Bio 213 GENETICS (Fall 2000)
Problem Set 9: Transcription and Regulation (Eukaryotic)

9.1. *lacZYA* is placed downstream from a eukaryotic promoter and sent into yeast. ß-galactosidase is well expressed but lactose permease is not. Why not?

9.2. The initial transcript from a human globin gene is injected into *E. coli* (this is technically impossible, but never mind that). The injected transcript produces no globin. Give two reasons for the failure of the transcript to work in *E. coli*.

9.3. Which statements below can conceivably explain the fact that human red blood cells have no nucleus but, of course, are full of hemoglobin?

A. The hemoglobin gene promoter is very strong
B. Hemoglobin is degraded very slowly
C. Hemoglobin mRNA is degraded very slowly
D. Hemoglobin transcripts are rapidly spliced
E. Red blood cells have a sigma factor that recognize the hemoglobin gene

9.4. Put the following eukaryotic genetic elements in the spatial order in which they appear in DNA:

TATA. TATA box
TAF. TAF binding site
TSS. Transcriptional start site
ATG. start codon
E. Exon
A. poly-A site
I. intron

9.5. Put the letters representing the following eukaryotic genetic elements in the temporal order in which they would be used:

m7G. m^7-G cap
TAF. TAF binding site
TGA. Stop codon
TATA. TATA box
ATG. start codon
polyA. poly-A tail
TSS. Transcriptional start site
ISS. intron splice site

9.6. A popular method of cloning eukaryotic genes begins with the following steps:

Step 1: Total RNA is hybridized to a synthetic DNA polymer consisting solely of of thymine nucleotides.

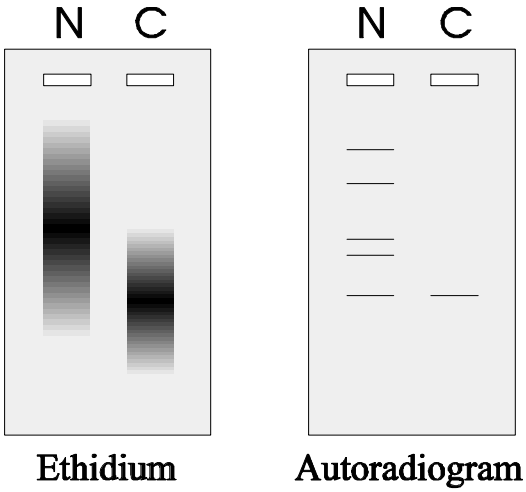
Step 2: the enzyme reverse transcriptase is added, along with dATP, dTTP, dCTP, and dGTP (reverse transcriptase, used by many RNA viruses, catalyzes the synthesis of DNA from a primer and an RNA template).

a. Why is poly dT used in step (1)?

b. What is the end product of step (2)? Draw a picture.

c. Suppose you wanted to clone a eukaryotic gene in *E. coli*, in order to study the function of the protein it encodes. Why might the method described above be preferred to simply cloning the gene from chromosomal DNA? (What would be the result of cloning a gene from chromosomal DNA?)

***9.7.** RNA is isolated from the nucleus and from the cytoplasm of a cell. The RNA is subjected to electrophoresis and then blotted. The filter (the blot) is probed with a radioactively labeled gene encoding hemoglobin (i.e. double stranded DNA cloned directly from the chromosome). The results are shown to the right.



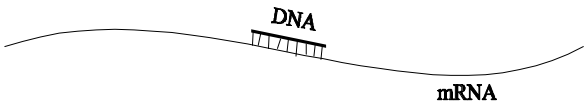
a. What is the significance of the multiple hybridizing bands in the lane carrying nuclear RNA?

b. Why is there only one band that hybridizes to the probe in the lane carrying cytoplasmic RNA?

c. Supposed instead you had probed with poly-dT. Draw a picture of the result would you then expect.

d. Whoops! Did I reverse the labels on the ethidium-stained panel? Why do/don't you think so?

***9.8.** In order to clone the gene for awful taste, you isolate mRNA from chicken liver. You hybridize to the mRNA a small piece of DNA that matches part of the middle of the gene (see picture at right).



Now you add reverse transcriptase (RT) plus nucleotides to synthesize a complementary strand. (RT synthesizes DNA from an RNA template). List those regions below that the newly synthesized piece of DNA must cover or overlap and those that the DNA cannot cover or overlap.

