

## 2006 7.012 Problem Set 5 KEY

\*\* Due before 5 PM on **THURSDAY**, November 9, 2006. \*\*

Turn answers in to the box outside of 68-120.

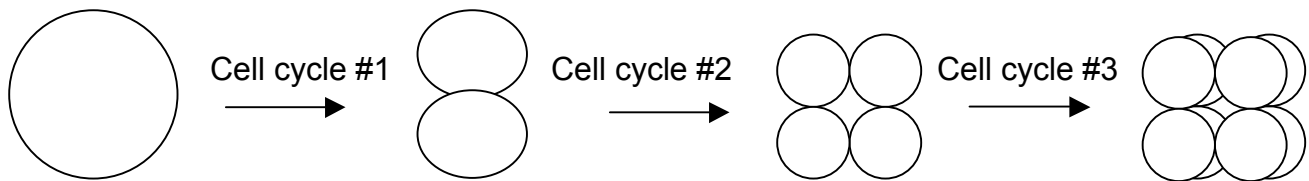
PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

**1.** You are studying the development of a new species of frog. A fertilized frog egg of this species (which is diploid) is produced when two haploid gametes (i.e. an egg from the mother frog and a sperm from the father frog) fuse.

**(a)** When these two gametes fuse, which gamete do you think contributes the vast majority of the cytoplasm and organelles?

**The egg contributes the vast majority of the cytoplasm and organelles. The egg is essentially a haploid animal cell containing cytoplasm and all the necessary organelles. The sperm however, carries virtually nothing but DNA.**

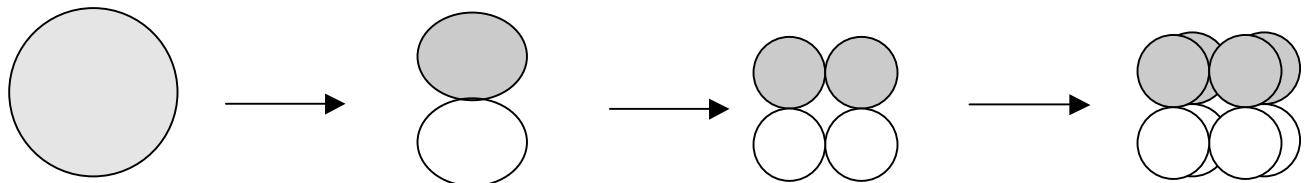
When a fertilized egg begins development, the outcome of the first three cell cycles look like this:



**(b)** Cell growth normally occurs in G1 stage and/or G2 stage of the cell cycle. Based on the above picture, do you think that the cell cycles occurring during this early phase of development involve a large amount of cell growth?

**No, this early phase of development doesn't involve a lot of cell growth. Although the cells are dividing, their total volume is not increasing, as shown in the picture. This lack of growth is standard for the first divisions that occur in early embryo development.**

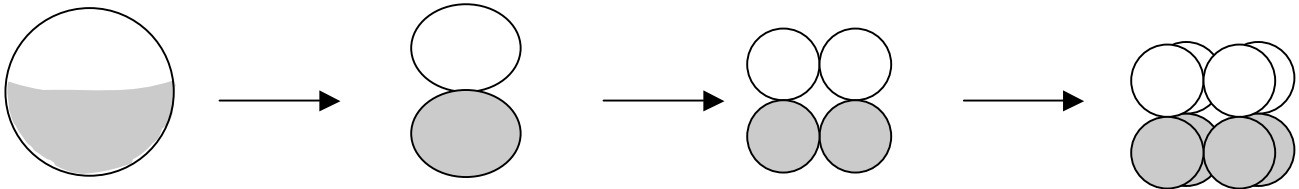
You examine the localization of a specific protein during each of these four early developmental stages. This protein is required for the formation of ectoderm. The location of the protein is indicated by gray shading.



**(c)** At the four cell stage, do you think that the four cells are equivalent in terms of the different fates their daughter cells can take on?

