Solution key- 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: *Short Range* **Ephrins:** *Long- Range*

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

You add fibronectin on one side of a strip and see the response of growth cone to this cue. If the cue is an attractant the growth cone will grow towards higher concentration gradient but if it is a repellant the growth cone will retract and the axon will not grow on the stripe. You should compared your results with the results of the control strip that shows how the neurons behave in the absence of any guidance cue.

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.

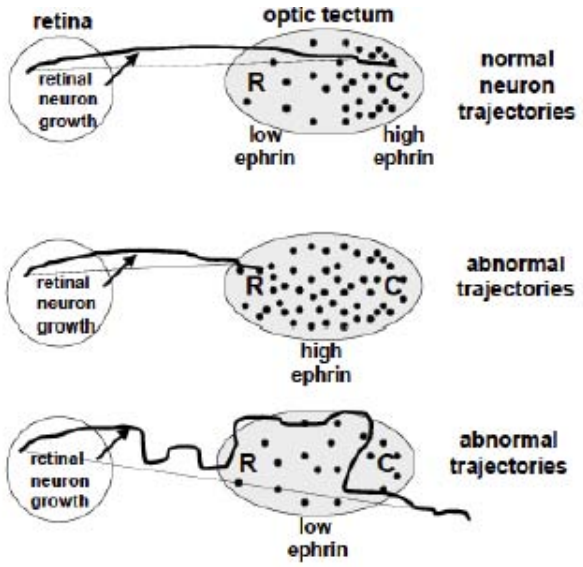
You can make null mutants that do not have the receptors for the specific guidance cues and compare their response to fibronectin and / or ephrins with that observed in normal situation. You can also make null mutants that do not make fibronectin and / or ephrins and compare their response to that observed in normal situation.

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)	*Microtubules are part of the cytoskeleton. Microtubules take part in many other processes: they are capable of growing and shrinking in order to generate force. In addition, organelles move along the microtubules to transport materials along the axon from the cell body to the terminal. Nocodazole would prevent axon elongation and thus prevent the neuron from reaching its target. Because microtubules are found in the central part of the growth cone, nocodazole could also prevent growth cone function.*
A collagen specific antibody that inhibits / disrupts extracellular matrix (ECM)	*The extracellular matrix is essential for the positional cues needed for axonal guidance. If the ECM is disrupted, the positional cues will be disorganized.*

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?

Growth cone or axon tip

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

Receptor will be at the growing end on the retinal axon, the growth cone of the retinal axon.

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

The data show that high levels of ephrin across the tectum cause the retinal neurons to stop rostrally, whereas low levels of ephrin across the tectum allow the retinal neurons to grow beyond the tectum. This indicates the a high concentration of ephrin is what signals the retinal neurons to stop, ***and this concentration is usually only present in the caudal tectum****.*

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

The retinal neurons would not be able to detect the positional cues, and would thus have an abnormal trajectory i.e. the axons will grow past the tectum.

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?

Cancer is a multi- step process that involves accumulation of many mutations in both tumor suppressor genes and oncogenes ***in the same cell****. These mutations accumulate throughout the life of a cell, either spontaneously or by exposure to carcinogens, by replication mistakes or by chromosomal translocations. Since the chances of accumulation of these mutations increase over time, the chances of an individual having cancer also increases with age.*

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

Cancer is caused by the accumulation of a set of mutations in a single somatic cell, either primarily due to exposure to carcinogens or as replication mistakes. As a side-note, gametes and gamete producing cells are well protected and have a limited number of cell divisions, so they are not readily mutated.

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 that the number of colonies on this plate is almost equal to those on a control plate that contains bacterial cells not incubated with B(a)P.

- **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 that that the number of His+ colonies appearing on this plate is 500 fold more compared to that observed in experiment 1.

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***

B (a)P is a promutagen, which is not cancer causing in its native form. However, as shown by Experiment 2 and Experiment 3, the metabolic enzymes present in the liver extract and different tissues of the experimental mice convert the B(a)P from its pro-mutagenic form into a metabolic form(s) that is mutagenic. Hence you see the development of solid tumor in experimental mice and reversion of His- bacterial cells to His+ bacterial cells in Experiment 3, which form colonies on the plate that has a selection growth media that lacks histidine.

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′

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.

Mutant #1, shows a point mutation that results in a missense mutation i.e. - 5'-CAA-3' (encoding Glu^{197}) is changed to 5'-CGA-3' (encoding Arg^{197}). Mutagen C can revert this point mutation.

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.

Mutant #3, shows a point mutation (deletion) that results in a frameshift altering the last five amino acids of enzyme E1. Mutagen D causes insertions that can correct the reading frame.

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. *Give one mechanism by which a non-mutagenic carcinogen such as alcohol can cause cancer.*
Excess consumption of alcohol results in liver damage. In order to repair this damage, the remaining cells in the liver undergo increased cell proliferation, which if pushed too much results in accumulation of mutations that ultimately result in 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 the wild- type phenotype (*Yes/ No*)? **Explain** your choice.

