

Midterm 3

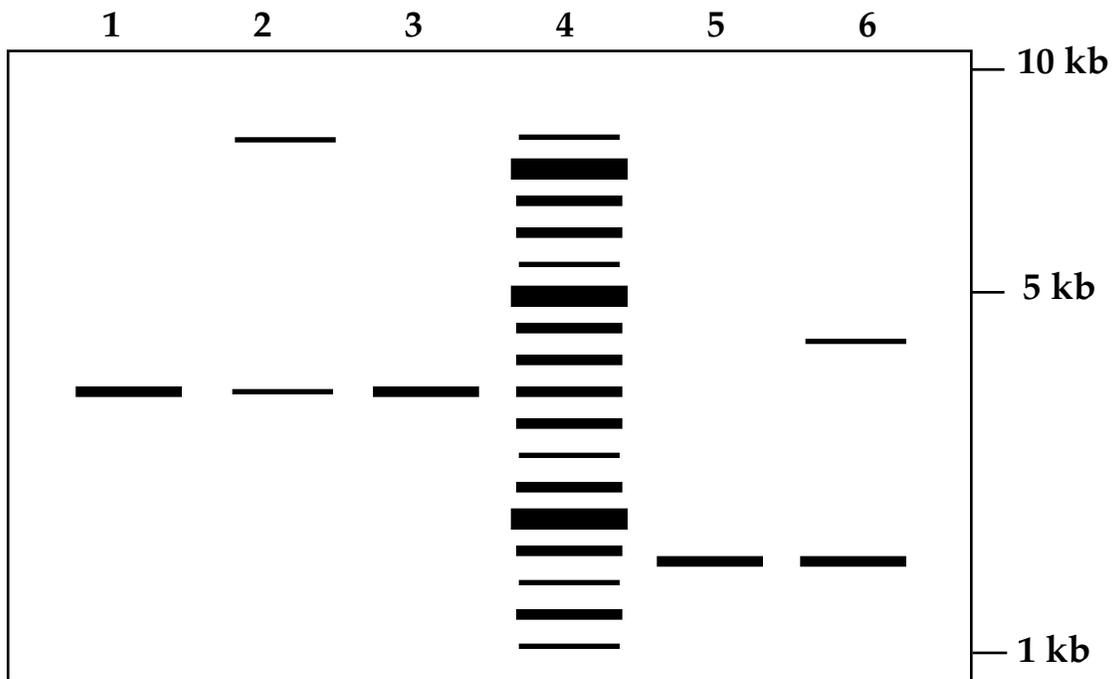
Question 1

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 *Eco* RI.
- 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)



- thick line** more than 2 copies/genome
- medium line** 2 copies/genome
- thin line** 1 copies/genome

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.

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.

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

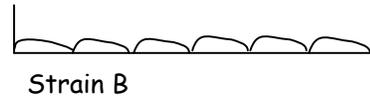
b) A mutation in the EPO receptor gene that resulted in the deletion of only the transmembrane domain of the EPO receptor.

c) 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.



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



Strain B

Part II

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

Cell Line	Mutation
WT	none (wild type DNA)
1	a deletion at the same region on both copies of chromosome 4
2	a point mutation in a gene on only one copy of chromosome 7

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

Cell Line	Cancer Gene (oncogene or tumor suppressor gene)
WT	none
1	
2	

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

The receptors Robo and DCC are important for axon's ability to responding to guidance cues from Netrin and Slit proteins expressed near the brain midline. The mammalian model is summarized below.

- Netrin is secreted from the midline and bound by DCC on the neuron. This attracts the neuron toward the midline.
- Robo is expressed on neurons once they have crossed the midline.
- Slit is secreted from the midline and bound by Robo on the neuron. This repulses the neuron from the midline.
- DCC receptors on the neurons expressing Robo become non-responsive to Netrin binding.