**No, all four of these cells are not equivalent. The protein required for ectoderm formation is only found in the upper two cells, therefore only these two cells can become ectoderm. The lower two cells can go on to become mesoderm or endoderm at this point, but cannot become ectoderm.**

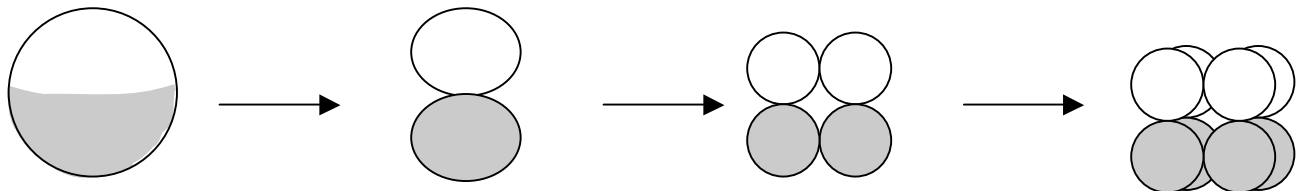
You examine the localization of a specific mRNA during each of these four early developmental stages. The location of the mRNA is indicated by gray shading.



**(d)** Given that these frog embryos do not begin their own transcription until part of the way through the blastula stage, when do you think this mRNA was produced and by whom?

**This mRNA was produced by the mother during the formation of her eggs from her germline cells. Since the egg provides all components of the cell (except the haploid complement of chromosomes that comes from the sperm), any mRNA present before the embryo can carry out transcription must come from the mother's egg's cytoplasm.**

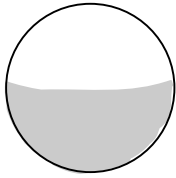
You examine the localization of a different specific protein during each of these four early developmental stages. The location of the protein is indicated by gray shading.



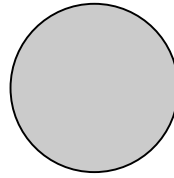
**(e)** Draw two different patterns for what you might see if you examined the sub-cellular localization pattern of the mRNA that encodes this protein in the single-celled fertilized egg. One of the two possibilities should imply that this gene is regulated at the level of translation. Draw the two potential patterns of mRNA localization as shading in the two eggs provided for you below.

**The pattern on the left represents post-transcriptional regulation. We only see the mRNA present where the protein is present, so after transcription the mRNA is localized to the bottom half of the cell. As the cell divides, only the daughter cell resulting from the bottom half of the single cell will contain this mRNA.**

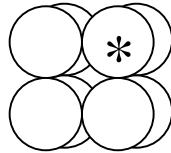
**The pattern on the right represents translational regulation. The mRNA is distributed equally throughout the cell, but it is only translated in the bottom half of the cell. As the cell divides, only the daughter cell resulting from the bottom half of the cell will have the protein that is produced when this mRNA is translated.**



OR



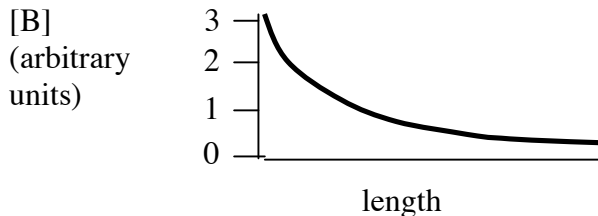
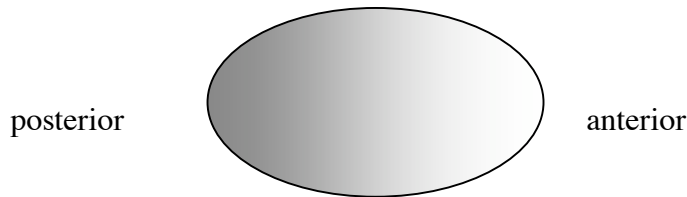
In this picture at the 8-cell stage, the cell marked with a star gives rise to only ectoderm.



(f) In one or two sentences, describe what experiment you would do to arrive at this conclusion, and what result you would get from this experiment that would allow you to make this conclusion.

**You would do fate mapping, and inject a dye into that one cell only, and then let the 8-celled embryo grow up into an embryo with different tissue types. The result you should get is that only ectodermal cells contain the dye.**

**2.** You are a developmental biologist studying pattern formation in fruit flies. In your efforts to understand how body patterns of gene expression in flies are regulated, you have recently identified a DNA-binding protein, B, which is present in a gradient in the embryo, as drawn below.



