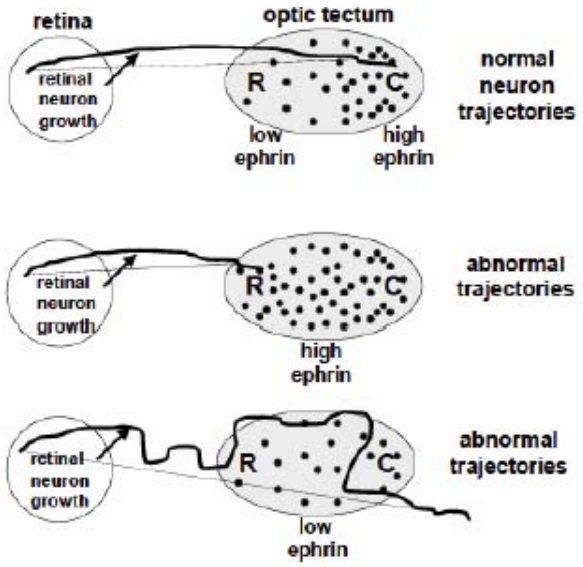
Question 1 continued

c) Ephrin is a ligand found in the optic tectum in a gradient, with more caudally (C), than rostrally (R). Temporally located retinal neurons (T) normally grow to the caudal tectum, as depicted below. When ephrin concentration is high throughout the tectum, retinal neurons stop in the rostral region (R). However, when ephrin concentration is low throughout the tectum, temporal retinal neurons grow past the tectum.



i. What part of the neuron grows, as neurons find their path?

ii. The receptor that binds ephrin is the Eph receptor. Where is the Eph receptor most likely to be expressed in the schematic on the left?

iii. Using the data in the schematic, explain why during normal development, the temporal retinal neurons grow to the caudal tectum.

iv. What would happen to the growth of retinal neurons in the absence of the Eph receptor? **Explain**.

Question 2

Cancer is caused by accumulation of two or more mutations in the same cell that affects its proliferation and survival.

a) Why does a person's chance of having cancer increases with age?

b) Why is cancer mostly considered a genetic disease of somatic cells?

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Question 2 continued

c) The Ames test is very often used to evaluate the mutagenic potential of a chemical agent. The test employs a strain of bacteria that have mutation in a gene(s) that codes for an enzyme(s), which is involved in the synthesis of the amino acid histidine. These bacterial strains are therefore His– (cannot synthesize histidine) and they grow only in the growth medium that contains histidine. However, a compensatory mutation to this gene(s) may cause the His- bacterial strain to revert to a His+ strain that can grow in the medium that lacks histidine.

You want to test the mutagenic potential of benzo(a)pyrene (B(a)P) found in cigarettes smoke. You want to perform the following experiments using this carcinogen.

- **Experiment 1:** You incubate the His- bacterial cells with B(a)P and plate them on cell culture plates with media that lack histidine. After incubating the plate overnight at 37°C, you observe NO colonies on this plate.
- **Experiment 2:** You inject B(a)P in mice every alternative day for one month. You carefully observe these mice overtime and see that that they develop a solid tumor.
- **Experiment 3:** You first incubate B(a)P with liver extract that contains metabolic and detoxifying enzymes and then add the His- bacterial cells to the incubation mix. You plate the bacterial cells on cell culture plates with media that lack histidine and incubate them overnight for 24 hrs at 37°C. You find abundant colonies appearing on this plate.

Circle the option from the choices below that best characterizes B(a)P and **explain** why you selected this option.

Mutagen **Carcinogen but not a mutagen** **Promutagen**

d) The following are three bacterial mutants that have different mutations in the DNA sequence that encodes the C-terminus of an enzyme (200 amino acids long) that is required for Histidine biosynthesis and that **requires Gln^{197}** for its function. The DNA sequence corresponding to the **last five amino acids** of the wild- type and three different mutant versions (*1*/ *2*/ *3*) of this enzyme is included within the sequence below. ***Please Note:*** *A codon chart is provided on the last page of this problem set.*