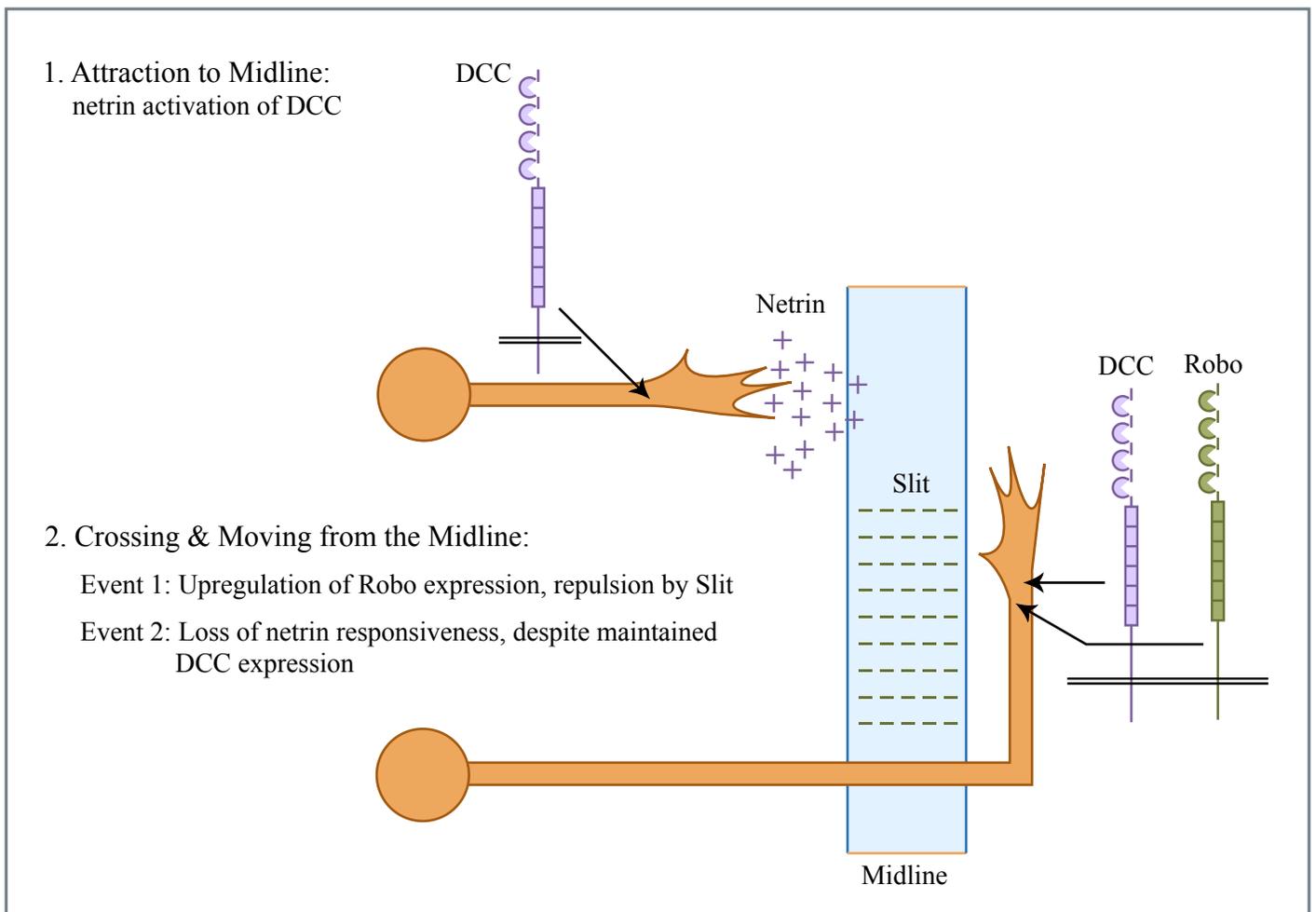


Figure by MIT OCW.

a) In this model of axon guidance it is important that Netrin is...

a (short/long) range **attractant** and a (short/long) range **repellant**

b) You're interested in studying this interaction so you use a convenient model organism, the beloved fruit fly. You make mutants in fruit flies that have the *robo* or *slit* genes knocked out (nonfunctional). Midlines outlined by dashed line. The midline sections of these mutants are shown below adjacent to the wild type. The nerves are stained and shown to cross the midline in the Wild type neuron.