B binds to the promoter of the X gene and helps RNA polymerase bind there. X protein, when produced, binds to the promoter of Z and impedes the binding of RNA polymerase there.

(a) Into which specific category of proteins does protein B fall, given its function?

**Protein B is a transcriptional activator.** Transcriptional activators are DNA-binding proteins that bind to promoter sequences and recruit RNA polymerase there. This greatly increases transcription of the gene to which protein B is bound (which is gene X here).

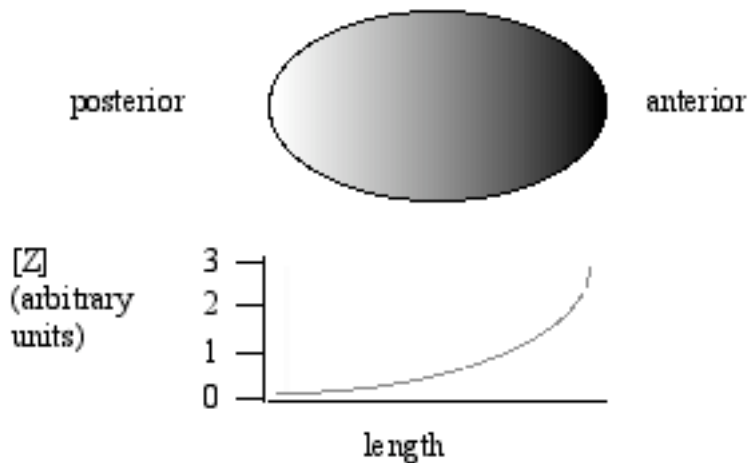
(b) Into which specific category of proteins does protein X fall, given its function?

**Protein X is a transcriptional repressor.** Transcriptional repressors are DNA-binding proteins that bind to promoter sequences and inhibit RNA polymerase from binding there. If RNA polymerase cannot bind to the promoter of a gene, that gene cannot be transcribed (in this example, protein X affects RNA polymerase binding to gene Z).

(c) At what level of gene regulation is the X gene regulated? Your choices are: transcriptional, post-transcriptional, translational, or post-translational.

**Gene X is regulated at the transcriptional level.** The presence of protein B causes gene X to be transcribed. If protein B is absent, gene X is not transcribed.

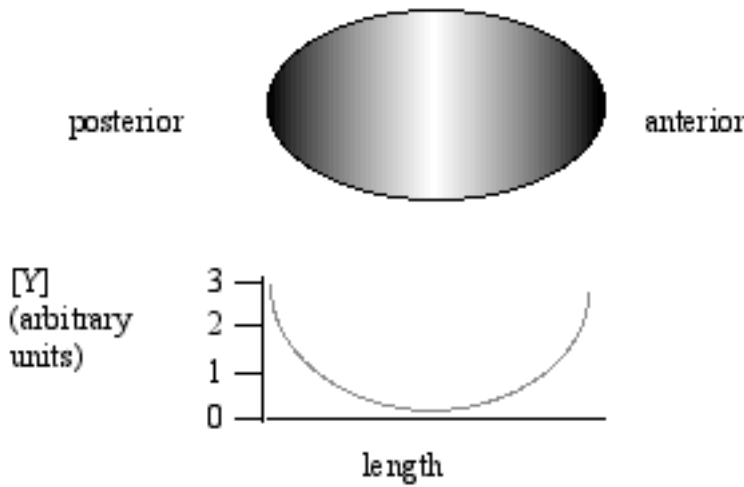
(d) Using the format used above, draw a line in the graph to indicate what you think the levels of Z look like across the embryo:



**Protein B activates the transcription and translation of protein X. Thus the distribution of protein X in the embryo looks like the distribution of protein B. Protein X represses the transcription of protein Z. Therefore, the distribution and concentration of protein Z in the embryo will be the opposite of Protein X. When [X] is high, [Z] is low, and when [X] is low, [Z] is high.**

Proteins X and Z both bind to the promoter region of gene Y, and both proteins impede RNA polymerase from binding there. Both X and Z must be bound to the Y promoter to impede RNA polymerase from binding.