Wild-type: 5′-ATTGCCAAAGATTAGGATGATAAAT-3′
3′-TAACGGTTTCTAATCCTACTATTTA-5′

Mutant #1 5′-ATTGCCGAAGATTAGGATGATAAAT-3′
3′-TAACGGCTTCTAATCCTACTATTTA-5′

Mutant #2 5′-ATTGCCAGAGATTAGGATGATAAAT-3′
3′-TAACGGTCTCTAATCCTACTATTTA-5′

Mutant #3 5′-ATTGCAAAGATTAGGATGATAAAT-3′
3′-TAACGTTTCTAATCCTACTATTTA-5′

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Question 2 continued
You treat mutants 1, 2 & 3 separately with two mutagens; mutagen C causes point mutations in comparison to mutagen D that results in frame-shift mutations.

i. Which bacterial mutant (*choose from 1, 2 or 3)* can revert to wild- type following the treatment with mutagen C? **Explain** why you selected this option.

ii. Which bacterial mutant (*choose from 1, 2 or 3*) can revert to wild- type following the treatment with mutagen D? **Explain** why you selected this option.

e) Although most carcinogens are mutagenic there are examples of some non- mutagenic carcinogens. **Alcohol** is one such example and excess consumption of alcohol is very often related to liver cancer. **Explain** how alcohol over- consumption may cause liver cancer.

Question 3
a) Many of the mutations that cause cancer involve oncogenes, whereas others involve tumor suppressor genes. Suppose that you had the ability to introduce the wild- type copy of a gene into a transformed cell.

i. If the cell is transformed due to mutation of a tumor suppressor gene, would you expect that adding the wild- type copy of the mutated gene would restore the cell's wild- type phenotype (*Yes/ No*)? **Explain** your choice.

ii. If the cell is transformed due to an oncogenic mutation, would you expect that adding the wild- type copy of the mutated gene would restore the cell's wild- type phenotype (*Yes/ No*)? **Explain** your choice.

b) Cancer is caused by accumulation of mutations in the same cell that affect its proliferation and survival. In the table below, you introduce a single copy of the **mutant gene** into an immortalized/ non- tumorigenic cell line. For each gene, s*tate the phenotype of the cell that has received a single copy of the mutant gene by choosing from* ***transformed*** *(characteristics of cancer cells) or* ***untransformed*** *(characteristics of wild-type cells) phenotype. Consider introduction of each gene separately.*

Gene	Normal function of encoded protein	A copy of the mutant Gene that is introduced in the cell line	Phenotype of the resulting cell that has received **one copy** of the mutant gene (*transformed/ untransformed*)?
ras	A G protein that stimulates growth signaling pathway	Ras that shows constitutive (always on) GTPase activity	
APC	A protein that inhibits the growth-signaling pathway	Functionally inactive APC gene product	
c-jun	A transcription factor that increases the expression of growth-promoting genes	Loss-of-function of c-jun gene product	

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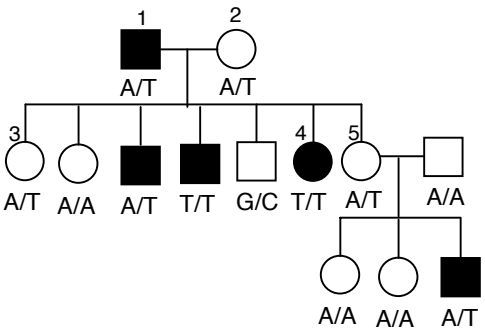
Question 3 continued

c) MSH2 is a gene commonly associated with **familial nonpolyposis colorectal cancer**. This gene encodes a protein that is involved in correcting the mismatched nucleotides. Briefly explain why the individuals who are heterozygous for the loss-of- function mutation of MSH2 gene (MSH2+/MSH2-) from birth are very likely to develop colon cancer very early in their lives.

d) The following pedigree represents the inheritance of **predisposition to colon cancer** that is caused by a mutation in the **MSH2 gene**. Give the most likely **inherited genotype** at the MSH2 locus for each of the following individuals. ***Please Note:*** *Use MSH2+ for the wild-type allele and MSH2- for the mutant allele. You may ignore the SNP information for this part of the question. Also note that people marrying into the family only have wild- type copies of MSH2 gene.*



Individual	Inherited genotype
1	
2	
3	
4	

e) You continue to study this family and are surprised when individual 5 has a child that develops colon cancer at an early age. You analyze a **tightly linked** SNP marker for the members of this family. The alleles of SNP for each individual are shown in the pedigree.

i. Assuming no recombination between SNP and the MSH2 locus, what allele of SNP is linked to the "MSH2" allele in individual 1?

ii. Assuming no recombination between SNP and the MSH2 loci, how can you **explain** the **phenotype** of individual 5?

