Welcome to the Third Genetics Exam!

BIOL 213 GENETICS: Exam November 13, 2000

RULES OF THE GAME: Same old rules. Open book, open notes, closed people.

ANSWER SHEET: Same old answer sheet. Turn it in plus any accompanying papers. Keep the questions.

MEANING OF WORD-RESTRICTIONS: Same old restrictions. Write little, write neatly. Save the spillover for another sheet.

WEIGHTS OF QUESTIONS: Same old parenthetical numbers before each question.

MULTIPLE CHOICE QUESTIONS: Same old ambiguity: more than one answer may be correct, or none may be correct.

NEED A FACT? Same old advice: If you need to make an assumption, state it and go on. If you need a clue, ask.

The Questions

1. (1 pt) If you have neither received nor given aid regarding this exam, nor have you gained or given knowledge concerning a previous or future administration of this exam, then sign your name. Otherwise sign someone else's name.

2. (1 pt) True/false: You realize that Thanksgiving is less than 12 days away, and if you just take it one day at a time everything will be OK, and the sun shines behind every cloud, and suicide is the coward's way out.

3. (2 pts) As judged by the examples presented in this course, gene expression is regulated to a great extent at the level of:

- **A.** Transcriptional initiation
- **B.** Translational elongation
- **C.** Transpositional termination
- **D.** Transcendental meditation

4. (9 pts) F'*lac* plasmids of different genotypes are mated into *E. coli* of different genotypes, as shown below. For each resulting strain, predict both the ß-galactosidase phenotype (**I** if ß-galactosidase can be induced by IPTG, **C** if activity is constitutive, and **N** if there is no activity under any circumstances) and the growth phenotype (**Lac**$^+$ if the strain can grow on lactose as the sole carbon source and **Lac**$^-$ if it cannot). If a genotype is not given for a gene or element, then it is presumed to be wild-type (+).An i^S gene encodes a repressor that is unable to bind allolactose. "*rbs*" signifies "ribosome binding site".

4a. $i^+ z^- y^-$ F'($i^- z^+ y^-$) **4b.** $i^+ o^c z^+ y^-$ F'($i^S z^- y^+$) **4c.** i^+ *rbs*$^+ z^- y^+$ F'(i^- *rbs*$^- z^+ y^-$)

5. (12 pts) Consider the section in your textbook on positive control of the *lac* operon (pp.403-404) in light of Fig. 1 on the next page. For each of the statements below taken from the book, provide two responses: (1) Indicate whether the data from the graphs, taken together, is supportive (**S**), contradicting (**C**), or uninformative (**U**) with respect to the entire statement. (2) If all the graphs are supportive or uninformative, indicate which graph (**A, B, C,** or **D**) is strongest in support. If at least one contradicts the statement, indicate which graph is strongest in opposition. If none are supportive or contradicting, draw a happy face.

5a. (Par.1,Sent.3) *If both lactose and glucose are present, synthesis of ß-galactosidase is not induced until all the glucose has been utilized.*

5b. (Par.3,Sent.1) *When glucose is present in high concentrations, the cAMP concentration is low…*

5c. (p.403, second to last line) *We now know that when a bacterium is exposed to glucose. . . cAMP is no longer available to bind to the CAP. Therefore, the unoccupied CAP does not bind to the CAP site. This causes the transcription [of the lac operon] to decrease.*

5d. Presuming the data of Fig. 1 to be accurate and broadly reflective of reality, is the model shown and described in the text the true basis for the diauxic effect?

6. (16) Reconsider Fig. 1 in light of the hypothesis depicted in Fig. 2, both on the next page. Only part of the model is shown in Fig. 2, that which is pertinent to the questions below.

6a. Indicate for each of the four graphs (panels **A** through **D**) whether it is supportive (**S**), contradicting (**C**), or uninformative (**U**) with regards to the hypothesis. In each case, explain your answer with respect to the most salient feature of the graph (no more than 20 words for each answer).

6b. Presuming the data of Fig. 1 to be accurate and broadly reflective of reality, is the model shown and described in Fig. 2 the true basis for the diauxic effect?

7. (12) Draw a picture of a eukaryotic cell (using the outline provided in the answer sheet or a similar outline of your own devising) that depicts the functioning of the elements listed below in the expression of hexokinase, an enzyme that catalyzes the phosphorylation of glucose. Use ovals to represent proteins. Use lots of labels.