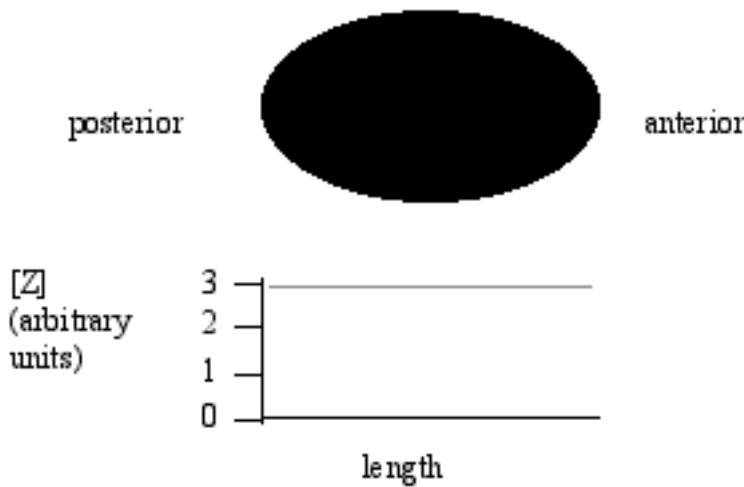
(e) Using the format used above, draw a line in the graph to indicate what you think the levels of Y look like across the embryo:



We would see high levels of protein Y on each end of the embryo and low levels of protein Y in the middle of the embryo. This is because both X and Z are needed to repress the transcription of Y. At the posterior end of the embryo we have high [X] but low [Z]. At the anterior of the embryo we have high [Z] but low [X]. The only place where the both X and Z are present together is in the middle of the embryo. This is where we will see low levels of Y because this is the only part of the embryo where there is enough of both X and Z proteins to repress the transcription of Y.

You make a strain of fruit fly by genetic engineering that lacks the promoter region in front of the X gene on both homologs. Draw the patterns of Z and Y expression you would expect in this mutant embryo in the graphs below.

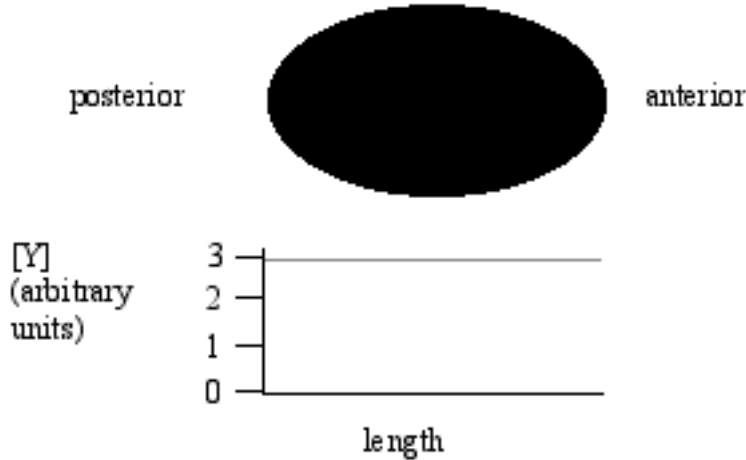
(f) Using the format used above, draw a line in the graph to indicate what you think the levels of Z look like across this mutant embryo:



Protein Z would be found at high levels throughout the embryo. The function of X is to repress the transcription of Z. If gene X does not have a promoter region, RNA polymerase cannot bind and transcribe the gene for X. If there is no protein X, then gene Z

will not be transcriptionally repressed anywhere in the embryo and protein Z can be made throughout.

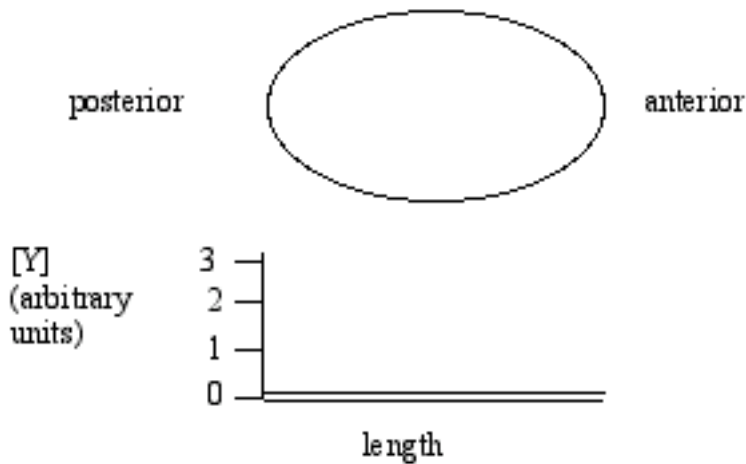
(g) Using the format used above, draw a line in the graph to indicate what you think the levels of Y look like across this mutant embryo:



