Name: _____

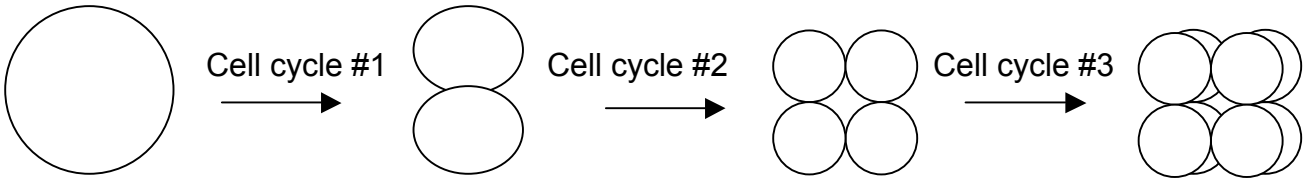
2006 7.012 Problem Set 5

** 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.

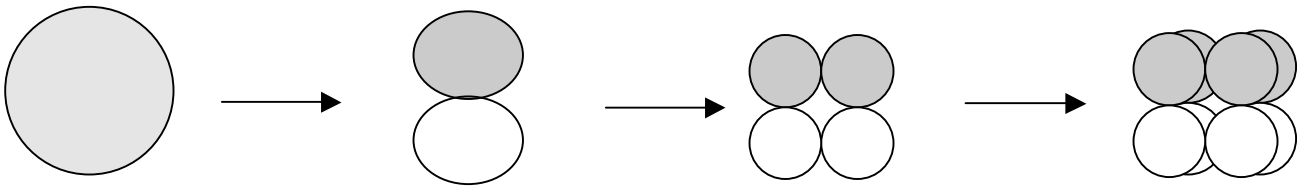
(a) When these two gametes fuse, which gamete do you think contributes the vast majority of the cytoplasm and organelles?

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?

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?

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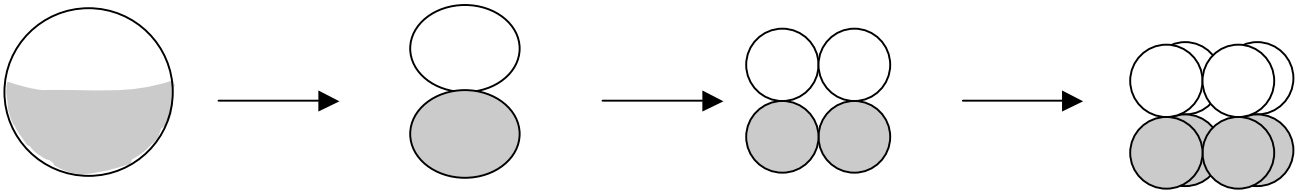
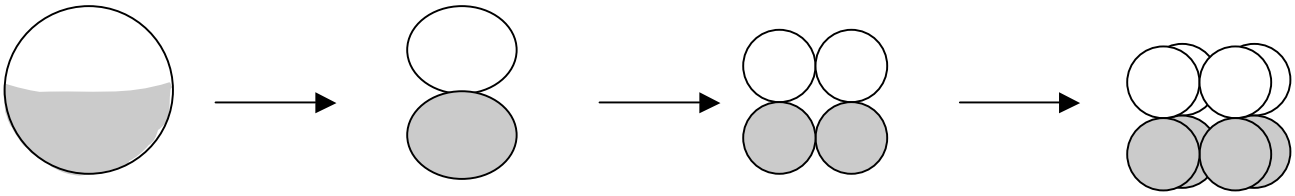
You examine the localization of a specific mRNA during each of these four early developmental stages. The location of the mRNA is indicated by gray shading.



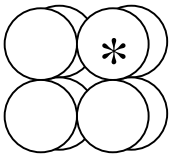
(d) Given that these frog embryos do not begin their own transcription until part of the way through the blastula stage, when do you think this mRNA was produced and by whom?

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.

