

**7.013 Problem Set 8 FRIDAY May 7th 2004****Problem 1**

Exposure to mutagenic substances increases the risk of cancer. These substances can be found in some foods, dyes, cigarette smoke and industrial pollutants. You suspect that a this food additive, "Spicyo's" that upsets your stomach may be causing cancer. In order to gather more data, you decide to examine the mutagenicity of "Spicyo's".

One of the commonly used tests for mutagenicity is the Ames test, in which a bacterial strain ( $\text{His}^-$ ) cannot grow if there is no histidine in the medium. This bacterium cannot make its own histidine because the gene coding for a protein in the histidine biosynthesis pathway has a premature stop codon. If one were to mutate the stop codon back to the normal codon, the bacterium would now be able to make its own histidine and grow on medium lacking histidine.

a) First, you plate  $\text{His}^-$  bacteria on media with or without histidine.

How many colonies would you expect on each plate? (none, very few, moderate, many)

Medium with histidine \_\_\_\_\_

Medium without histidine \_\_\_\_\_

b) When you plate  $\text{His}^-$  bacteria on medium without histidine, you occasionally see colonies come up with a low but constant frequency. You reason that these few colonies must have new mutations in their already mutant *his* gene that reverted them back to wildtype (changed the premature stop codon to a normal codon).

How does this happen? What may be the mechanism by which these bacteria get mutations so that they revert back to wildtype with a low constant frequency?

c) Now to test the mutagenicity of Spicyo's! You mix it with  $\text{His}^-$  bacteria and plate on both kinds of plates. How many colonies would you expect on each plate if Spicyo's is highly mutagenic? (none, very few, moderate, many)

Medium with histidine \_\_\_\_\_

Medium without histidine \_\_\_\_\_

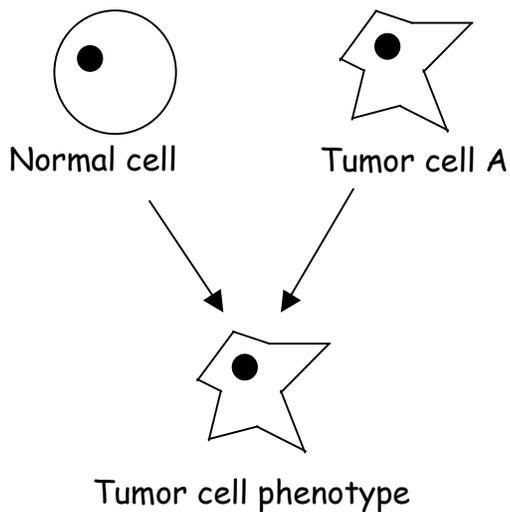
## Problem 2

You are a physician treating several patients with cancer. You would like to know which mutations caused the tumors in two patients A and B. You isolate tumor cells from these patients and grow the cells in culture. The tumor cell lines, A and B, divide rapidly in culture.

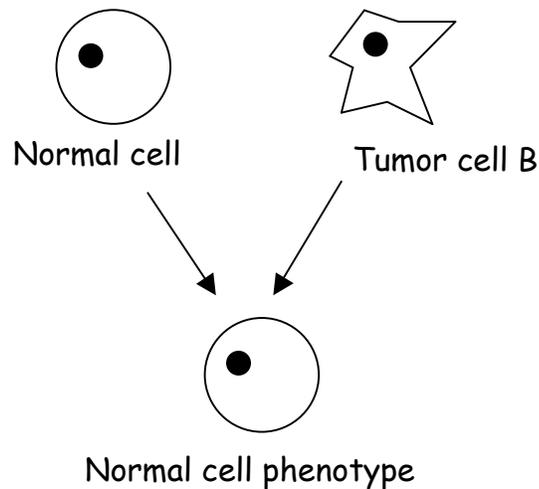
You carry out cell fusion experiments in which you fuse each of the tumor cells with a normal cell and examine whether the fused cells behave like tumor cells.

### Experiment

1.