TGA: stop codon
ISS: intron splice site
PR: positively acting transcriptional activator
NR: negatively acting transcriptional activator
Ser: serine residue required for binding glucose
A: poly-A addition site
TSS: transcriptional start site
ATG: start codon
m7G: m^7G cap
TATA: TATA box

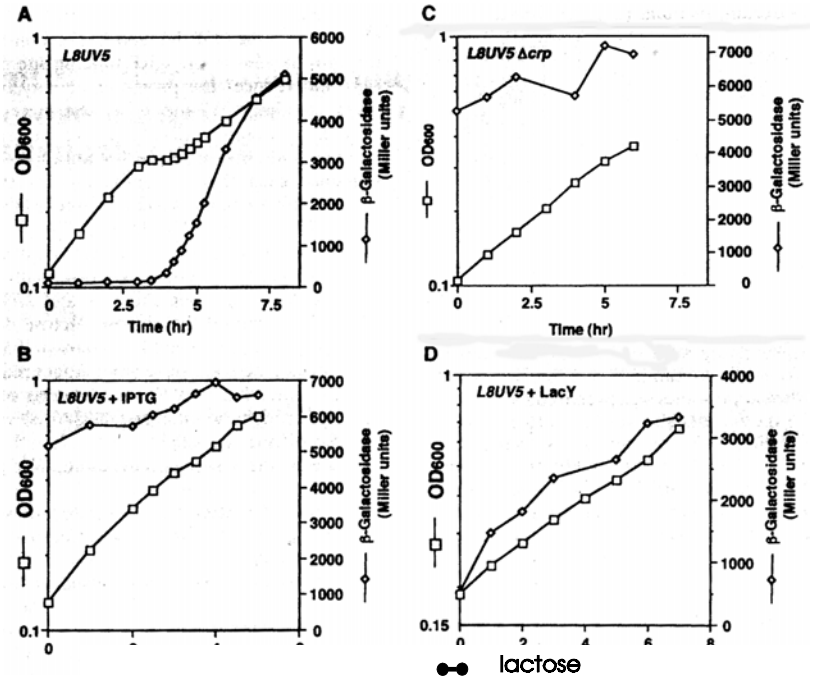

Figure 1: Diauxic growth by mutant *E. coli* (for Questions 4 and 5. An *E. coli* strain carrying the *L8* and *UV5* mutations was grown with a mixture of glucose and lactose. Square symbols represent measurements of the optical density of the suspension, indicative of the amount of cell growth. Diamond symbols represent measurements of ß-galactosidase activity. (**A**) No addition. (**B**) IPTG added at 0 hrs. (**C**) Strain also has *crp* mutation and is unable to make CRP protein. (**D**) Strain carries a plasmid with a *lacY* gene. This strain makes significantly more Lac permease than normal. Data from Kimata et al (1997).

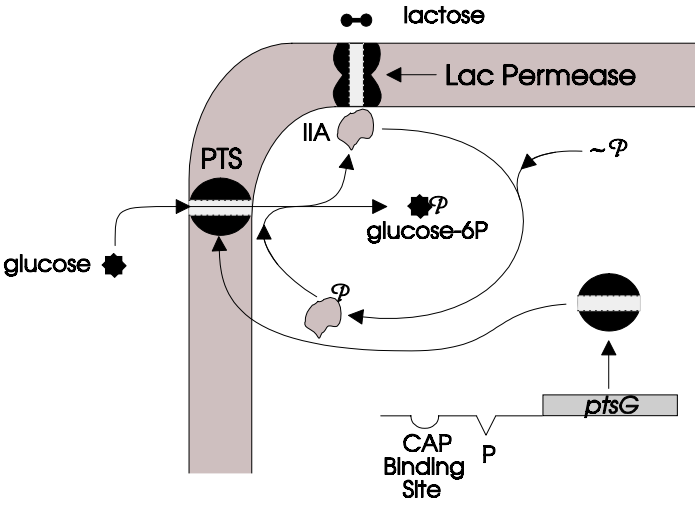

Figure 2: Hypothesis to explain glucose effect (for Question 5. Glucose enters *E coli* either through a specialized permease (PTS) that couples entry to phosphorylation of the sugar: Glucose cannot enter without phosphate being added to it. The phosphate (P) received by glucose is donated by the phosphorylated IIA protein. Unphosphorylated IIA protein (protein that has donated its phosphate to glucose) binds to and inhibits the function of the Lac permease. Phosphorylated IIA protein has no effect on the Lac permease. The PTS permease is encoded by *ptsG*, which is positively regulated by the binding of CRP protein near its promoter.