Protein Y would be found throughout the embryo at high levels. Although Z is present everywhere, X is also necessary in addition to Z to inhibit RNA polymerase from binding to the Y promoter. Since X cannot be made in this embryo, the transcription of Y is not repressed and protein Y is present throughout the cell.

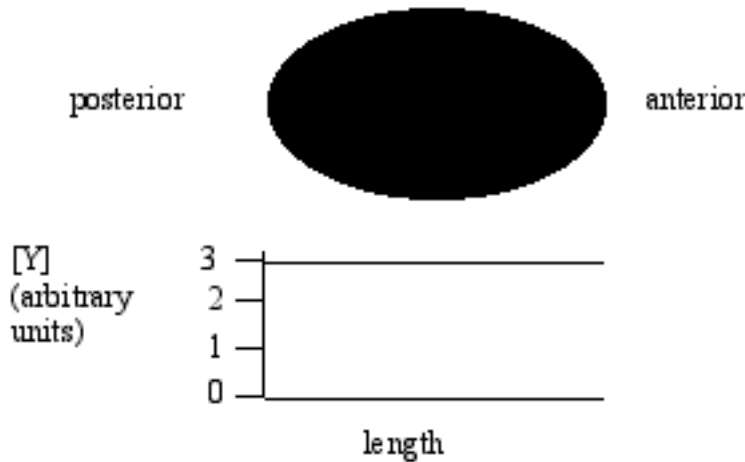
You make a strain of fruit fly by genetic engineering that is expressing B protein at equal levels (at 3 “arbitrary units”) evenly across the entire embryo. Draw the patterns of Z and Y expression you would expect in this mutant embryo in the graphs below.

(h) Using the format used above, draw a line in the graph to indicate what you think the levels of Z look like across this mutant embryo:



Protein Z is present at extremely low levels throughout the embryo. Since B is expressed at high levels evenly throughout the embryo, X is expressed at high levels throughout the embryo. Protein X represses the transcription of Z, so when [X] is high, [Z] is very low.

(i) Using the format used above, draw a line in the graph to indicate what you think the levels of Y look like across this mutant embryo:



We would see high levels of protein Y throughout the embryo. Since Z is absent, one of the necessary repressors of Y expression is missing. Transcription of Y is not inhibited and protein Y will be present through the entire embryo in equal amounts.

**3.** The eye cancer retinoblastoma is usually caused by mutations in the Rb gene. The wild-type function of Rb is to keep proteins that promote the cell cycle inactive unless the cell is supposed to be growing and dividing. Rb does this by binding and inhibiting the transcriptional activator E2F. When free from Rb, E2F turns on transcription of genes that promote passing through the “R point” (restriction point) of the cell cycle, at which point the decision of the cell to go through S, G<sub>2</sub>, and M is irreversible.

(a) Is E2F a tumor suppressor gene or an oncogene?

E2F is an oncogene. Normally E2F promotes the cell to enter the cell cycle. When E2F is expressed at the inappropriate time or in an uncontrolled manner, the cell will progress unchecked through the cell cycle. This unchecked proliferation of cells is cancer.

(b) Would a mutation in the Rb gene that disrupts the physical interaction between the Rb and E2F proteins promote the development of cancer?

Yes, this mutation would promote the development of cancer. Rb physically interacts with E2F to inhibit E2F from turning on genes that allow the cell to enter the cell cycle. If Rb can no longer interact with E2F, E2F is free to always turn on these genes that allow the

cell to continue through the cell cycle. The cell can then grow and divide in an uncontrolled fashion, resulting in cancer.