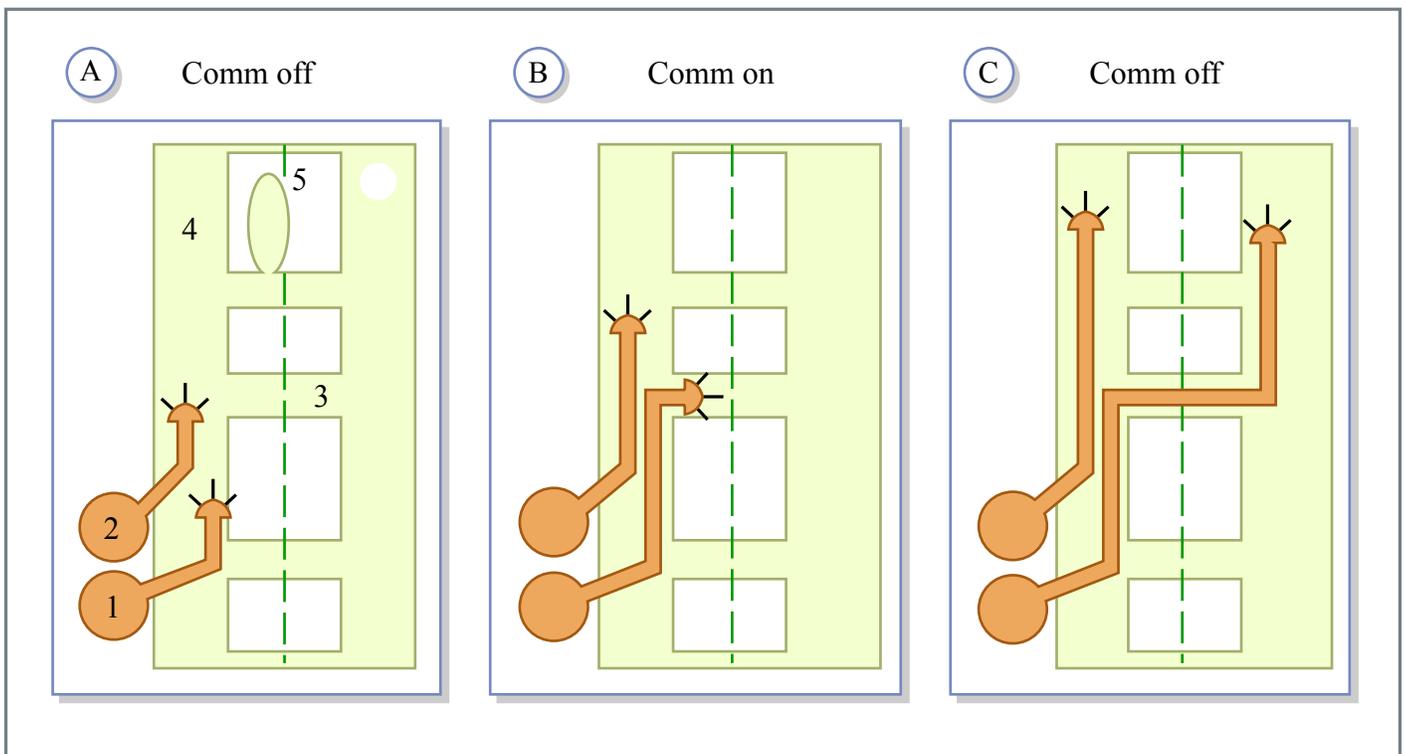
Figure removed due to copyright reasons. Please see Figure 4 in Kidd, Thomas, Kimberly S. Bland, and Corey S. Goodman. "Slit Is the Midline Repellent for the Robo Receptor in Drosophila." *Cell* 96 (March 19, 1999): 785–794. Copyright 1999 by Cell Press.

Wild type neuron Slit knockout neuron Robo knockout neuron

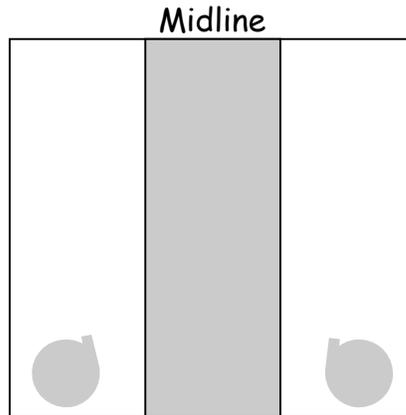
i) Explain the mechanism that would give mutant "Slit knockout" neurons this pattern?

ii) Why is the "Robo knockout" pattern similar to the mutant Slit knockout neurons?

c) In fruit flies there is another intracellular protein, called *Commissureless* (*Comm*), that must be expressed for these neurons to cross the midline. The figure below shows different time points from early (A), to later (B), to even later (C). In figure B, only neuron 1 is expressing the *comm* gene, transiently.