A. Start codon
B. TATA box
C. Poly-A tail
D. Any nontranslatable region
E. First exon
F. First intron
G. Upstream enhancer

9.9. Fill in the chart below, relating what would be the effect on *lacZ* transcription in the presenece of a small amount of lactose if there were a point mutation at one of the bases shown below the X-axis. The sequence is that of the *lac* operon from 66 bases prior to the start of *lacZ* to 9 bases within *lacZ* (the same sequence is given below the chart in a larger font for those without microscopic vision). The level of transcription should be expressed relative to the wild-type *lac* operon under the same conditions, where 1.0 indicates that the level of transcription was unchanged by the mutation.

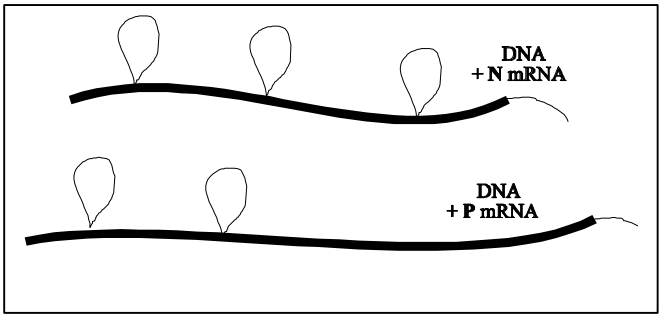
GCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTA
TGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATG

9.10. People with the recessive disease, Purple Tongue Syndrome, are unable to make the enzyme Blue Pigment Decolorase (BPD). You isolate mRNA from a person (P) afflicted with the disease and from a normal person (N) and do the following:

i. Mix excess **P** mRNA with cloned DNA carrying the BPD gene and, separately, mix mRNA with the same DNA.

ii. Raise the temperature of the mixtures, and then cool to allow RNA-DNA hybridization to occur.

iii. For each mixture, locate by electron microscopy heteroduplex molecules. "Heteroduplex" here refers to hybrid molecules in which one strand is mRNA and the other BPD DNA.



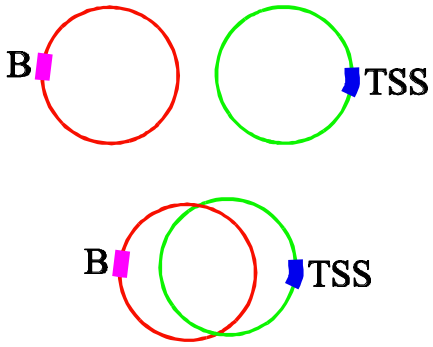
The cartoon at the right is supposed to be what you see by electron microscopy. Double-stranded regions are represented by thick lines, and single-stranded regions are represented by thin lines.

a. The single-stranded loops are composed of which: RNA or DNA? Why?

b. What is the single-stranded region at one end of the heteroduplex?

c. What is the molecular cause of Purple Tongue Syndrome?

9.11. Wedel et al [Science (1990) 248:486-489] studied how postive acting transcription factors work by analyzing the requirements for binding in vitro (in a test tube) of RNA polymerase to the position near the start of transcription. Two situations were examined (shown at right). In the first, a plasmid carrying the binding site (**B**) for a positive acting transcriptional regulator was mixed with a separate plasmid carrying the transcriptional start site (**TSS**) plus 32 bases upstream. In the second, these two plasmids were linked together (like in a chain).



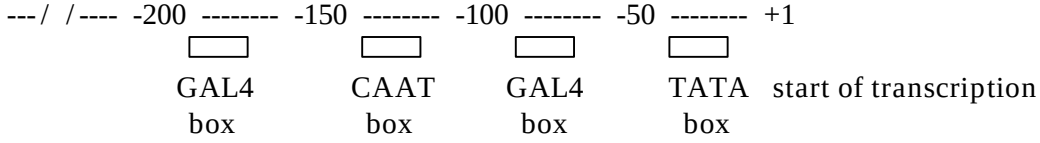
The authors added RNA polymerase, the positive acting transcriptional regulator, plus other necessary components to the two plasmids and measured transcription. They found that transcription was significantly higher when the plasmids were linked than when they were not. What does this result say about what is required for binding sites to enhance transcription?

