Solution key - 2010 7.012 Problem Set 6

Question 1

Working as a UROP, you are asked to complete some experiments that involve several different viruses, including **R**ous **s**arcoma **v**irus retrovirus (RSV)**.** After a long day, you place your viral samples in the refrigerator, but you forget to label them. As a 7.012 student, you have recently heard Prof. Weinberg's lecture on cancer viruses, so you know you can identify the tubes.

a) The type of genomes found in viruses can vary. Some viruses have single (ss) or double-stranded (ds) DNA genomes; others have single (ss) or double-stranded (ds) RNA genomes.
Given the data below, identify **all** possible types of genomes for each virus.

Virus	% A	% T	% U	% C	% G	Type of genome, i.e., ds-DNA
1	18	18	0	32	32	*ds-DNA (ss-DNA also possible)*
2	25	0	25	25	25	*ds-RNA (ss-RNA also possible)*
3	35	15	0	15	35	*ss-DNA*

The experiment in part (a) allows you to identify all of the unlabeled viruses except 2. The remaining 2 viruses are both single-stranded RNA viruses. You know that one virus is the Norwalk virus, and the other is RSV.

> The Norwalk virus causes intestinal illness. Norwalk and Norwalk-like viruses are increasingly being recognized as leading causes of food borne disease in the United States. The Norwalk virus is a + strand RNA virus that is surrounded by an icosahedron-shaped capsid composed of two different polypeptides.
>
> RSV is a retrovirus that also has a + strand RNA genome that encodes four genes; gag (encodes the capsid protein), pol (encodes the reverse transcriptase), env (encodes the envelop glyco-protein) and src (encodes a tyrosine kinase enzyme).

b) Given the information above…

i) Do you expect that the Norwalk virus would encode its own RNA polymerase? Explain why or why not.

Yes, this virus must encode its own polymerase as the host cell will not have an RNA dependent RNA polymerase. However, it does not have to package and deliver this polymerase to the host cell because it has the same polarity as an mRNA and the host cell translation machinery can recognize the viral RNA and translate it. The viral polymerase is then used during replication of the viral genome, which proceeds through the (+)RNA --> (-)RNA --> (+)RNA process.

ii) Reverse transcriptase is considered which of the following?

~~A DNA directed DNA polymerase~~
A DNA directed RNA polymerase
~~A RNA directed DNA polymerase~~
A RNA directed RNA polymerase

iii) Why is it essential that the RSV encodes Reverse transcriptase?

The enzyme needed to copy the viral genome must be able to copy RNA into a cDNA before it can integrate into the genome of the host cell. The host cell will not have an enzyme that can carry out these functions.

c) You have a cell line that can be infected by these two viruses. What is a cell line?

A cell line is an immortalized cell, which continuously grows in culture. All the cells in the culture are originating from a single cell and therefore have the same genetic make-up as each other.

Question 1, continued
To determine which virus is which, you grow your cell line on two identical plates (plates A and B) in a medium that contains C^{14}-labeled uracil, P^{32}-labeled phosphates and S^{35}-labeled methionine. You infect the cells grown on plate A with the virus from tube #1 and the cells grown on plate B with the virus from tube #2 and allow the viruses to reproduce and make new viral particles. You then isolate the radiolabeled virus particles from the infected cells in each plate and determine the radioisotopes that they incorporate. You find that both the viruses incorporated **all** three radio- isotopes.

d) Which viral molecule or molecules are labeled with each of the following?

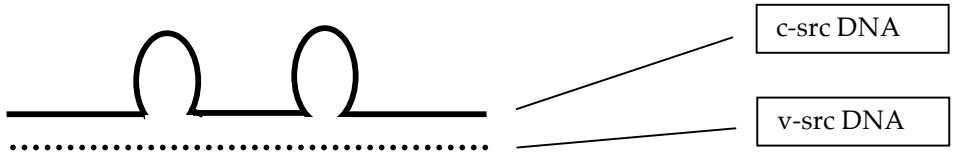
C^{14}-labeled uracil : *RNA*
P^{32}-labeled phosphates: *DNA/ RNA (proteins also acceptable)*
S^{35}-labeled methionine : *Proteins*

e) You purify the proteins from each virus. You find that virus 1 and virus 2 are each composed of two major proteins. However, one of the proteins purified from virus 1 is a transmembrane protein. Which virus is most likely RSV, virus 1 or virus 2? **Explain** your answer.
Most likely Virus 1 is RSV since it contains a transmembrane protein that should be a part of its envelop.

f) Given what you know, the newly synthesized **functional RSV particles** are released by

- lysing the host cell.
- budding without host cell lysis.
- both by host cell lysis and budding.
- Can't tell

g) The following schematic represents a DNA duplex that is the result of the hybridization of the viral src DNA (v-src) with the genomic cellular (c-src) DNA of the target cells.



- Label the dashed and the solid lines in the schematic above as a v-src DNA or c-src DNA.

- Explain why regions of non-hybridization exist between the v-src DNA and the c-src DNA.