(c) Which kind of mutation in the Rb gene would promote the development of cancer -- a loss-of-function mutation or a gain-of-function mutation?

**A loss-of-function mutation in the Rb gene would promote the development of cancer. Rb's normal function is to inhibit cells from going through the cell cycle. The loss of Rb's function, to bind to and inhibit E2F, would allow E2F to promote entrance into the cell cycle all the time, resulting in the uncontrolled proliferation of cells.**

(d) People who inherit an Rb<sup>-</sup> allele generally develop retinoblastoma at a much earlier age than people with retinoblastoma who do not inherit any Rb<sup>-</sup> alleles. Why is this?

**They only need to accumulate mutations and lose function in one of their two Rb alleles (the Rb<sup>+</sup> allele) because they already have one nonfunctional allele (the Rb<sup>-</sup> allele). Once they have two nonfunctional alleles, no functional Rb protein is made and E2F cannot be inhibited. People who do not inherit any Rb<sup>-</sup> alleles must lose function in both of their Rb<sup>+</sup> alleles in order to develop retinoblastoma.**

(e) Which one specific mutation must people who inherit an Rb<sup>-</sup> allele undergo before their cells can become cancerous?

**Their second allele, which is Rb<sup>+</sup> at birth, must have a random loss-of-function mutation occur in it. A loss-of-function mutation in the other homologous version of Rb would now lead to an absence of Rb protein, and the development of cancer.**

(f) You are studying a female retinoblastoma patient who has sporadic retinoblastoma (i.e. no one else in her family has ever had retinoblastoma). For each cell described below, state how many total alleles of the Rb gene are in that cell, and how many are wild-type vs mutant:

-- an eye cell from the tumor: **2 alleles, both mutant**

-- a skin cell: **2 alleles, both wild-type**

-- an egg cell: **1 allele, wild-type**

**An eye cell is a somatic cell so it has two alleles for every gene, one on each homologous chromosome. For retinoblastoma to develop, both alleles must be mutant. A skin cell is also a somatic cell so it has 2 alleles. These alleles are both wild-type because the patient has a sporadic form of retinoblastoma. This means that she did not inherit any Rb mutations from her parents; all mutations in Rb occurred within her own eye cells after birth. An egg cell is a gamete, so it only contains 1 allele from one homologous chromosome. This allele is wild-type because the mutations in Rb only occurred in the patient's one eye cell that led to the development of the tumor.**

(g) You are studying a female retinoblastoma patient who has inherited retinoblastoma (i.e. one of her two parents had retinoblastoma). For each cell described below, state how many total alleles of the Rb gene are in that cell, and how many are wild-type vs mutant:

-- an eye cell from the tumor: **2 alleles, both mutant**

-- a skin cell: **2 alleles, one wild-type and one mutant**

-- an egg cell: **1 allele, there is an equal chance that it is mutant or wild-type**

**An eye cell is a somatic cell so it has two alleles for every gene, one on each homologous chromosome. Because the patient has inherited retinoblastoma, she inherited one mutant allele from a parent and then the other allele incurred a loss-of-function mutation only in the eye cell that became a tumor. A skin cell is a somatic cell so it has two alleles for every gene. One allele is mutant, which was inherited from the parent with retinoblastoma. The other allele is wild-type and was inherited from the unaffected parent. A loss-of-function mutation did not occur in this allele in the skin cell. An egg cell is a gamete so it contains one allele from only one homologous chromosome. Because the patient has one wild-type and one mutant allele inherited from each of her parents, the egg cell has an equal chance of containing the wild-type allele OR the mutant Rb- allele.**

(h) The female patient who has inherited retinoblastoma gets two retinal tumors removed before the age of 10, and then never develops another eye tumor again. She is now 35 and wants to have a child. How has the woman's surgery that treated her cancer affected her child's chance of developing retinoblastoma? Your choices are that her child's chances are now: higher, the same, lower, zero.