Yes, Mutations in tumor suppressor genes involve a loss of both of the alleles of the gene. Hence introduction of a normal copy of the mutated gene could restore the cell's normal phenotype.

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.

No, Oncogenic mutations involve a gain of function of one of two alleles of the same gene, and create a transformed phenotype that is dominant. Hence introduction of a normal copy of the gene would not restore the cell's normal phenotype.

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 **<u>mutant gene</u>** into an immortalized 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.*

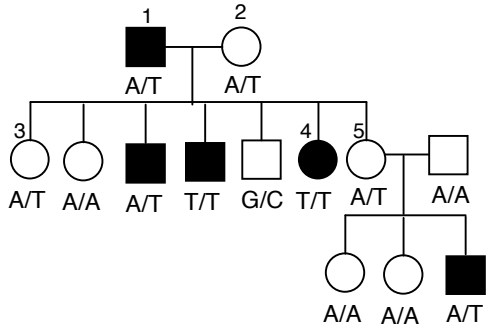
Gene	Normal function of encoded protein	A copy of the mutant Gene that is introduced in the cell line	Phenotype of the resulting cell that has received one copy of the mutant gene (*transformed/ untransformed*)?
ras	A G protein that stimulates growth signaling pathway	Ras that shows constitutive (always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

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.

All the cell types in this individual, right from birth, only have one functional copy of the MSH2 gene including the cells that make the colon (Genotype: (MSH2+/MSH2-). There is >90% probability that one of these cells will show a loss-of- heterozygosity (LOH) and acquire a genotype MSH2-/MSH2- . This cell will not be able to repair any DNA damage and hence will get transformed over time. The uncontrolled proliferation of this cell along with the accumulation of mutations will ultimately lead to the development of colon cancer.

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	*MSH2+/MSH2-*
2	*MSH2+/MSH2+*
3	*MSH2+/MSH2+*
4	*MSH2+/MSH2-*

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? *T allele*

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

He did not get the 2nd mutation that would have converted MSH+ allele to MSH- allele resulting in a loss of heterozygosity. But this individual is still an obligate carrier and pass on the mutant allele to the next generation.

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.

p53 is a tumor suppressor gene product that inhibits cell division when active. Since E6 protein of HPV inactivates p53, it is no longer capable of inhibiting cell division. Hence, the cells carrying mutations will go through uncontrolled cell proliferation resulting in the formation of tumor.

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.

p53 is a protein that is activated in response to DNA damage. Once activated it can initiate the apoptosis pathway. A tumor composed of cells that are p53-/p53- would be less sensitive to DNA damage because it cannot activate the apoptosis pathway, whereas a cell that is p53+/p53- can partially activate the apoptosis pathway and those cells will die as a result of treatment.

b) Gardasil represents a growing number of immunotherapies that may be employed to effectively treat specific types of cancers with minimal side effects. The major capsid protein of HPV can spontaneously assemble to form virus like particles (VLPs) that resemble HPV virions. 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.

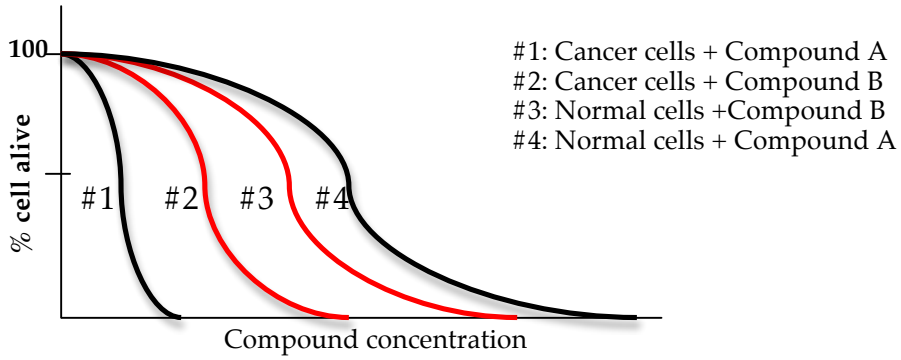
Since Gardasil contains the antigens of HPV, it can mount a humoral immune response.

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 drugs.

Most of these chemotherapeutic agents target the cancer cells since they are actively dividing cells compared to the normal cells. However, normal cells that are actively dividing, such as hair cells, blood cells and gut cells, are also targeted by these treatments resulting in hair loss and nausea. Therefore, they all result in very similar side-effects.

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.



Compound A will be a better choice since it has a larger therapeutic index i.e. The effective concentration of compound A that is required to kill the cancer cells is far less compared to the concentration needed to kill the normal cells.

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

Vincrisine prevents the formation of mitotic spindle and hence inhibits cell proliferation.