The v-src is a cDNA copy of the cellular src gene and hence lacks the introns. The v-src will hybridize with the portions of the genomic c-src DNA that correspond to exons whereas the portions of genomic c-src DNA that correspond to introns will remain un- hybridized and loop out.

h) The RSV can infect the host cells and cause sarcoma, which is a cancer of connective tissues. Circle all the correct options from below. RSV can cause sarcoma by…

-activating the c-src genes of the host cells.

-integrating its **original genome** (i.e. without any replication) , immediately after infection, into the genome of the host cells.

-making a cDNA copy of the complete viral genome that integrates into the genome of the host cells.

-integrating the cDNA copy of the v-src gene into the genome of the host cells.

Question 1, continued

i) Based on your answers above, you would classify v-src gene as which of the following?

an Oncogene, a Proto-oncogene, or a Tumor suppressor gene.

Explain your choice.

It is an oncogene since the v-src represents the activated form of src or a gain-of-function mutation that results in uncontrolled cell proliferation or sarcoma.

Question 2

a) Normal cells in culture display contact inhibition. What is contact inhibition?

This is the ability of the cells to stop dividing any further when they come in contact with a neighboring cell.

b) Exposure of cells to various chemicals can cause cells to lose contact inhibition. Cells that have lost contact inhibition can form a pile of cells called a focus and such cells are referred to as *transformed*.

- Explain at a molecular level how the chemical acts to transform cells.

A chemical may transform a cell by causing mutations in the genome. If these mutations result in a constitutively active oncogene or the loss of function of both alleles of a tumor suppressor gene then the control on cell division is lost. Some chemicals may also transform a cell by altering the function of a gene product i.e. phosphorylating and activating src tyrosine kinase resulting in uncontrolled cell proliferation. Such chemicals are not mutagens but may still act as a carcinogen.

- Do the cells of a single focus represent a monoclonal or a polyclonal cell population? **Explain** why you selected this option.

A focus is the result of a single cell that divides uncontrollably. All the cells in a focus are the descendants of a single transformed cell and hence have the same genetic makeup i.e. they are monoclonal.

c) You want to determine the carcinogenic potential of three different chemicals: A, B and C. You incubate a human cell line with these chemicals individually and look at their ability to form foci. You also inject these chemicals separately into mice and look for the development of cancer and mutations in DNA. The results are tabulated below. *Note: You may assume that your results are real and not an experimental artifact.*

Chemical	Foci formation?	Cancer in mice?	Mutations in DNA
NONE	No	No	No
A	Yes	Yes	Yes
B	No	Yes	Yes
C	Yes	Yes	No

- Which of these chemicals (A/B/C) are carcinogens? Circle **all** that apply.
- Which of these chemicals (A/B/C) are mutagens? Circle **all** that apply.
- Give an explanation for why chemical "B" might cause cancer in mice and yet not transform the cell line?

Chemical B is most likely a promutagen, which does not cause cancer in its original/native form. However, when it is internalized by the living organisms, it may be metabolized by certain enzymes in the body to a form that can act as a mutagen. Alternatively, it may be a chemical that transforms cells by altering the function of a gene product i.e. phosphorylating and activating src tyrosine kinase resulting in uncontrolled cell proliferation.

Question 2, continued

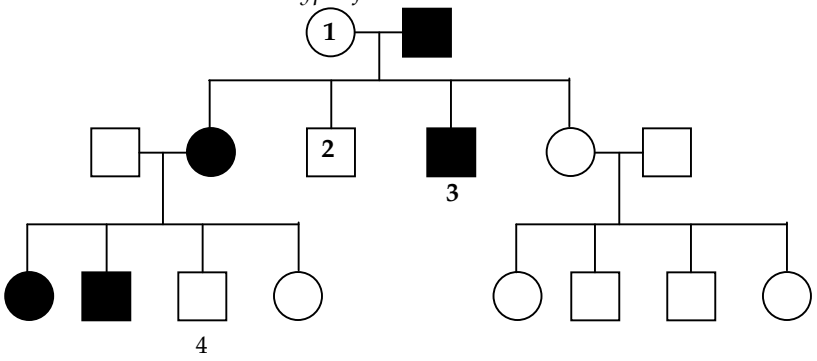
d) In cells in culture, a transformed phenotype can be seen in cells that have mutations in proto-oncogenes and tumor suppressor genes. Classify each of the following genes of interest either as a proto-oncogene or tumor suppressor gene. Also state whether you expect to see a transformed phenotype in cells that are heterozygous for a loss-of-function (LOF) mutation in the gene of interest.

Genes mutated	Normal function of encoded protein	Proto-oncogene or a tumor suppressor gene?	Would phenotype associated with LOF mutation be present in a cell heterozygous for a mutation in that gene? *(Note: Assume haplo sufficiency)*
Bax	Promotes cell death	*Tumor suppressor*	*No*
PDGF	Growth factor receptor protein	*proto-oncogene*	*No*
APC	Halts cell cycle	*tumor suppressor*	*No*
Her-2	Promotes cell to enter cell cycle upon activation	*Proto- oncogene*	*No*
myc	Acts as a transcription factor to promote cell division	*proto- oncogene*	*No*

e) Cell lines are often used to test the oncogenic potential of the viruses. If cancer is a multi-step process, why can the introduction of a single active viral oncogene transform these cells?
Unlike the normal cells, the cell lines are immortal i.e. they can divide indefinitely and they are made so through the accumulation of mutations. Therefore the introduction of a single viral oncogene into a cell line essentially reflects the addition of one more mutation to a series of mutations that were preexisting in the cell line. Hence the transformation of a cell line by adding a single active viral oncogene does not counteract the statement that "cancer is a multi step process".

