

Biochemistry/ MCB 497C                      Instructor: Hardison  
Final Examination  
Spring 1994  
May 04, 1994

This examination has 44 questions for a total of 200 points. The first 43 are multiple choice and are worth 4 points each. Please answer these on the enclosed answer sheet. **BE SURE TO WRITE YOUR NAME AND STUDENT NUMBER ON THE ANSWER SHEET AND ENCODE YOUR NUMBER!**

The final question (Problem 44, worth a total of 28 points) requires you to write answers on the last sheet of the exam. Put your name and student number on this last sheet, and turn in **BOTH** the answer sheet and this last sheet. We will grade the last series of questions and encode those values on the answer sheet, which will then be machine graded. Good luck!

Use this information on the genetic code and restriction enzyme cleavage sites as required during the exam.

<u>Restriction endonuclease</u>	<u>Recognition sequence</u>
BamHI	G ' GATCC
EcoRI	G ' AATTC
HindIII	A ' AGCTT
KpnI	GGTAC ' C
PstI	CTGCA ' G

Choose the **correct** response in questions 1-10.

1. Mutations in the *lac* operator lead to

- a. constitutive expression of the operon.
- b. inducible expression of the operon.
- c. noninducible expression of the operon (low level expression in the presence or absence of inducer).
- d. activated expression of the operon in response to the inducer.

2. A culture of *E. coli* is growing in media with glucose as the sole carbon source. After tryptophan is added, the cells will continue to grow and divide about every 30 min. If the mRNA and protein for tryptophan synthetase are unstable relative to the cell division time (e.g. if they have a half-life of about 5 min), which of the following will be observed for tryptophan synthetase activity in the culture after addition of tryptophan?

- a. A slow decrease in activity, decreasing about 2-fold every generation.
- b. An abrupt decrease in activity.
- c. A slow increase in activity.
- d. A stable level of activity.

3. Bacteria that are lysogenic for phage are immune to further infection with phage because

- a. the prophage causes degradation of the receptor on the cell surface.
- b. the prophage encodes a restriction-modification system that degrades incoming DNA.
- c. the CII protein encoded on the prophage will activate transcription from  $P_{RE}$  on the incoming phage, forcing it to produce the repressor and thus blocking infection.
- d. the prophage makes the repressor, which binds to  $O_R$  and  $O_L$  and blocks lytic infection from the incoming phage.

4. In the leader region of the *trp* mRNA, what would be the effect of increasing the distance between sequences 2 and 3?

- a. The operon would be constitutively expressed.
- b. No attenuation would be observed.
- c. Attenuation would be observed only at high concentrations of tryptophan.
- d. Attenuation would be observed even at low concentrations of tryptophan.

5. The mutants  $cI^-$  and  $cII^-$  produce no lysogens, so they make clear plaques. If they are coinfecting into *E. coli*, will they produce turbid plaques, and if so which phage will be found in the resulting lysogen?

- a. No turbid plaques would be formed.
- b. The two phage would complement to form turbid plaques;  $cII^-$  phage will be found in the lysogen.
- c. The two phage would complement to form turbid plaques;  $cI^-$  phage will be found in the lysogen.
- d. The two phage would complement to form turbid plaques; both  $cI^-$  and  $cII^-$  phage will be found in the lysogen.

6. After the *lac* repressor binds the inducer,

- a. it dissociates from the operator and is free in solution.
- b. it is degraded by specific proteases.
- c. it dissociates from the operator and rebinds to nonspecific sites on the DNA.
- d. it remains bound to the operator and facilitates the transition of the transcription complex from closed to open.

7. The *lac* repressor prevents transcription of the *lac* operon by

- a. binding to an operator centered on the -10 promoter sequence and preventing binding of RNA polymerase.
- b. binding to an operator centered on the -35 promoter sequence and preventing recognition by the  $\sigma$  factor.
- c. competing with cAMP-CAP for its binding site and thereby preventing activation.
- d. binding to an operator centered on the sequence at +11 and holding the complex between RNA polymerase and the promoter in the closed conformation.