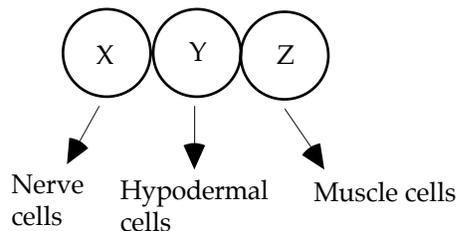
What would an animal with a deletion of both *comm* genes look like? Draw your answer in the box below elongating the axons of the neurons on either side of the midline. (Assume Netrin, DCC, Robo and Slit still expressed normally.) *Axon growth cones never enter midline.*



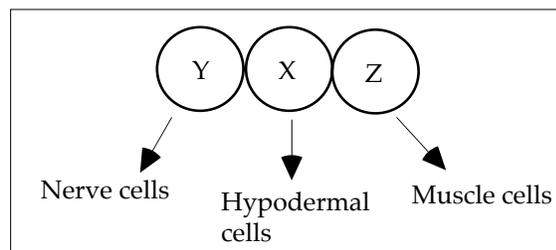
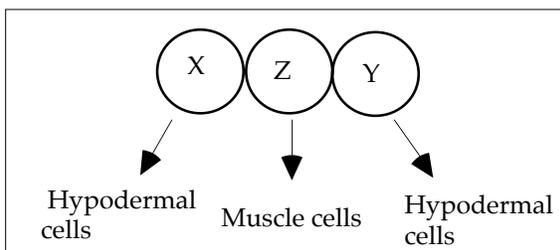
- d) *Comm* is a (Negative/Positive) regulator of the Robo receptor.
- e) Give one example of how intracellular *Comm* may act on the receptor Robo.

Question 5

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.

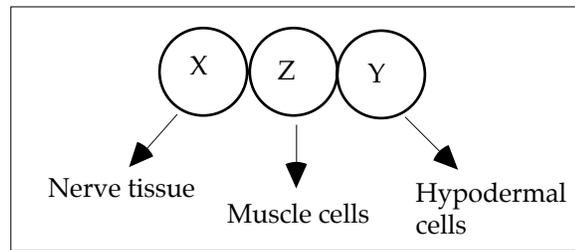
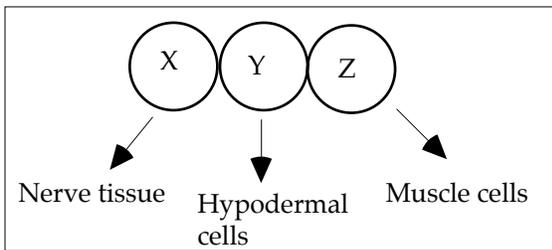


a) Based on the above information, what is the fate of cell X?

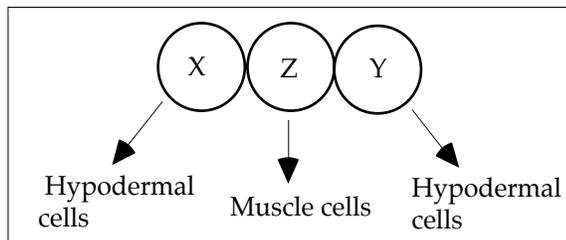
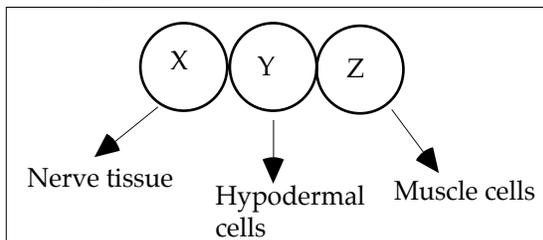
b) Based on the above information, to which cell types does cell X have the potential to give rise?

c) 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?

d) Briefly explain why X cells lacking the GTP-binding function of the G protein coupled to this receptor yield the following results.