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



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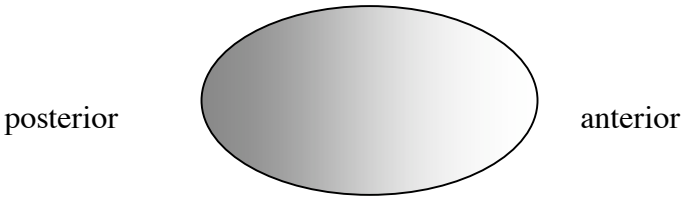
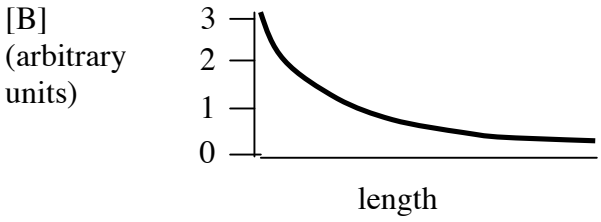
(f) In one or two sentences, describe what experiment you would do to arrive at this conclusion, and what result you would get from this experiment that would allow you to make this conclusion.

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?

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

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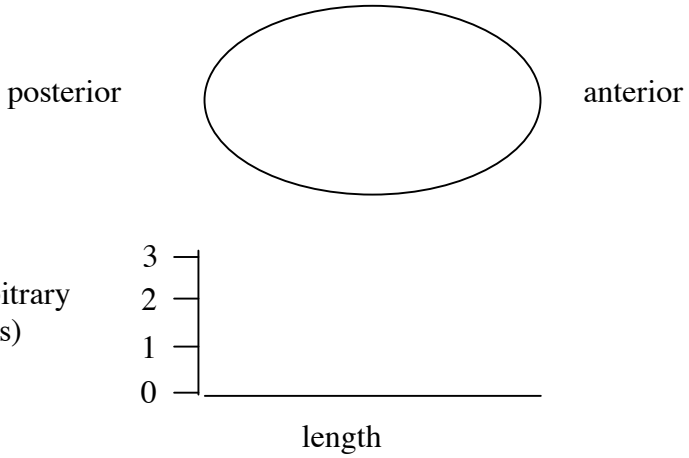
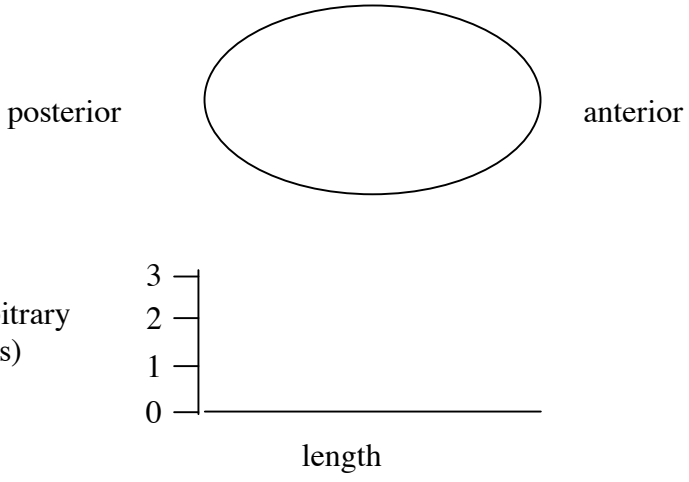
(c) At what level of gene regulation is the X gene regulated? Your choices are: transcriptional, post-transcriptional, translational, or post-translational.

(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:



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:



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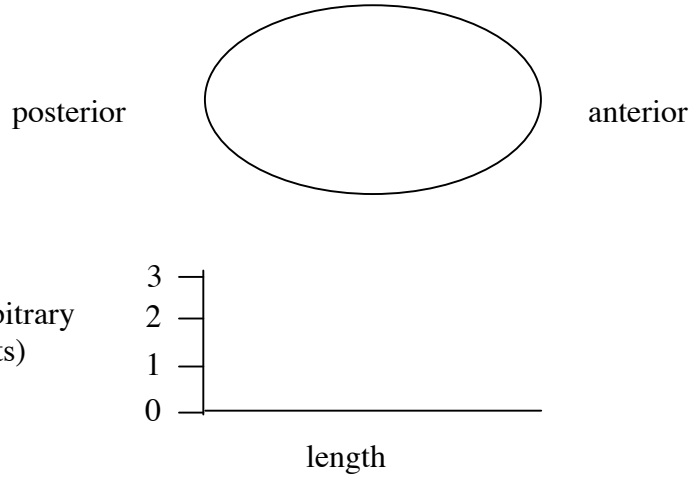
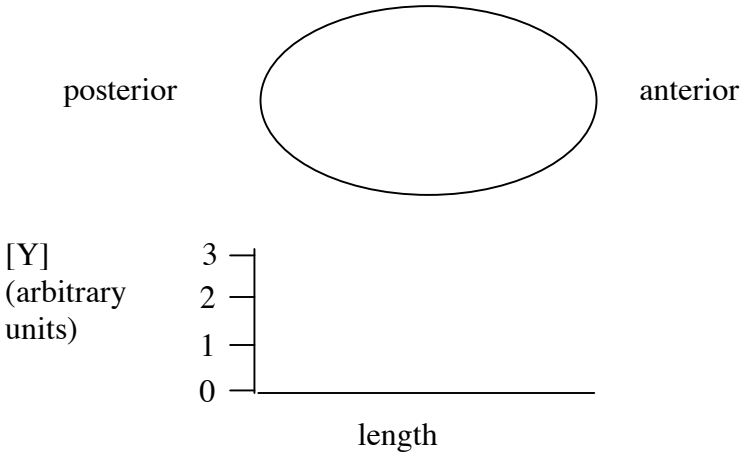
You make a strain of fruit fly by genetic engineering that lacks the promoter region in front of the X gene on both homologs. Draw the patterns of Z and Y expression you would expect in this mutant embryo in the graphs below.