8. The ratio  $[RDs]/[Ds]$ , is the the concentration of a hypothetical repressor bound to its specific site on DNA divided by the concentration of unbound DNA, or the ratio of bound DNA to free DNA. When the measured  $[RDs]/[Ds]$ , is plotted versus the concentration of free repressor  $[R]$ , the slope of the plot showed that the ratio  $[RDs]/[Ds]$ , increased by 60 for every increase of  $1 \times 10^{-11}$  M in  $[R]$ . The binding constant  $K_s$  for association of the repressor with its specific site is

- a.  $6 \times 10^{12} \text{ M}^{-1}$
- b.  $1.7 \times 10^{-13} \text{ M}$
- c.  $6 \times 10^{-10} \text{ M}$
- d.  $2 \times 10^{13} \text{ M}^{-1}$

9. The transcription factor Sp1 binds to DNA by

- a. Cys<sub>2</sub>-His<sub>2</sub> zinc fingers.
- b. Cys<sub>2</sub>-Cys<sub>2</sub> zinc fingers.
- c. a helix-turn-helix.

d. a leucine zipper.

10. The dimerization domain of the transcription factor AP1 is

- a. a Cys<sub>2</sub>-His<sub>2</sub> zinc finger.
- b. a helix-loop-helix.
- c. a helix-turn-helix.
- d. a leucine zipper.

11. Which of the following functions have **NOT** been ascribed to a locus control region?

- a. Generate an open, active domain of chromatin.
- b. Terminate transcription from adjacent genes.
- c. Insulate from negative effects of adjacent sequences (negative position effects)
- d. Enhance transcription of genes within the domain in a developmentally regulated manner.

12. Which of the following statements about N protein (the product of the *N* gene) is **NOT** true?

- a. N activity requires the host protein NusA.
- b. N action requires sequences on the DNA called *nutL* and *nutR*.
- c. N protein acts to promote  $\lambda$ -dependent termination.
- d. N protein can relieve the polarity of certain amber mutations.

Use the following information to answer the next 6 questions (13 to 18). The regulatory scheme is imaginary but illustrative of some of the models we have discussed.

The protein surfactin is produced in the lung to provide surface area for efficient gas exchange in the alveoli. Let's suppose that expression of the surfactin gene is induced in lung cells by a new polypeptide hormone called pulmonin. Induction by pulmonin requires a particular DNA sequence upstream of the surfactin gene; this is called PRE for pulmonin response element. Proteins that bind specifically to that site were isolated, and the most highly purified fraction that bound to the PRE contained two polypeptides. A cDNA clone was isolated that encoded one of the polypeptides called NFL2. Antisera that specifically recognizes NFL2 is available.

The mechanism of the induction by pulmonin was investigated by testing various cell fractions (nuclear or cytoplasmic) from uninduced or pulmonin-induced lung cells in two assays. The presence or absence of NFL2 polypeptide was determined by reacting with the anti-NFL2 antisera, and the ability to bind to the PRE DNA sequence was tested by an electrophoretic mobility shift assay. In a further series of experiments, the NFL2 polypeptide was synthesized in vitro by transcribing the cDNA clone and translating that artificial mRNA. The product has the same amino acid sequence as the native polypeptide and is referred to below as "expressed cDNA." The expressed cDNA (which is the polypeptide synthesized in vitro) was tested in the same assays, before and after treatment with the cytoplasmic and nuclear extracts and also with a protein kinase that will phosphorylate the expressed cDNA on a specific serine.