9.12. The process of splicing involves the piecing together of separated (polypeptide/RNA/DNA) sequences to form the mature (DNA/mRNA/protein). Splicing can be regulated by the differential use of _____.

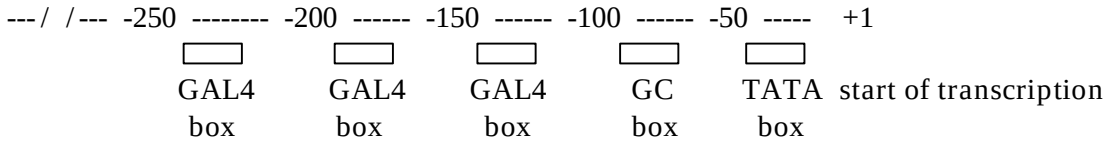
9.13. Prove to someone that a split gene really doesn't break the colinearity between DNA, mRNA, and protein.

***9.14.** As we know, galactose induces several genes in yeast. The induction occurs at the level of transcription and involves the GAL4 transcription factor. When galactose is absent, GAL4 cannot interact with its DNA binding site. When galactose is present, GAL4 binds and activates transcription. The upstream regions of the galA and galB genes are shown below:

galA



galB



For each of the following mutations in a haploid strain of yeast, determine the level of expression for galA and galB. Use +++ to indicate a high inducible expression, ++ to indicate a moderate inducible expression, + to indicate a constant low expression, +/- to indicate a constant very low expression, and - to indicate no expression under any condition.

Mutation	**Expression of *gal*A**	**Expression of *gal*B**
Deletion of TBF (TATA Box Binding Factor) Gene		
Deletion of –300 to –150 upstream of *gal*A gene		
Deletion of –300 to –150 upstream of *gal*B gene		
Deletion of –300 to –100 upstream of *gal*B gene		
Deletion of –200 to –50 upstream of *gal*A gene		
Deletion of GAL4 gene		

9.15. A cell in the heart, a cell in the bicep muscle, and a cell in the pancreas have the same set of genes. All three cells express some of the same genes, the heart cell and bicep muscle cell express some genes that the pancreas cell does not, and each cell type expresses genes not expressed in the other cell types. Explain how all this could happen.

9.16. You isolate one maize cDNA clone corresponding to a gene turned on only during leaf development, and another cDNA clone that is turned on in the shoot tip and off in developing leaves.

a. When you isolate the genomic DNA clones for each of the genes and sequence them, you discover that they both contain binding sites for the transcription factor KNOTTED-1 upstream of their promoters. How do you interpret this new information?

b. Maize mutants have been found in which the leaves have strange structures somewhat resembling shoot tips all over their surface. The mutations have been mapped to the KNOTTED-1 gene. What has happened to the KNOTTED-1 gene? How can you test your hypothesis?

***9.17. Acetylcholinesterase III (AchIII) is an enzyme expressed in the first neurons of the developing Drosophila embryo. The enzyme is composed of two subunits, A and B, encoded by the *acl*A and *acl*B genes respectively. You conduct the following crosses. Give an explanation for the data you obtain.

Female Parent	Male Parent	Presence/absence of AchIII activity in embryos
*acl*A+ *acl*A+ *acl*B- *acl*B-	*acl*A- *acl*A- *acl*B+ *acl*B+	Present
*acl*A- *acl*A- *acl*B+ *acl*B+	*acl*A+ *acl*A+ *acl*B- *acl*B-	Absent
*acl*A+ *acl*A- *acl*B+ *acl*B-	*acl*A+ *acl*A- *acl*B+ *acl*B-	Present in 3/4, absent in 1/4

***9.18. Last spring, while admiring some oak flowers you are surprised to find one of the flowers actually moving around and eating other flowers. It turns out to be a caterpillar that looks remarkably like a group of oak flowers. You bring it back to the lab along with some oak flowers and watch it develop. Later in the summer, you find on the same oak tree a caterpillar eating some oak leaves that looks just like a twig. You bring it back to the lab and watch it develop. Both caterpillars metamorphose into adult moths that look identical. They are the same species!

You wonder whether the two caterpillar morphologies are due to a difference in a single gene, so you cross the two adult moths (call them "flower" and "twig", based on their caterpillar phenotype). Plenty of eggs are laid and soon you have hundreds of tiny, but hungry F1 caterpillars to feed. Luckily, you have some oak flowers stored in the freezer. The caterpillars eat away and when they are big enough to study with your own eyes, you find that all of them resemble oak flowers.