8. (12 pts) Let's return to the regulation of hunchback expression during *Drosophila* embryogenesis. You may wish to refer back to part D of the class notes for 11/8 and to Problem 9.19.

8a. What will the gradient of hunchback <u>protein</u> look like in an embryo from a cross of a wildtype father with a mother homozygous mutant for both bicoid and nanos? Draw your answer on the graph provided on the answer sheet.

8b. In 20 words or less, how do you interpret the following information? Nanos inhibits the translation of hunchback mRNA and a few other mRNAs (call them P, Q, and R), but has no effect on the vast majority of mRNAs. A mutant is found in which nanos does not inhibit the translation of hunchback mRNA, but still inhibits the translation of P, Q, and R mRNAs. The mutation maps in or very near the hunchback gene, but the encoded hunchback protein is identical to that of wildtype flies.

8c. Wreden et al. (Development [1997] 124:3015-23) wanted to understand how nanos inhibits the translation of hunchback mRNA. They carried out two related

experiments. First, they looked at the fate of the native hunchback mRNA and its encoded protein within *Drosophila* eggs and embryos. Second, they made their own hunchback mRNAs in a test tube by incubating the hunchback gene with ribonucleotides and a cocktail of proteins (RNA Pol II, TBF, TAFs, and other transcription factors). Sometimes, the cocktail included PolyA Polymerase, the enzyme involved in addition of a polyA tail. They injected the in vitro-synthesized (IVT) hunchback mRNA into *Drosophila* embryos and studied the fate of the mRNA and its encoded protein. In 20 words or less, draw as many conclusions as you can from the data shown.

	Amount in posterior end		**Relative length of Hb mRNA recovered from posterior end**
Hb mRNA analyzed	**Hb mRNA**	**Hb protein**	
1a. Native mRNA in egg from wt mom	high	very low	short (no polyA tail)
1b. Native mRNA in embryo from wt mom	high	very low	short (no polyA tail)
1c. Native mRNA in egg from *nos*$^-$ mom	high	very low	short (no polyA tail)
1d. Native mRNA in embryo from *nos*$^-$ mom	high	high	long (long polyA tail)
2a. IVT mRNA with no polyA tail added in vitro injected into embryo from wt mom	high	very low	short (no polyA tail)
2b. IVT mRNA with long polyA tail added in vitro injected into embryo from wt mom	high	very low	short (no polyA tail)

9. (3 pts) In 15 words or less, does splicing go against the idea of genome equivalency during development? Why or why not?

10. (8 pts) This question deals with flower development in *Arabidopsis*, as described in Problem 9.20. You may wish to refer to your work on Problem 9.20 in answering these questions.

10a. What will be the floral gene expression pattern and whorl phenotypes in a A$^-$B$^-$ double mutant?

10b. The floral gene expression pattern and whorl phenotype of a A$^-$C$^-$ double mutant is shown below, where Le denotes a leaf-like state.

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Whorl 1	2	3	4
Gene Expression Pattern		B	

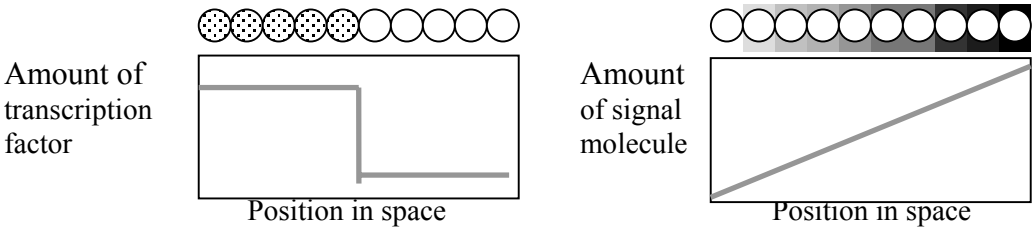
Organ	Le	Le	Le	Le

In 30 words or less, what is the default state of floral organ development and what does this data combined with the data in Problem 9.20 say about the determination of the petal and stamen states?

