Quiz 3 on Wednesday, May 1\textsuperscript{nd}  
11:05 - 11:55

Review Session 4/29 from 7-9 pm in 10-250

Tutoring Session 4/30 from 4-6 pm in 26-002

<table>
<thead>
<tr>
<th>Last Names</th>
<th>A through B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>and W through Z in 10-250</td>
</tr>
</tbody>
</table>

and

<table>
<thead>
<tr>
<th>Last names</th>
<th>C through V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in Walker Gymnasium</td>
</tr>
</tbody>
</table>

This will be a closed book exam.  
Bring IDs.
Question 1

A) You infected mice with mouse mammary tumor virus (a retrovirus). After a period of time, most infected mice had developed breast tumors, whereas uninfected mice did not. You isolated cell lines from over 50 independent tumors. You demonstrated that all of these lines had virus integrations in the same chromosomal location. Can one conclude that the virus integrates into cellular DNA at only one site? Explain.

B) The ras oncogene is involved in a variety of human and animal cancers. DNA was isolated from a number of normal and cancerous tissues.

- Cellular DNA was digested with EcoRI.
- Digested DNA was separated by gel electrophoresis and transferred to a nitrocellulose membrane.
- The membrane was probed with the radioactive labelled cloned ras DNA and then the membrane was exposed to x-ray film.
- The resulting autoradiograph is shown below.

1) white blood cells from a healthy human
2) human lymphoma cells (cancerous)
3) human bladder carcinoma cells (cancerous)
4) human sarcoma cells (cancerous)
5) blood from a healthy mouse
6) mouse myeloma cells (cancerous)
Question 1 continued

a) How do you explain the presence of sequences complementary to the oncogene in the DNA from healthy human and mouse samples? Why don't they have cancer?

b) Why is the hybridizing band from sample 1 a different size than that from sample 5?

c) For each cancer examined above, based on the autoradiogram, choose the most likely mechanism of transformation and explain your choice:

1) point mutation within the gene
2) chromosomal rearrangement involving the gene
3) gene amplification
4) oncogenic retroviral insertion.
Question 2

Erythropoietin (EPO), a protein growth factor secreted by the kidneys, is essential for the terminal differentiation of red blood cells (erythrocytes) in the bone marrow. EPO binds to a transmembrane EPO receptor found on erythroid precursor cells.

a) Is EPO an example of an autocrine, paracrine or endocrine signal?

b) The following homozygous mutations were made only in the hematopoietic stem cell lineage. How would these mutations affect (increase, decrease, or not change) the number of red blood cells formed, compared to the wild-type situation? Briefly explain your reasoning for each mutation. Consider each mutation independently.

i) A mutation in the EPO gene that resulted in the deletion of only the signal sequence of the EPO protein.

ii) A mutation in the EPO receptor gene that resulted in the deletion of only the transmembrane domain of the EPO receptor.

iii) A mutation in the EPO receptor gene that resulted in the deletion of only the cytoplasmic domain of the EPO receptor.
Question 3

Part I

As a premier cancer biologist, you often plate cells in dishes, feeding them serum with growth factors and allowing them to grow for 2 weeks. Sometimes after incubation of strains you observe the following when looking at the side of a culture dish.

![Strain A and Strain B](image)

a) Which plate shows abnormal cells? Explain.

b) Predict the behavior of these cell lines if grown without added growth factors by drawing what the plates will look like after incubation without growth factors. Simply modify the existing figure below for your answer. (Note: one cell from each strain is initially deposited in each dish.)

![Strain A and Strain B](image)

Part II

A fellow researcher gives you two cancerous cell lines to examine and determine possible mutations. The results are shown below.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>none (wild type DNA)</td>
</tr>
<tr>
<td>1</td>
<td>a deletion at the same region on both copies of chromosome 4</td>
</tr>
<tr>
<td>2</td>
<td>a point mutation in a gene on only one copy of chromosome 7</td>
</tr>
</tbody>
</table>

c) Based on this data above, identify the type of cancer gene that is mutated in each of the cell lines.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Cancer Gene (oncogene or tumor suppressor gene)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>none</td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
Question 3 continued

You learn that cell line 1 is a skin cancer cell line. The region you identified as deleted on chromosome 4 in these cells normally contains a gene called \( p16 \).

d) What is the role of the \( p16 \) gene product in the normal cell based on the information above?