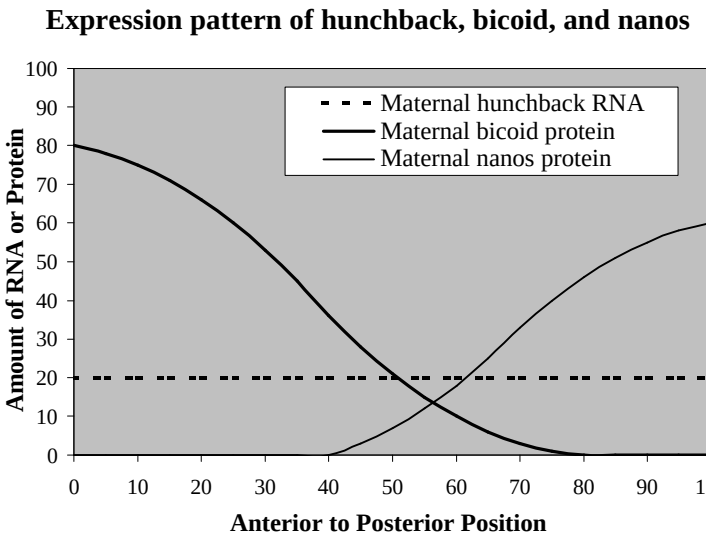
a. Give two possible explanations for your observations so far.

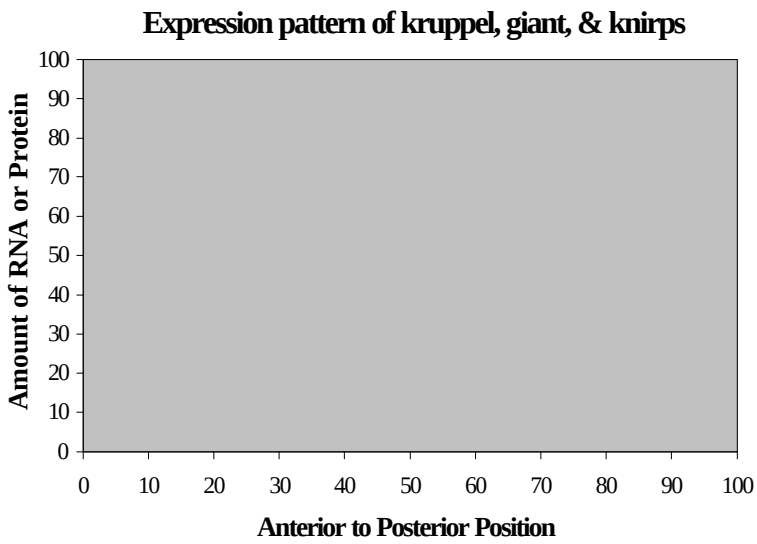
b. In order to test your hypotheses, you conduct further experiments. Based on the results shown below, give an explanation in terms of regulation of genes involved in cuticle formation.

Parental Moths	Food Given to Tiny F1 Caterpillars	Cuticle Phenotype of F1 Caterpillars When Larger
“Flower” x “Flower”	oak flowers	“Flower”
“Flower x Flower”	oak leaves	“Twig”
“Twig x Twig”	oak flowers	“Flower”
“Twig x Twig”	oak leaves	“Twig”
“Twig x Twig”	artificial diet (basic nutrients)	“Flower”
“Twig x Twig”	artificial diet + oak flowers	“Flower”
“Twig x Twig”	artificial diet + oak leaves	“Twig”

***9.19.** In order to better understand how the Drosophila anterior-posterior axis is determined, here are certain rules and a starting point that you will use to determine the expression pattern of 3 gap genes (Kruppel, knirps, & giant) in wildtype and mutant flies.