2.



a) Based on these experiments, indicate whether each tumor cell (A and B) has a mutation in a tumor-suppressor gene or a mutation in a proto-oncogene, and what kind of mutation (activating, inactivating, dominant and/or recessive) produced the mutant phenotype of the tumor cell?

b) You would like to know if the tumors from two other patients C and D respond to radiation therapy. You irradiate the tumor cell growing in culture and observe whether the cells are killed by treatment. Tumor cells taken from patient C do not seem to be affected by the treatment, but the cells from patient D, like normal cells, stop dividing and then die by apoptosis (programmed cell death). Briefly explain how radiation treatment of cells inhibit cell growth and induces apoptosis.

c) Based on the results from the radiation treatment, would you expect p53 to be functional in tumor cells from patients C and D? Explain your reasoning.

### Problem 3

Retinoblastoma is a tumor that forms in the eye and is caused by mutations in the *Rb* gene. Children that have inherited one defective copy of the gene from their parents develop tumors in both eyes, whereas other children who develop retinoblastoma but don't inherit a defective copy only develop the tumor in one eye.

a) What class of cancer-causing gene is the *Rb* gene?

b) Why do heterozygous children are much more likely to develop tumors in both eyes?

c) Many other types of cancers also contain mutations in the *Rb* gene. Since you know this you decide to sequence the *Rb* gene from a panel of tumors. Interestingly you do not find any mutations in the *Rb* gene in these tumors. You then look at the Rb protein in cells from these tumors and find that it is fully phosphorylated in all phases of the cell cycle. Briefly explain why the constitutive phosphorylation of the Rb protein would lead to tumor formation?

#### Problem 4

HIV is a retrovirus that infects  $T_H$  cells of the immune system thereby causing AIDS. HIV genome is a single stranded RNA molecule. Two copies of this RNA genome are packaged with two molecules of the retroviral enzyme, reverse transcriptase within a protein capsid. This capsid is further packaged into an envelope derived from the plasma membrane of the host cell in which the virus was formed. The surface of the envelope is covered with an envelope glycoprotein called gp120.

a) HIV specifically infects specifically the helper T cells of the immune system. If the virus must bind to a cell surface receptor on the T helper cells in order to gain entry into them, what protein on T helper would you predict serves as a receptor for the virus?

b) Once infection takes place the RNA genome has to be made into double-stranded DNA. This process is mediated by reverse transcriptase. Once a double stranded DNA copy of the HIV genome is made, it is integrated into the host cell genome. What are the three steps required to produce double stranded DNA from the single strand RNA genome?

c) The central dogma states that



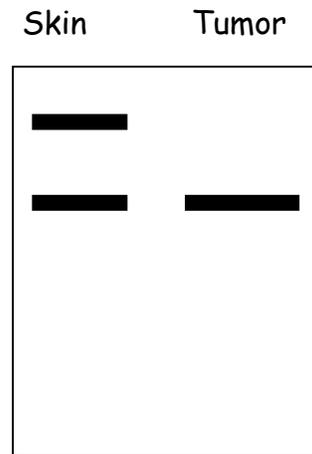
Which one of these steps of the central dogma does the reverse transcriptase goes against?

d) The drug that was most commonly used to combat AIDS was Azidothymidine (AZT). The structure of AZT is very similar to thymidine except that in AZT that in AZT the 3' hydroxyl has been replaced by an azido ( $N_3$ ) group. Which process in the life cycle of HIV do you think AZT inhibits?

e) AZT resistant forms of HIV have been isolated. These mutant viruses have mutations in one of the viral genes. In which viral gene do you think the mutations are most likely to be found?

### Problem 5

A schwannoma (a tumor of the scwhann cells in the nervous system) is surgically removed from a person with one germline mutation neurofibromatosis type 2 (*NF2*) gene (heterozygous for *NF2* gene). DNA is isolated from the tumor, cut with a restriction enzyme, and hybridized with a polymorphic DNA probe closely linked to the *NF2* locus. The following Southern blot pattern is seen, along with the pattern from analysis of skin DNA from the same person.



a) How many copies of the *NF2* gene are present in each of the cells of the tumor mass? Does this suggest *NF2* acts as an oncogene or a tumor suppressor?

b)

i) What are some of the mechanisms by which the one copy of the *NF2* gene can be lost in the tumor cells?

ii) What is the formal definition of this process? *Loss of heterozygosity*

c) If the father of this person also has tumors associated with *NF2* (therefore he is also heterozygous), would you expect the father's or mother's allele to have been the one lost in the tumor of this person?