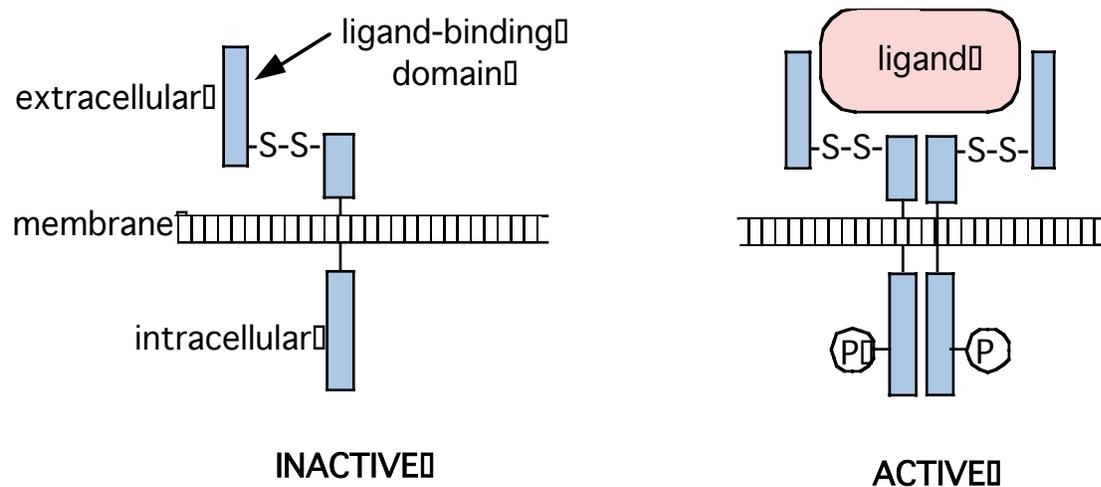


e) Briefly explain why X cells lacking the GTPase activity of the G protein yield the following results.



Question 6

The bos/seven receptor is required for differentiation of a particular cell, called R7. It is a receptor tyrosine kinase with the structure below. As a monomer, the protein is inactive. Binding of ligand causes the receptor to dimerize, causing phosphorylation of the intracellular domain, activating the protein. During processing of the protein, the extracellular domain is cleaved and a disulfide bridge forms between two cysteines, tethering the ligand-binding domain to the rest of the protein.



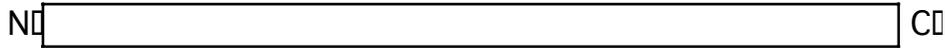
a)

i) How would receptor activity be affected by changing one of the two cysteines shown above to an alanine? Explain.

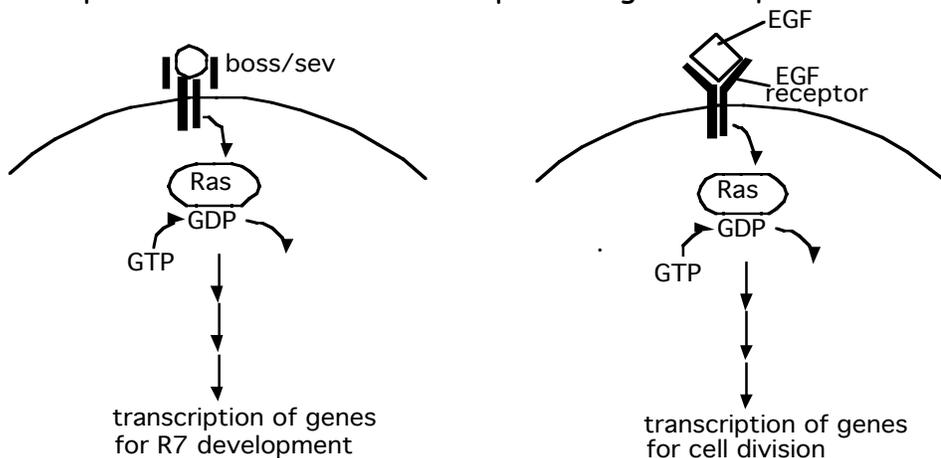
ii) What effect would this mutation have on the differentiation of R7?

b) Name three amino acids that would be likely to be found in the transmembrane domain. What property do those amino acids have in common, and why do they cause the transmembrane domain to stay in the membrane?

d) Draw a schematic of the receptor tyrosine kinase (discussed above) prior to any cleavage or modification using the template below. Include the domains of this protein that are required for targeting to and insertion in the plasma membrane. Also label the intracellular and extracellular domains.



e) Activation of the above receptor causes Ras to exchange GDP for GTP, thereby activating it. This activated Ras can activate a signal transduction cascade, which ultimately results in the transcription of genes required for R7 differentiation. In different cells in the same animal, Ras can be activated by an activated growth factor receptor. This leads to transcription of genes required for cell division.



i) How is it possible for the activation of Ras to lead to transcription of different sets of genes?