<u>Line</u>	<u>Source of protein and Type of treatment</u>	<u>React with anti-NFL2</u>	<u>Bind to PRE DNA</u>
1	Uninduced cell cytoplasmic extract = unind. CE	+	-
2	Uninduced cell nuclear extract = unind. NE	-	-
3	Induced cell cytoplasmic extract = ind. CE	-	-
4	Induced cell nuclear extract = ind. NE	+	+
5	Induced cell nuclear extract + phosphatase	+	-
6	Expressed cDNA	+	-
7	Expressed cDNA + ind. CE	+	-
8	Expressed cDNA + unind. NE	+	-
9	Expressed cDNA + ind. CE + unind. NE	+	+
10	Expressed cDNA + unind. CE + unind. NE	+	-
11	Expressed cDNA + protein kinase + ATP	+	-
12	Expressed cDNA + protein kinase + ATP + unind. NE	+	+
13	Expressed cDNA + protein kinase + ATP + ind. CE	+	-

Based on these data, an affinity column was made with the expressed NFL2 cDNA as the ligand and used to test binding of proteins from nuclear extracts. When the column was pretreated with protein kinase + ATP (so that NFL2 was phosphorylated), a ubiquitous nuclear protein called UBF3 was bound from nuclear extracts from both induced and uninduced cells. If the NFL2 ligand was not phosphorylated, no binding of nuclear proteins was observed.

To confirm that NFL2 really was part of the protein complex on PRE, antibodies against NFL2 were shown to react with this protein-DNA complex. Furthermore, antibodies against phosphoserine, but not antibodies against phosphotyrosine, reacted with the specific PRE-protein complex.

Answer questions 13 to 18 based on the above observations. Choose the correct response.

13. Where is the NFL2 polypeptide? (Use data in lines 1-5.)

- a. Nucleus of uninduced cells and cytoplasm of induced cells.
- b. Cytoplasm of uninduced cells and nucleus of induced cells.
- c. Nucleus of uninduced cells and nucleus of induced cells.
- d. Cytoplasm of uninduced cells and cytoplasm of induced cells.

14. Where is the activity that will bind to the PRE site in DNA? (Use data in lines 1-5.)

- a. Cytoplasm of uninduced cells and nucleus of induced cells.
- b. Nucleus of uninduced cells.
- c. Nucleus of induced cells.
- d. Cytoplasm of induced cells.

15. From the data in lines 6-13, in vitro synthesized NFL2 (the expressed cDNA)

- a. will bind to the PRE site.
- b. will bind to the PRE site after phosphorylation (treatment with protein kinase + ATP).
- c. is degraded by the cytoplasmic extract from induced cells (ind. CE).
- d. must be phosphorylated and interact with something in the nucleus in order to bind the PRE site.

16. Binding to the PRE site

- a. requires only NFL2.
- b. requires phosphorylated NFL2 plus UBF3.
- c. requires nonphosphorylated NFL2 plus UBF3.
- d. requires only UBF3.

17. Which cell compartment has the protein kinase that acts on NFL2?

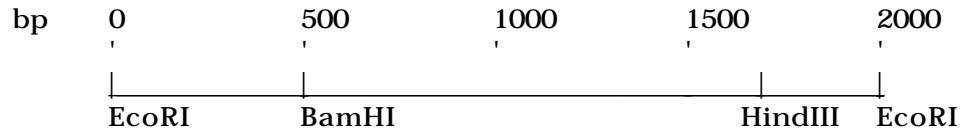
- a. Induced cell cytoplasm.
- b. Uninduced cell nucleus.
- c. Uninduced cell cytoplasm.
- d. Cannot tell from these data.

18. Which model for pulmonin induction of the surfactin gene best fits the data given?  
After exposure of lung cells to pulmonin,

- a. NFL2 in the cytoplasm is dephosphorylated, translocates to the nucleus and binds to the PRE.
- b. a heterodimer of UBF3 and NFL2 is phosphorylated (on a serine of NFL2), UBF3 dissociates, and NFL2 translocates to the nucleus where it binds the PRE.
- c. NFL2 in the cytoplasm is phosphorylated by a kinase that is activated by the pulmonin treatment. Phospho-NFL2 translocates to the nucleus where it binds UBF3 and subsequently the heterodimer binds to the PRE.
- d. a proteolytic activity of NFL2 is activated. This protease translocates into the nucleus and degrades UBF3, thereby liberating a protein that binds to the PRE. Prior to induction, this latter protein was bound to UBF3 and blocked from binding to the PRE.