Question 4

a) p53 is a tumor suppressor gene that is mutated in 50% of the cancers including cervical cancer.

i. The Human papilloma virus (HPV) has been implicated as a risk factor for cervical cancer. The E6 protein of HPV binds to and inactivates p53. **Explain** why the virally encoded E6 protein can result in cells that form a tumor.

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Question 4 continued

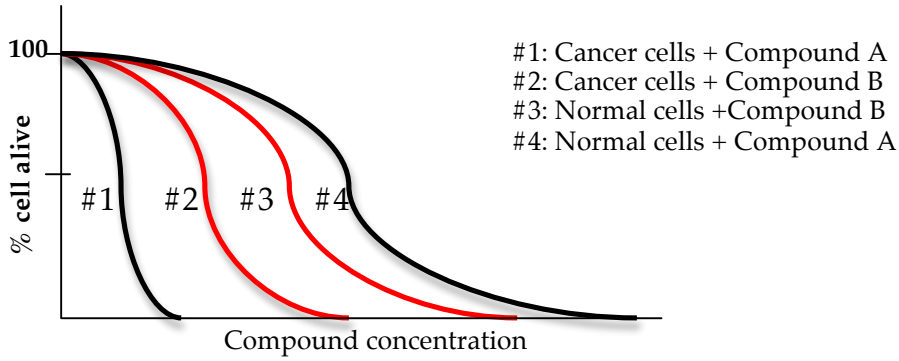
ii. It is possible to create a knock out mouse model where both the alleles of p53 gene are deleted (p53-/p53-). Such mice can survive for 4-6 months but are highly prone to developing cancer. Would you predict that a tumor composed of cells that are p53-/p53- (a tumor suppressor gene) would be more or less sensitive to radiation than a tumor that is p53+/p53-? **Explain** your answer.

b) Gardasil is a preventive vaccine against HPV infections,which are the most common cause of cervical cancers. The major capsid protein of HPV can spontaneously assemble to form virus like particles (VLPs) that resemble HPV. Gardasil contains recombinant VLPs. These VLPs can induce an immunological response that prevents HPV infection but they do not cause cancer. **Explain** why Gardasil can induce an immune response in an individual.

c) Radiation and chemotherapy are very often used individually or in combination to treat different types of cancers.

i. Both radiation and chemotherapeutic drugs often have side effects such as diarrhea, constipation, mouth sores, hair loss, nausea, and blood-related side effects. **Explain** why the side effects are the same for radiation and a variety of different chemotherapeutic drugs.

ii. Prior to being used for treatment, each chemotherapeutic drug is extensively screened. During drug screening you identify two compounds A and B that have the potential to kill cancer cells and normal cells as shown by the following graph. Which compound (*choose from compound A or compound B) is* a better candidate for cancer treatment? **Explain** why you selected this option.



d) Vincristine (a microtubule inhibitor) is often used to treat different cancers. Briefly **explain** how vincristine can help treat a wide – variety of cancers.

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Question 5

a) Chronic myeloid leukemia (CML) is a hematological cancer of myeloid origin that occurs predominantly in adults. Most of the CML patients show a chromosomal translocation that results in the formation of the Bcr-Abl fusion gene or Philadelphia Chromosome. This gene encodes for Bcr-Abl tyrosine kinase protein.

i. Consider a patient who has CML, and answer the following questions.

- Would the Philadelphia chromosomal translocation be present in all of the cells in the patient's blood system?
- How many independent times did the Philadelphia chromosomal translocation occur in the patient?
- Could the patient pass CML onto his/her kids? **Explain** your choice.
- Would all the cancer cells in this patient have the same gene expression profile (*Yes*/ *No*)? **Explain** your choice.

ii. CML patients are effectively treated with Gleevec, which inhibits Bcr-Abl tyrosine kinase activity by binding to its ATP binding pocket.