You obtain another cell line (cell line 3) that has one wild-type copy of chromosome 4 and one mutant copy of chromosome 4 (as described above in cell line 1).

e) Will cell line 3 display a cancerous phenotype when grown in the presence of growth factors? Yes/No (Circle one.)

Explain briefly.

f) Will cell line 3 display a cancerous phenotype when grown in the absence of growth factors? Yes/No (Circle one.)

Explain briefly.

Part III

g) Cell line 2 is a breast cancer cell line that expresses a mutant version of a receptor protein called KIT. Choose from the following options to explain the role of KIT in normal cells. Circle one.

- Activation of KIT causes cells to undergo apoptosis.
- Activation of KIT promotes progression through the cell cycle.
- Activation of KIT has no effect on the cell cycle.
- Activation of KIT causes cells to enter G0.

h) Specifically how could a point mutation in the gene encoding the KIT receptor cause the abnormal behavior depicted in Part I.
Question 4

In a developing organism, three cells, X, Y, and Z, that lie adjacent to one another give rise to cells that form nerve cells, hypodermal cells and muscle cells, respectively, as shown below:

A series of transplant experiments were also done with these cells, to give the following rearrangements. The results show that the Z cell signals immediately adjacent cells to become hypodermal cells.

a) Based on the above information, what is the fate of cell X?

b) Based on the above information, to which cell types does cell X have the potential to give rise?

c) For which cell(s) (X, Y and/or Z) can you infer that development is regulative? Briefly justify your reasoning.

d) The same cell-surface receptor that is associated with a G protein signal transduction pathway is found on X cells and Y cells; this receptor is not present on Z cells. What is the most likely function of this receptor?
e) Briefly explain why X cells lacking the GTP-binding function of the G protein coupled to this receptor yield the following results.

[Diagram showing cell types and their interactions]

f) Briefly explain why X cells lacking the GTPase activity of the G protein yield the following results.

[Diagram showing cell types and their interactions]
Answers
Question 1
A) One cannot conclude that the virus is able to integrate at only one site. However, one might propose that the virus can only cause cancer when it integrates into a certain chromosomal location or next to a specific gene. In fact, viruses can integrate many places throughout the genome. The reason you only observed integration events at one site is because you have examined only those events that cause tumors. Perhaps the integration of the virus next to a proto-oncogene can cause it to become oncogenic, possibly by activating expression of the oncogene in the wrong place or at the wrong time.

B) a) The sequences that are complementary to the probe in normal cell DNA correspond to the cellular proto-oncogene. The individuals from which the material came don’t have cancer because they have not acquired the mutations necessary to turn the proto-oncogene into an oncogene.

b) Random sequence variation between mouse and human DNA alters the restriction map around the gene. The two species diverged from a common ancestor during the process of evolution, and DNA sequence variation has been accumulating since. Some of these variations occur in restriction enzyme sites.

c) -lane 2: a chromosomal rearrangement or a point mutation at one of EcoRI restriction sites are the most probable mechanisms because one copy of the gene has changed its location with respect to at least one of the flanking EcoR1 sites.

-lane 3: a point mutation within the coding sequence of the gene is the probable mechanism of transformation because there is no obvious change in the Southern blot--none of the restriction sites have been altered.

-lane 4: gene amplification has created many copies of the gene which probably are present in several tandem arrays in the sarcoma DNA.

-lane 6: retroviral transduction has brought an extra copy of the oncogene into the cell. Since the smaller fragment is still present in two copies per cell, there has probably not been any change in the "resident" proto-oncogenes.

Question 2
a) It is an example of endocrine signaling because the hormone erythropoietin is sent from the kidney via the blood to the bone marrow.

b) i) No effect on red blood cell (RBC) formation. If the EPO signal sequence were deleted, EPO would remain in the cytoplasm of the hematopoietic stem cell. EPO, however, is only made by kidney cells and thus the mutation should not affect RBC formation in the bone marrow.

ii) Red blood cell formation would decrease. Since the CFC-E cell is derived from the hematopoietic stem cell, a deletion of the transmembrane sequence would result in the secretion of the EPO-R outside of the CFC-E cells, and, as a result, it would not be able to signal the CFC-E cells to grow and differentiate to form erythrocytes.
iii) Red blood cell formation would decrease. If the EPO-R receptor lacked its phosphorylation site, it cannot be activated. After binding EPO, the activated EPO-R could not signal the CFC-E cell to differentiate into erythrocytes.