Question 3

The following human pedigree shows inheritance of a specific type of cancer. *Note: The shaded squares and circles represent the individuals that have this type of cancer.*



a) Looking only at the pedigree, this disease appears to have what mode of inheritance?
Autosomal dominant

b) For individual 3, you find that the blood samples are heterozygous at the locus associated with the disease, *i.e.*, they carry one wild type allele of the gene and one mutant allele of the gene associated with this type of cancer. Is this consistent with what you would predict given your answer to (a) above?
Yes.

Question 3, continued

c) If you check the genotype of the tumor cells from individual 3, you find that they are homozygous for the disease allele. Explain why the pattern of alleles is different in the blood sample and the tumor sample isolated from individual 3.

This indicates that this cancer results from the loss of both alleles of a tumor suppressor gene, not the acquisition of an oncogene. Thus the apparent autosomal dominant mode of inheritance represents the inheritance of ***predisposition*** *to this type of cancer. The cancer phenotype is correlated with being a carrier of the disease allele because in an individual that is Dd, the chance of a subsequent mutation in the good copy of the disease allele is very high.*

d) For individual 4, the blood samples are heterozygous, carrying both the wild-type allele and a mutant allele of the gene associated with this type of cancer. However, this individual **unexpectedly did not develop** cancer. Explain why.

This individual was lucky enough and did not have the 2nd mutation that would have resulted in the loss-of heterozygosity (LOH) of the tumor suppressor gene and therefore the development of cancer.

Question 4

a) Would you describe the transformed phenotype seen in cells that have acquired an oncogene as dominant or recessive? Explain your answer.

The transformed phenotype of a cell carrying an oncogene is dominant. The cells receiving the oncogene have their own wild-type copies of the proto-oncogene, thus when they acquire an oncogene, they are heterozygous. If the transformed phenotype is present in the heterozygote, by definition it is dominant.

b) Would you describe the transformed phenotype seen in cells as a result of mutation in tumor suppressor genes as dominant or recessive? Explain your answer.

The transformed phenotype seen with the loss of a tumor suppressor function requires that both copies of the tumor suppressor gene be mutated and is therefore recessive.

c) Not all mutations in a proto-oncogene result in the formation of an oncogene.

- Give an example of a proto-oncogene. *C-src, Her-2,c-abl, etc. (Many choices here)*

- Given an example of a mutation in this proto-oncogene that would result in an onocogene.

Amplification of the Her-2 gene that result in excessive production of active Her-2 protein through dimerization. (Many choices here)

- Given an example of a mutation in this proto-oncogene that would NOT result in an oncogene.

Mutations that result in the formation of src tyrosine kinase that lacks its kinase domain and can therefore not be phosphorylated or activated. (Many choices here)

d) Radiation therapy can be used to treat tumors.

- Explain how radiation therapy works to treat a tumor.

Radiation therapy works by massively damaging the DNA of the rapidly dividing cancer cells. With extensive DNA damage, cell will often initiate a pathway for apoptosis or programmed cells death.

- Would you predict that a tumor composed of cells that are $p53^-/p53^-$ 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 these cells cannot activate the apoptosis pathway. A tumor composed of cells that are $p53^+/p53^-$ can activate the apoptosis pathway and those cells will die as a result of treatment.

Question 4, continued

e) Chemotherapeutic drugs often have side effects such as diarrhea, constipation, mouth sores, hair loss, nausea, and blood-related side effects.

- Chemotherapeutic drugs have a wide range of structures and functions, yet many elicit the same side effects. Explain why the side effects are the same for 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 almost very similar side-effects.

- Describe what is meant by the "therapeutic window" of a drug used in chemotherapy, and how it relates to the side effects seen in a patient.

The therapeutic window is a measure of the difference between the concentration of a drug that is required to kill the cancer cells (effective dose) and the concentration of the drug that affects normal cells. A drug with a larger therapeutic window will have fewer side effects at the effective dose.

f) The Human papilloma virus (HPV) has been implicated as a risk factor for cervical cancer. The E7 protein of HPV can bind to pRB and prevent pRB from inhibiting the cell cycle. In contrast another HPV protein, namely E6 binds to p53 and induces destruction of p53. Explain why the virally encoded E6 and E7 proteins can result in cells that form a tumor.

Both RB and p53 are tumor suppressor genes that encode proteins that inhibit cell cycle or promote cell death. Both E6 and E7 bind to and inactivate the p53 and RB proteins. Therefore the p53 and RB proteins can no longer inhibit the cell cycle. This results in uncontrolled cell proliferation or tumor.