(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:



(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:



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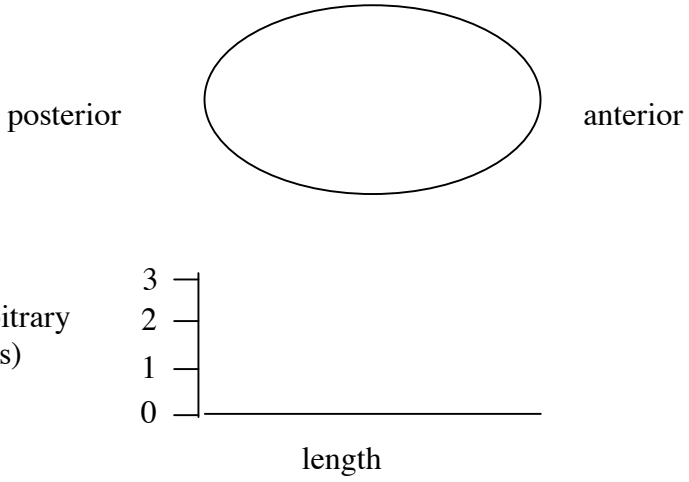
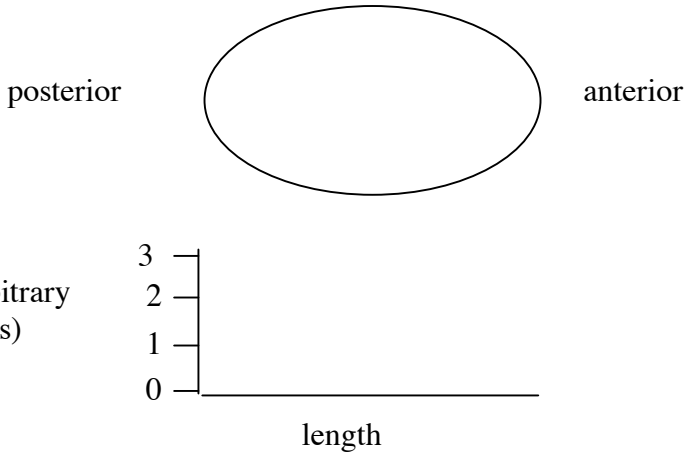
You make a strain of fruit fly by genetic engineering that is expressing B protein at equal levels (at 3 “arbitrary units”) evenly across the entire embryo. Draw the patterns of Z and Y expression you would expect in this mutant embryo in the graphs below.

(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:



(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:



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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, G2, and M is irreversible.

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

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

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

(e) Which one specific mutation must people who inherit an Rb– allele undergo before their cells can become cancerous?

(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:

-- a skin cell:

-- an egg cell:

(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:

-- a skin cell:

-- an egg cell:

(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.

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?

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

(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?

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

(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	
Mutant property of cell	*No TGF-beta present in the environment*	*TGF-beta is present in the environment*
None (cell is wild-type)		
The cell lacks TGF-beta receptors		
The TGF-beta receptor always thinks it is bound to TGF-beta		
Smad cannot be phosphorylated		
The promoter of both copies of p16 is deleted		

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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?

(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?

(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.

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?

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

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