**Question 3**

**Part I**

As a premier cancer biologist, you often plate cells in dishes, feeding them serum with growth factors and allowing them to grow for 2 weeks. Sometimes after incubation of strains you observe the following when looking at the side of a culture dish.

![Strain A](image1) ![Strain B](image2)

a) Which plate shows abnormal cells? Explain.

*The plate on the left. Strain A shows no contact inhibition. Normal cells stop growing when they touch each other. Abnormal cells pile up.*

b) Predict the behavior of these cell lines if grown without added growth factors by **drawing** what the plates will look like after incubation **without** growth factors. Simply modify the existing figure below for your answer. (Note: one cell from each strain is initially deposited in each dish.)

![Strain A](image3) ![Strain B](image4)

**Part II**

A fellow researcher gives you two cancerous cell lines to examine and determine possible mutations. The results are shown below.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>none (wild type DNA)</td>
</tr>
<tr>
<td>1</td>
<td>a deletion at the same region on both copies of chromosome 4</td>
</tr>
<tr>
<td>2</td>
<td>a point mutation in a gene on only one copy of chromosome 7</td>
</tr>
</tbody>
</table>

c) Based on this data above, identify the type of cancer gene that is mutated in each of the cell lines.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Cancer Gene (oncogene or tumor suppressor gene)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>none</td>
</tr>
<tr>
<td>1</td>
<td>TSG</td>
</tr>
<tr>
<td>2</td>
<td>ONCOGENE</td>
</tr>
</tbody>
</table>
**Question 3 continued**

You learn that cell line 1 is a skin cancer cell line. The region you identified as deleted on chromosome 4 in these cells normally contains a gene called *p16*.

d) What is the role of the *p16* gene product in the normal cell based on the information above?

It is a gatekeeper of the cell cycle (the brake linings) preventing progression through cell cycle unless all checks out. It inhibits cell proliferation.

You obtain another cell line (cell line 3) that has one wild-type copy of chromosome 4 and one mutant copy of chromosome 4 (as described above in cell line 1).

e) Will cell line 3 display a cancerous phenotype when grown in the presence of growth factors?  **Yes/No** (Circle one.)

Explain briefly.

No it the p16 mutation is recessive to wild-type. Basically the mutant chromosome 4 gives a recessive cancerous phenotype. Cell line 3 has a WT copy of chromosome 4 which is sufficient to give the WT (NORMAL) phenotype.

f) Will cell line 3 display a cancerous phenotype when grown in the absence of growth factors?  **Yes/No** (Circle one.)

Explain briefly.

It will not grow. THERE ARE NO GROWTH FACTORS. Cell line 3’s cancerous phenotype is recessive. (Not simply that the cells wouldn't grow because cell line 3 contains a WT copy of the p16 gene, and enough p16 protein is present to block proceeding through the cell cycle given the absence of growth factors.)

**Part III**

g) Cell line 2 is a breast cancer cell line that expresses a mutant version of a receptor protein called KIT. Choose from the following options to explain the role of KIT in normal cells. Circle one.

- Activation of KIT causes cells to undergo apoptosis.
- Activation of KIT promotes progression through the cell cycle.
- Activation of KIT has no effect on the cell cycle.
- Activation of KIT causes cells to enter G0.

h) Specifically how could a point mutation in the gene encoding the KIT receptor cause the abnormal behavior depicted in Part I.

Any mutation in the receptor that would cause it to be constitutive, ligand-independent activation, dimerization, always active, etc. would be enough to cause the cancer phenotype.
Question 4

a) To become nerve cells

b) To become either nerve cells or hypodermal cells.

c) Both cells X and Y, because for each cell potency > cell fate. Cells X and Y require signaling by Z cells to develop into hypodermal cells; without this signal, they become nerve cells.

d) Both X and Y cells have the potential to become nerve cells or hypodermal cells. This receptor is involved in receiving the inducing signal sent by the Z cell to follow the hypodermal cell lineage.

e) The G protein cannot be activated in these X cells, and thus the signal transduction pathway is not active in these X cells. These X cells are never induced to become hypodermal cells.

f) X cells require the signal for induction. In these X cells the G protein signal transduction pathway, once activated, remains active and induction occurs.