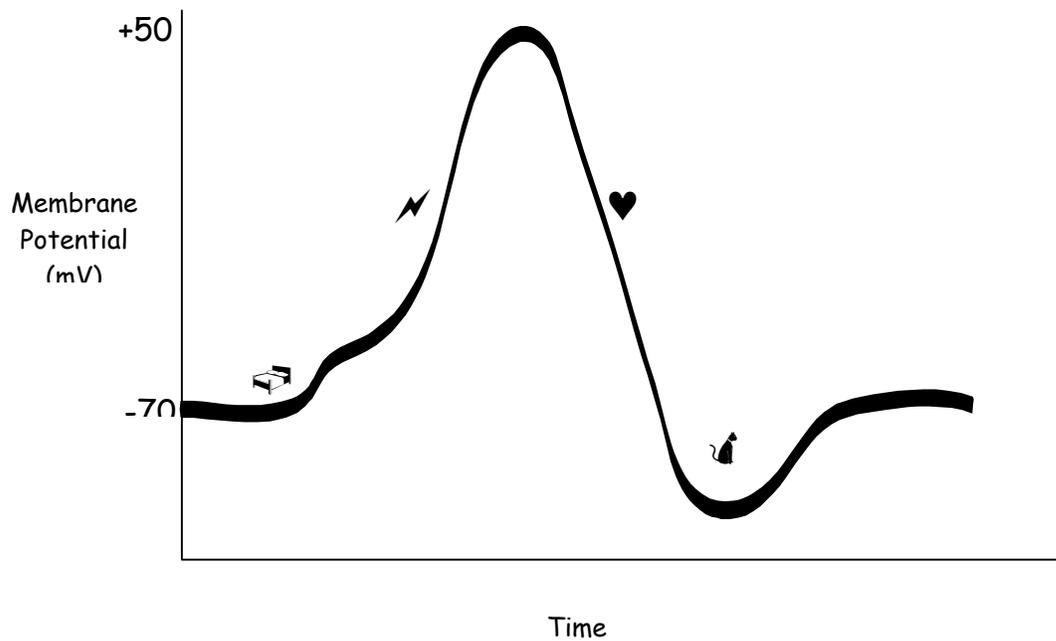
ii) Given that these cells exist in the same animal, name one component in the pathway that could be mutated to give each of the following results (consider each situation independently). Describe how the mutant component differs from the wild-type component, and whether it is a loss-of-function or gain-of-function mutation.

- You never see differentiation of R7 cells.

- You see uncontrolled cell proliferation.

Question 7

The following is a plot of an action potential measured at a single spot along an axon. Four points are highlighted along the curve, 📡, ⚡, ♥, 🐱.



a) On the table below, identify which ion (Na^+ , K^+ , Ca^{++} , Cl^-) is undergoing the greatest net flow across the membrane at the points indicated and state the direction that the ion is moving (into the cell or out of the cell).

Point	Ion	Direction (in/out)
⚡		
♥		

b) The membrane potential is -70 mV at points 📡 and 🐱, on the plot above. Which of the voltage-gated ion channels is closed at point 📡, but open at point 🐱?

c) What dictates the closing of the voltage-gated channel that is open at point ⚡?

d) There are at least three states in which the voltage-dependent Na^+ channel exists.