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? No, *it is restricted to myeloid cells in the blood.*

- How many independent times did the Philadelphia chromosomal translocation occur in the patient? *Only once, and all the cancerous cells originated as the clones of this mutated cell.*

- Could the patient pass CML onto his/her kids? Explain your choice. *No, this mutation has occurred in somatic cells and not in the germline cells.*

- Would all the cancer cells in this patient have the same gene expression profile (*Yes*/ *No*)? **Explain** your choice. *Yes, within a specified time span all cancer cells are being produced from the clonal expansion of the transformed cell that was produced due to the Bcr-Abl chromosomal translocation. But over time the cancer cells will acquire further mutations resulting in the generation of new clones.*

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.

Gleevec binds to the ATP binding site of Bcr-Abl kinase thus preventing ATP from binding to it. If a patient stops taking Gleevec, then the Bcr-Abl kinase will bind to ATP and result in uncontrolled cell proliferation.

- 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 results in Gleevec resistance.

These mutations alter the kinase domain or ATP binding domain of Bcr-Abl kinase preventing Gleevec from binding to it.

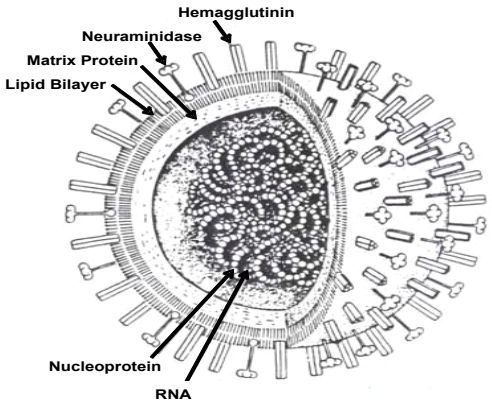
- The Gleevec resistant clones however respond to another drug, Spryzel. If the patients are given both Gleevec and Spryzel together at the beginning of treatment, the generation of drug-resistant clones can be delayed. **Explain** why this may be so.

Multiple separate mutations are far less likely to occur simultaneously than the single mutations needed to confer resistance to either drugs alone.

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, they 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.

Some less aggressive forms of breast cancer are not associated with an overexpression of the Her2 gene, so they are not sensitive to Herceptin. Often the aggressive forms of breast cancers, are associated with an amplification of the Her 2 gene. The amplification of the Her2 gene correlates with the increased expression of receptor on cell surface, which increases the proliferation signal that is critical for tumor development. Herceptin is a monoclonal antibody that binds to the Her2 receptors that are expressed on the surface of cancerous cells thereby flagging these cells for destruction by the immune system. At the same time Herceptin blocks the dimerization of receptors (which is needed for their activation) the downstream signaling by the Her2 receptors thereby preventing tumor proliferation. It also promotes receptors internalization and their degradation or proteolysis.

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**.
Budding: the presence of the lipid bilayer in the virion shows that this particle was derived from the host cell membrane.

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.
Its genome has the opposite polarity compared to the RNA of the host cell that it this virus infects. So this virus has to bring in its own RNA dependent RNA polymerase into the host cell at the time of infection which converts the minus RNA strand to + RNA strand that can then be recognized and translated by the host cell machinery.

ii. **Explain** how the virus overcomes this issue and replicates its genome in the host.
It brings in its own RNA dependent RNA polymerase into the host cell at the time of infection.

c) **Explain** why we need to develop a new vaccine against the flu virus almost each flu season.
This is a segmented virus with multiple RNA strands. If two different viral strains (Strain A and Strain B) simultaneously infect a cell the progeny virus may have mixture of genome segments some coming from strain A and others from strain B. Hence they may have new antigens for which we need a new vaccine. Besides, the RNA dependent ***RNA polymerase has no proofreading activity*** *thus increasing the frequency of mutation that leads to the production of new strains.*

Question 6 continued

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	*Yes*	*Hemagglutinin since they are localized on the surface and are involved in binding of the virus to the receptors on target cells. So antibodies produced against these surface antigens will neutralize these antigens and prevent the virus from binding to the target cells*
Matrix protein	*No*	*No since these is not a part of the cell surface protein and will not be available to bind to the antibodies developed against them.*
Nucleoproteins		
Neuraminidase	*Yes*	*Neuraminidase since they are localized on the surface and are involved in the entry of the virus into the target cells. So antibodies produced against these surface antigens will prevent the virus to enter into the cells.*

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?

The gp120 protein on the surface of HIV envelop binds to the CD4 receptor on the surface of T helper cells and this ligand-receptor binding event is the first step of 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?

The gp41 protein on the surface of virus binds to a chemokine receptor (CCR) on the surface of T helper cells. If a person shows a homozygous mutation for the CCR gene (CCR-/ CCR-) he/she will not have the chemokine receptor and will not contract AIDS even after repeated exposure 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?

AZT is a thymidine analogue (a nucleotide used in the synthesis of DNA). Therefore AZT interferes with the synthesis of DNA from RNA by reverse transcriptase. This enzyme incorporates AZT more effectively into the growing DNA chain and this blocks the further elongation of the chain because the growing end has no 3'-OH group on the deoxyribose sugar. So the viral concentration decreases over time with response to the treatment.

7.013 Introductory Biology
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