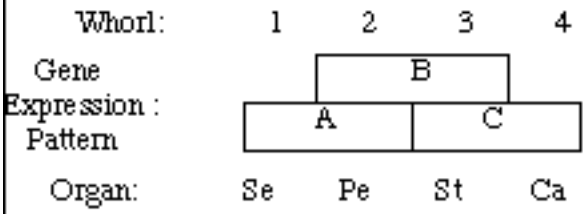
1. Bicoid activates hunchback gene expression in embryo (1 unit of bicoid leads to 1 unit of hunchback mRNA)
2. Nanos inhibits translation of hunchback mRNA in embryo (1 unit of nanos inhibits 1 unit of hunchback mRNA)
3. 1 unit of hunchback protein is produced for each unit of translatable hunchback mRNA from combined maternal & embryo pools
4. From 25 units up, hunchback activates Kruppel gene expression (2 units of Kruppel mRNA is produced for each unit of hunchback over 25 and up to 75; above 75 acts the same as 75)
5. From 75 units up, hunchback inhibits Kruppel gene expression (4 units less of Kruppel mRNA is produced for each unit of hunchback over 75)
6. From 10 units up, hunchback activates knirps gene expression (4 units of knirps mRNA is produced for each unit of hunchback over 10 and up to 35; above 35 acts the same as 35)
7. From 35 units up, hunchback inhibits knirps gene expression (2 units less of knirps mRNA is produced for each unit of hunchback over 35)
8. From 0 units up, hunchback activates giant gene expression (5 units of giant mRNA is produced for each unit of hunchback over 0 and up to 20; above 20 acts the same as 20)
9. From 20 units up, hunchback inhibits giant gene expression (5 units less of giant mRNA is produced for each unit of hunchback over 20)

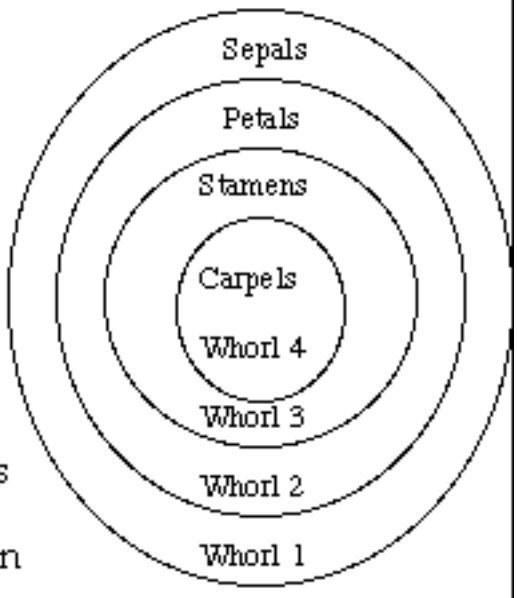




9.20. In *Arabidopsis thaliana*, the organs of a flower develop in a simple four whorl pattern as shown on the right. Genes A, B, and C are expressed in an overlapping pattern shown below.

Wildtype





Based on the floral gene expression patterns and whorl phenotypes found in the mutants shown below,

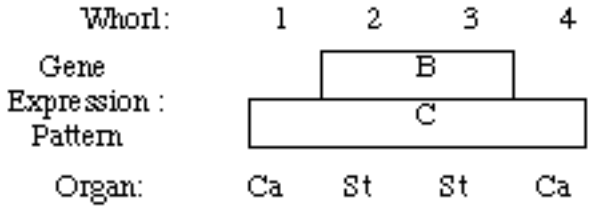
a. what are the roles of the A, B, and C genes respectively, in organ determination?

b. develop a model explaining the expression patterns of genes A, B, and C.

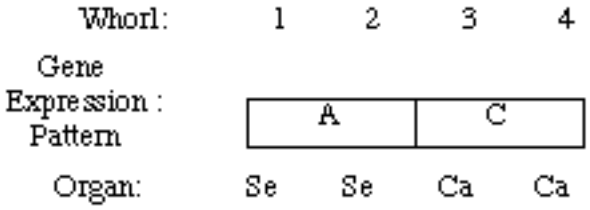
c. what is the most likely cellular activity of the products of genes A, B, and C?

d. where do you think gene D is expressed and what is its role in flower development?

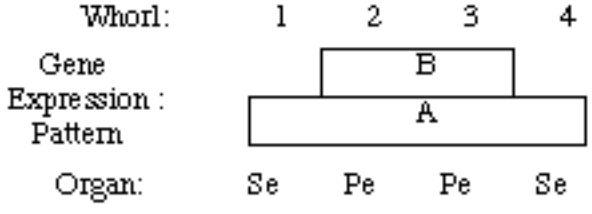
A⁻ mutant



B⁻ mutant



C⁻ mutant



D⁻ mutant