**It is the same. The surgery has not affected the chance of her child developing retinoblastoma. The surgery was performed on the somatic cells of the retina. 50% of her gametes still contain a wild-type allele of Rb and 50% of her gametes contain the mutant Rb allele. The surgery did not affect her gametes so the chances did not change.**

**4.** There is an extracellular protein signal called TGF-beta that gets sent by certain cells in the body to other cells. The cells that receive this signal express TGF-beta receptors, which localize to the plasma membrane. When TGF-beta binds to its transmembrane receptor, that receptor is activated. The active receptor is a serine/threonine kinase, and it phosphorylates amino acids in a transcriptional activator protein called Smad. When Smad is phosphorylated, it undergoes a conformational change that allows it to form a dimer and move into the nucleus. There, the Smad dimer binds to the promoter of a gene called p16 and recruits RNA polymerase to the gene's promoter. p16 is a protein that, when produced, binds to and inhibits cyclin/CDK complexes.

(a) Is the gene encoding the TGF-beta receptor a tumor suppressor gene or an oncogene?

The TGF-beta receptor gene is a **tumor suppressor gene**. This is true because, when TGF-beta interacts with its receptor, it activates a signaling pathway whose goal is to inhibit the cell cycle. When the TGF-beta receptor cannot transduce its signal to the inside of the cell (when it loses its function) the cell cycle is not inhibited and uncontrolled cell proliferation occurs. A tumor suppressor gene is a gene whose normal function is to inhibit the cell cycle.

(b) Would a mutation that makes Smad think that it is phosphorylated all of the time promote the development of cancer?

**No, this mutation would not promote the development of cancer. This is true because, if Smad were phosphorylated all the time, p16 would be expressed all the time, and p16 inhibits the cell cycle. Uncontrolled cell proliferation would not occur. Rather, the cell cycle would always be inhibited.**

(c) You make a mutant version of Smad in which the amino acid that is normally phosphorylated (a serine) is changed to an aspartate. This mutant version of Smad acts as if it is always phosphorylated. Why do you think this mutation makes Smad think it is always phosphorylated?

**This mutation makes Smad think it is always phosphorylated because phosphorylation gives the protein a negative charge and aspartate is a negatively charged amino acid. The negative charge is what promotes the conformational change so that the protein exists as a dimer. Either the covalent modification (phosphorylation) or the substitution of serine with a negatively charged amino acid (Asp) will cause this conformational change.**

(d) Which type of mutation in p16 would promote the development of cancer, a loss-of-function mutation or a gain-of-function mutation?

**A loss-of-function mutation in p16 would promote the development of cancer. The normal job of p16 is to inhibit the cell cycle, so if p16 can no longer carry out this function, the cell can proliferate in an uncontrolled fashion, resulting in cancer.**

(e) You properly generate a hybrid gene that produces a fusion of Smad to GFP when expressed. Would you expect to see green fluorescence in the cytoplasm or in the nucleus if the cells containing the Smad-GFP fusion were grown under the following conditions, in cells with the following mutant properties? Fill in each block of this table with the words "cytoplasm" or "nucleus."

GROWTH CONDITIONS

<i>Mutant property of cell</i>	<i>No TGF-beta present in the environment</i>	<i>TGF-beta is present in the environment</i>
None (cell is wild-type)	<b>Cytoplasm</b>	<b>Nucleus</b>
The cell lacks TGF-beta receptors	<b>Cytoplasm</b>	<b>Cytoplasm</b>

The TGF-beta receptor always thinks it is bound to TGF-beta	<b>Nucleus</b>	<b>Nucleus</b>
Smad cannot be phosphorylated	<b>Cytoplasm</b>	<b>Cytoplasm</b>
The promoter of both copies of p16 is deleted	<b>Cytoplasm</b>	<b>Nucleus</b>

**If the cell is wild-type, without TGF-beta the receptor is not activated and Smad is not phosphorylated and it can't move into the nucleus. It will stay in the cytoplasm. When TGF-beta is present, the receptor is activated and Smad is phosphorylated, dimerizes and moves into the nucleus.**

**If the cells lack TGF-beta receptors, in both cases there are no receptors to activate. Smad can never be phosphorylated so it can never dimerize and it will never move into the nucleus. It will stay in the cytoplasm in both cases.**

**If the cell's TGF-beta receptors always think they are bound to TGF-beta, then in both cases the receptors will be activated. Smad will always be phosphorylated so it will always dimerize and move into the nucleus.**