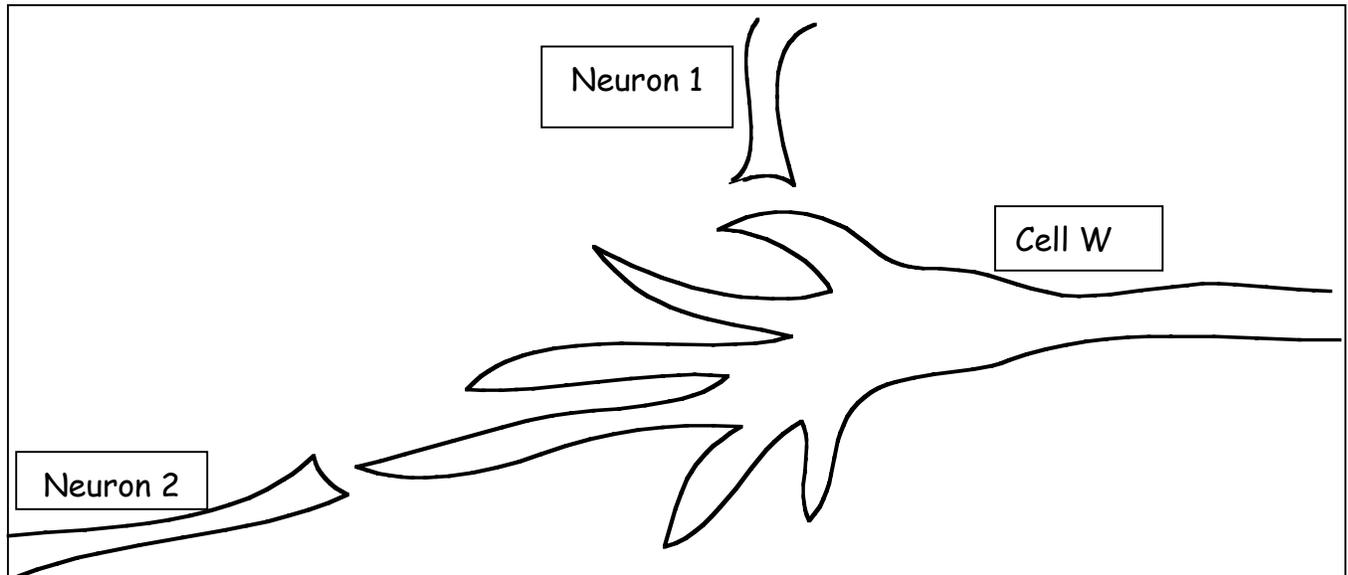
At ♥ on the above plot, the majority of voltage-dependent Na^+ channels would be in which state? Circle the best answer.

Open

Closed

Inactivated

Two different pre-synaptic neurons, neuron 1 and neuron 2, synapse onto cell W as shown below. When neuron 1 is stimulated, the membrane of cell W is locally depolarized. When neuron 2 is stimulated, the membrane of cell W is locally depolarized to exactly the same extent as seen with neuron 1.



e) Circle the one correct statement below.

- ❖ If stimulated equally, neuron 1 is more likely to result in an action potential in cell W than neuron 2.
- ❖ If stimulated equally, neuron 2 is more likely to result in an action potential in cell W than neuron 1.
- ❖ If stimulated equally, neuron 1 and neuron 2 are equally as likely to result in an action potential in cell W.

f) If you were exposed to a toxin that irreversibly blocked voltage-gated Ca^{++} channels, indicate whether the following statements would be TRUE or FALSE.

T	F	Secretory vesicles filled with neurotransmitters would stay in the nerve.
T	F	Your muscles would end up in a rigid contraction.
T	F	Secretory vesicles filled with neurotransmitters would fuse with the plasma membrane.

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 that 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 *EcoRI* 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) 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.

b) 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.

c) 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



Strain B

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



Strain B

Part II

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

Cell Line	Mutation
WT	none (wild type DNA)
1	a deletion at the same region on both copies of chromosome 4
2	a point mutation in a gene on only one copy of chromosome 7

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

Cell Line	Cancer Gene (oncogene or tumor suppressor gene)
WT	none
1	TSG
2	ONCOGENE

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) In this model of axon guidance it is important that Netrin is...

a (short/long) range **attractant** and a (short/long) range **repellant**

b)

Figure removed due to copyright reasons. Please see Figure 4 in Kidd, Thomas, Kimberly S. Bland, and Corey S. Goodman. "Slit Is the Midline Repellent for the Robo Receptor in *Drosophila*." *Cell* 96 (March 19, 1999): 785–794. Copyright 1999 by Cell Press.

Wild type neuron Slit knockout neuron Robo knockout neuron

i) Explain the mechanism that would give mutant "Slit knockout" neurons this pattern?

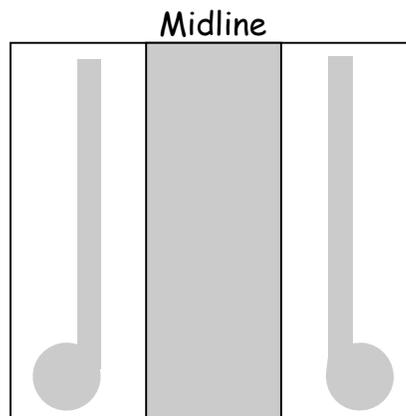
Without Slit, neurons are still attracted by Netrin and would not leave the midline area.

ii) Why is the "Robo knockout" pattern similar to the mutant Slit knockout neurons?