11. (9 pts) The *bg* gene codes for an enzyme of the same name (how boring). We know nothing about the expression pattern of the *bg* gene, but we should be able to predict the phenotype (BG enzyme activity present or not) for the progeny of different crosses under different assumptions. If you think all progeny should act the same, include the word ALL. If not, state the fractions that should act each way. (Assume that bg^+ codes for active BG enzyme, while bg- does not).

	Prediction for BG enzyme activity in the embryo (or later) in progeny (Yes or No; all or fraction)			
Assumption	***bg*$^+$*bg*$^+$ mom x *bg*$^+$*bg*$^+$ dad**	***bg*$^+$*bg*$^+$ mom x *bg*$^-$*bg*$^-$ dad**	***bg*$^-$*bg*$^-$ mom x *bg*$^+$*bg*$^+$ dad**	***bg*$^+$*bg*$^-$ mom x *bg*$^+$*bg*$^-$ dad**
***bg* gene is maternal-effect**	11a	11b	11c	11d
***bg* gene is zygotic-effect**	11e	11f	11g	11h
***bg* gene is both maternal-effect and zygotic-effect**	11i	11j	11k	11l

12. (4 pts) Imagine a two-dimensional array of cells: 
There is an asymmetric distribution of a particular transcription factor (see left graph below) and an asymmetric distribution of the small signal molecule that activates the transcription factor (see right graph below). Draw the distribution of gene expression across the array of cells for a gene that is turned on by the activated transcription factor. Also, in 10 words or less, state the assumption behind the shape of your distribution.



13. (9 pts) The mammalian metallothionein gene encodes a small protein that binds many different metal atoms. This protein is important for binding excess metal atoms and preventing metal toxicity. The metallothionein gene is induced by increasing levels of soluble metal atoms and is also induced by steroid hormones. Shown below are the results of a deletion analysis of the region upstream of the metallothionein gene. Based on these results, draw a diagram of the region upstream of the metallothionein gene showing all pertinent regulatory sequences (be sure to indicate the role of each sequence).

Section **Deleted** Within the Upstream Region of Metallothionein Gene	Relative Level of Metallothionein mRNA Upon Addition of: Nothing	Metal (low level)	Metal (high level)	Steroid
No deletion (wildtype)	100	1000	1,000,000	1000
-1000 to -500	100	1000	1,000,000	1000
-1000 to -250	100	1000	1,000,000	100
-500 to -250	100	1000	1,000,000	100
-250 to -200	75	750	750,000	750
-200 to -175	100	500	100,000	1000
-200 to -150	100	200	10,000	1000
-150 to -100	75	750	750,000	750
-100 to -75	100	500	100,000	1000
-75 to -50	75	750	750,000	750
-50 to -25	100	500	100,000	1000
-25 to -10	75	750	750,000	750
-200 to –150 and –100 to –75	100	100	1000	1000
-200 to –150, -100 to –75 and –50 to –25	100	100	100	1000

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1	2	3

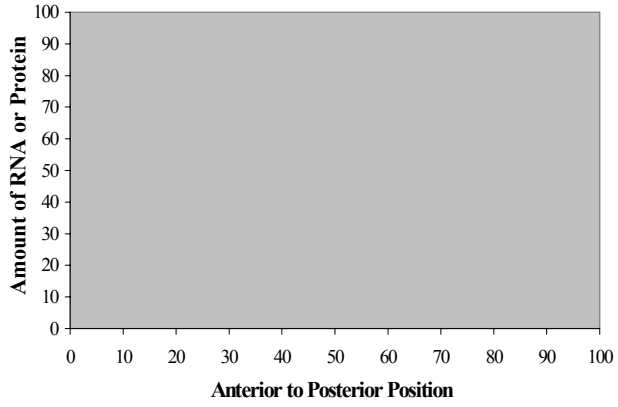
4a	4b	4c

5a	5b	5c	5d

6a (Panel A)	6a (Panel B)
6a (Panel C)	6a (Panel D)

6b

8a



8b

8c		**9**	
10a	**10b**		
11a	**11b**	**11c**	**11d**
11e	**11f**	**11g**	**11h**
11i	**11j**	**11k**	**11l**
12a 		**12b**	
13			
14 *Be sure to hand in your summary and the first page of the article*		**15** ***Space for quiz credit***	