- The CML patients relapse if they stop taking Gleevec. **Explain** why is this so.
- CML patients over time generate Gleevec-resistant clones that have mutations in their kinase domain. Briefly **explain** how the mutations in the kinase domain of Bcr-Abl fusion gene result in Gleevec resistance.
- The Gleevec resistant clones however respond to another drug, Spryzel. If the patients are given both Gleevec and Spryzel together at the beginning of treatment, the generation of drug-resistant clones can be delayed. **Explain** why is this so.

c) Approximately 25-30% of women with breast cancer over-express Her-2 receptor proteins. If these patients are treated with Herceptin antibody, specific for Her-2 receptors, the Her-2 over-expressing breast cancer cells are specifically attacked by the patient's own immune system. Additionally, these cells also show decreased proliferation. **Explain** how Herceptin mediates a dual effect.

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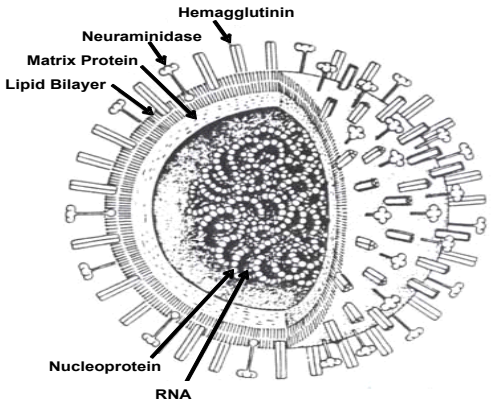
Question 6

Influenza virus is a **single minus stranded,** segmented, RNA virus that does not replicate via a DNA intermediate. The virus typically infects vertebrate epithelial cells. The following is a schematic of the influenza virus.



a) Based on the details provided in the schematic is it more likely that this virus escapes its host cell via a mechanism involving cell lysis or budding? **Explain**.

b) Influenza virus is unable to make more viral RNA within the host cells using exclusively the host cell proteins.

i. **Explain** why this is so.

ii. **Explain** how the virus overcomes this issue and replicates its genome in the host.

c) **Explain** why we need to develop a new vaccine against the flu virus almost each flu season.

d) You decide to generate a vaccine against Influenza virus that would elicit a **humoral response**. Which viral proteins are best candidates for designing vaccine?

Proteins	**Good candidate (Yes/No)?**	**Explain**
Hemagglutinin		
Matrix protein		
Nucleoproteins		
Neuraminidase		

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Question 6 continued (*Note: Part (e) is optional and will not be graded*)

e) Human immunodeficiency virus (HIV) is a retrovirus. Its genome is a single (+) stranded RNA that is packaged with the reverse transcriptase enzyme within a protein capsid. This is further packaged into an envelope that is derived from the plasma membrane of the host cell in which the virus had replicated. The surface of the envelope is covered with the envelope glycoprotein, called gp120.

i. HIV specifically infects the T- helper (T_H) cells of the human immune system. If the HIV enters the host cell by means of host receptor recognizing a viral protein, what would be the most likely **ligand** and its **corresponding receptor** during HIV infection?

ii. Some individuals are resistant to HIV infection even after repeated exposure. Assuming that these individuals express a normal level of the functional receptor that you have recognized above, how can you explain their resistance to HIV?

iii. In recent years, therapies have been developed to fight AIDS using nucleotide analogs. The drug used to combat AIDS is Azidothymine (AZT). The structure of AZT is very similar to thymidine except that in AZT, the 3'-OH group on the deoxyribose sugar is replaced by an azido (N_3) group. Which process of the life cycle of HIV do you think is inhibited by AZT?

Codon chart

	U	**C**	**A**	**G**
U	UUU Phe (F) UUC " UUA Leu (L) UUG "	UCU Ser (S) UCC " UCA " UCG "	UAU Tyr (Y) UAC " UAA ***Stop*** UAG ***Stop***	UGU Cys (C) UGC " UGA ***Stop*** UGG Trp (W)
C	CUU Leu (L) CUC " CUA " CUG "	CCU Pro (P) CCC " CCA " CCG "	CAU His (H) CAC " CAA Gln (Q) CAG "	CGU Arg (R) CGC " CGA " CGG "
A	AUU Ile (I) AUC " AUA " **AUG** Met (M)	ACU Thr (T) ACC " ACA " ACG "	AAU Asn (N) AAC " AAA Lys (K) AAG "	AGU Ser (S) AGC " AGA Arg (R) AGG "
G	GUU Val (V) GUC " GUA " GUG "	GCU Ala (A) GCC " GCA " GCG "	GAU Asp (D) GAC " GAA Glu (E) GAG "	GGU Gly (G) GGC " GGA " GGG "

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