Not having a ligand (Slit) or a Receptor (Robo) will block the cell signaling pathway controlling movement so they will give a similar pattern.

c) In fruit flies there is another intracellular protein, called Commissureless (Comm), that must be expressed for these neurons to cross the midline. The figure below shows different time points from early (A), to later (B), to even later (C). In figure B, only neuron 1 is expressing the *comm* gene, transiently.

What would an animal with a deletion of both *comm* genes look like? Draw your answer in the box below elongating the axons of the neurons on either side of the midline. (Assume Netrin, DCC, Robo and Slit still expressed normally.) ***Axon growth cones never enter midline.***



d) Comm is a (**Negative/Positive**) regulator of the Robo receptor.

e) Give one example of how intracellular Comm may act on the receptor Robo.

Comm might bind to the intracellular domain of Robo interfering with signaling, or Comm could interfere with something else downstream in the pathway. Could destroy the Robo receptor by marking it for degradation.

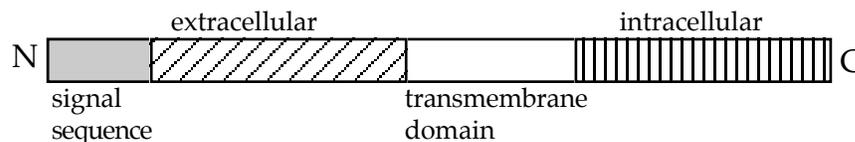
Question 5

- a) To become nerve cells
- b) To become either nerve cells or hypodermal cells.
- c) 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.
- d) 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.
- e) X cells require the signal for induction. In these X cells the G protein signal transduction pathway, once activated, remains active and induction occurs.

Question 6

- a) i) *This would eliminate the disulfide bridge tethering the ligand-binding domain to the rest of the protein. The receptor would be inactive.*
- ii) *This would prevent the differentiation of the R7 cell type.*

b) *Leucine, alanine, isoleucine, valine, phenylalanine, glycine, tryptophan are all hydrophobic amino acids. The hydrophobic effect causes these amino acids to cluster away from water and stay in the interior of the plasma membrane.*



- d)
- e) i) *Ras can activate several different proteins, each of which leads to a different signal transduction cascade. Different cells express different genes, and the specific protein that a given cell expresses will determine the outcome of Ras activation.*
- ii) • You never see differentiation of R7 cells.
A loss-of function mutation in the boss/sev receptor would prevent signaling through Ras and activation of the differentiation pathway.
- You see uncontrolled cell proliferation.
A gain-of function mutation in the EGF receptor such that it signals to Ras in the absence of growth factor would allow uncontrolled cell division.

Question 7

a) On the table below, identify which ion (Na^+ , K^+ , Ca^{++} , Cl^-) is undergoing the greatest net flow across the membrane at the points indicated and state the direction that the ion is moving (into the cell or out of the cell).

Point	Ion	Direction (in/out)
↗	Na^+	in
♥	K^+	out

b) The membrane potential is -70 mV at points ↗ and ♥, on the plot above. Which of the voltage-gated ion channels is closed at point ↗, but open at point ♥?

voltage-gated K^+ channel

c) What dictates the closing of the voltage-gated channel that is open at point ↗?

TIME

d) There are at least three states in which the voltage-dependent Na^+ channel exists.

At ♥ on the above plot, the majority of voltage-dependent Na^+ channels would be in which state? Circle the best answer.

Open

Closed

Inactivated

Two different pre-synaptic neurons, neuron 1 and neuron 2, synapse onto cell W as shown below. When neuron 1 is stimulated, the membrane of cell W is locally depolarized. When neuron 2 is stimulated, the membrane of cell W is locally depolarized to exactly the same extent as seen with neuron 1.

e) Circle the one correct statement below.

- If stimulated equally, neuron 1 is more likely to result in an action potential in cell W than neuron 2.
- If stimulated equally, neuron 2 is more likely to result in an action potential in cell W than neuron 1.
- If stimulated equally, neuron 1 and neuron 2 are equally as likely to result in an action potential in cell W.

f) If you were exposed to a toxin that irreversibly blocked voltage-gated Ca^{++} channels, indicate whether the following statements would be TRUE or FALSE.

<input checked="" type="radio"/> T	<input type="radio"/> F	Secretory vesicles filled with neurotransmitters would stay in the nerve.
<input type="radio"/> T	<input checked="" type="radio"/> F	Your muscles would end up in a rigid contraction.
<input type="radio"/> T	<input checked="" type="radio"/> F	Secretory vesicles filled with neurotransmitters would fuse with the plasma membrane.