**If Smad cannot be phosphorylated, then in both cases it can never dimerize and can never move into the nucleus. It will always be found in the cytoplasm.**

**If the promoter of p16 is deleted, the localization of Smad will not be altered. If TGF-beta is not present, then the receptor will not be activated. Smad will not be modified so it will not dimerize and cannot move into the nucleus. It will stay in the cytoplasm. If TGF-beta is present, the receptor can be activated and Smad will be phosphorylated and it will dimerize and move into the nucleus. The only problem is that now, the dimerized and phosphorylated Smad has no target gene to bind to, because the p16 promoter is deleted. Thus Smad will be in the nucleus, but it will be unable to carry out the function it is normally supposed to do there.**

**5.** There are two general categories of viruses that can cause cancer, slow-acting viruses and fast-acting viruses. Slow-acting viruses cause cancer over a longer time frame than do fast-acting viruses.

**(a)** Slow-acting viruses cause cancer due to an event in which they randomly insert their genome into a host gene. How do you think that the insertion of a viral gene into the inside of a host gene generally affects the activity of the product of that host gene?

The insertion of a viral gene into the inside of a host gene generally destroys the gene, causing a loss of function mutation. If a large stretch of DNA is inserted in the middle of a gene, its DNA sequence is changed, which will alter the mRNA sequence and the protein sequence made from that gene. Any protein product made from the gene will be grossly changed.

(b) Do you think that the host genes in cancerous cells that were affected by the insertion of the genomes of slow-acting viruses are generally oncogenes or tumor suppressor genes?

They are generally tumor suppressor genes. This is true because, when the viral gene is inserted, it is abolishing the function of the gene into which it has been inserted. Tumor suppressor genes normally act to inhibit the cell cycle, so when their function is lost, the cell cycle is no longer inhibited. The normal function of an oncogene is to encourage the progression through the cell cycle, so if that function is lost the cell cannot proceed through the cell cycle. This would not result in uncontrolled cell proliferation.

(c) Which of the following viral genes could be mutated such that a retrovirus that could potentially be a slow-acting virus now cannot cause cancer? Your choices are: viral reverse transcriptase, viral integrase, both, or neither.

**Both** of these viral genes could be mutated so that the slow-acting virus could not cause cancer. If reverse transcriptase is mutated so that it can no longer function, the retrovirus cannot make DNA from its RNA genome. If it cannot convert its genome into dsDNA, it cannot be integrated into the host's dsDNA genome. Therefore it cannot insert into and thereby disrupt tumor suppressor genes anymore. If integrase is mutated so that it can no longer function, then there is no way for the virus to integrate its genome into the host's genome. In either case, if no DNA is inserted into the genome, no host gene functions can be interrupted, and no cancer can result.

Fast-acting viruses that cause cancer do so by actively producing mRNAs and proteins from their own genomes that affect the activity of cellular proteins. The fast-acting cancer-causing virus HPV makes three proteins that influence host protein activity. The viral protein E5 physically interacts with the host PDGF receptor protein. (PDGF is one example of a typical growth factor protein.) The viral protein E6 physically interacts with the host p53 protein. The viral protein E7 physically interacts with the host Rb protein.

(d) Do you think the viral E5 protein inhibits or activates the host PDGF receptor protein?

**E5 activates** the host PDGF receptor protein. This growth factor protein can then signal the cell that it is time to grow and divide so that the cell will continue unchecked through the cell cycle.

(e) Do you think the viral E6 protein inhibits or activates the host p53 protein?

Name: \_\_\_\_\_KEY\_\_\_\_\_

**E6 inhibits the host p53 protein. p53 is a tumor suppressor gene whose normal function is to inhibit the cell cycle. If its action is inhibited then the cell can continue unchecked through the cell cycle leading to uncontrolled proliferation.**

**(f) Do you think the viral E7 protein inhibits or activates the host Rb protein?**

**E7 inhibits the host Rb protein. Rb is also a tumor suppressor whose normal function is to inhibit proteins that allow cells to proceed through the cell cycle. If Rb is inhibited, then it cannot inhibit the proteins that promote entrance into the cell cycle. This will result in uncontrolled cell proliferation.**