Use the following information to answer questions 19 to 21.

A gene that determines flower color in azuratum, called *sapphire*, is contained within a 2000 bp EcoRI fragment that has been cloned in a plasmid. As shown in the map below, the EcoRI fragment has a BamHI site 500 bp from the left EcoRI site and a HindIII site 300 bp from the right EcoRI site.



In order to determine the positions that correspond to the 5' and 3' ends of the *sapphire* RNA, the HindIII and BamHI sites were labeled at the 5' end (in one set of experiments) or the 3' end (in another set of experiments). The end-labeled EcoRI to BamHI fragments (500 and 1500 bp) and the EcoRI to HindIII fragments (1700 and 300 bp) were isolated, hybridized to *sapphire* RNA and treated with the single-strand specific nuclease S1. The sizes of the probe fragments protected from digestion in the RNA-DNA duplex are shown below (in nucleotides); a 0 means that the probe was not protected by RNA. The site that is labeled is indicated by an asterisk (\*).

<u>5' end-labeled probe</u>	<u>protected fragment</u>	<u>3' end-labeled probe</u>	<u>protected fragment</u>
EcoRI -BamHI * 500 bp	0	EcoRI -BamHI * 500 bp	300
*BamHI -EcoRI 1500 bp	1400	*BamHI -EcoRI 1500 bp	0
EcoRI -HindIII* 1700 bp	0	EcoRI -HindIII* 1700 bp	1500
*HindIII-EcoRI 300 bp	200	*HindIII-EcoRI 300 bp	0

19. What is the direction of transcription of the *sapphire* gene, relative to the map above?

- a. Left to right
- b. Right to left
- c. Cannot be determined from the data given
- d. Bidirectional transcription

20. What position on the map corresponds to the 5' end of the mRNA?

- a. 100
- b. 200
- c. 1500
- d. 1900

21. What position on the map corresponds to the 3' end of the mRNA?

- a. 100
- b. 200
- c. 1500
- d. 1900

22. Which of the following is required for binding aminoacyl-tRNAs to the A site of the bacterial ribosome during elongation?

- a. EF-Tu-GTP
- b. EF-Ts-GDP
- c. EF-Tu-GDP
- d. EF-G-GTP

23. Allowing for "wobble" between the 3rd position of the codon and the 1st position of the anticodon, how many tRNAs are required to recognize codons for leucine (Leu)?

- a. two
- b. three
- c. four
- d. six

24. A phosphoester transfer mechanism (or transesterification) is observed frequently in splicing and other reactions involving RNA. Which of the following statements about this mechanism is INCORRECT?

- a. The mechanism requires the cleavage of high-energy bonds from ATP.
- b. The initiating nucleophile for splicing of Group I introns (including the intron of pre-rRNA from Tetrahymena) is the 3' hydroxyl of a guanine nucleotide.
- c. The initiating nucleophile for splicing of nuclear pre-mRNA is the 2' hydroxyl of an internal adenine nucleotide.
- d. The individual reactions in the phosphoester transfers are reversible, but the overall process is essentially irreversible because of circularization (includes lariat formation) of the excised intron.

Use the following information to answer problems 25 and 26. Consider the replication of a circular viral DNA in infected cells. The infected cells were pulse labeled with [<sup>3</sup>H] thymidine for 1, 2, 3, 4, 5 and 6 min; it takes 6 min for the DNA molecules to be replicated in this system (from initiation to termination). Those DNA molecules that had completed synthesis at each time point were isolated, cut with a restriction endonuclease, and assayed for radioactivity in each fragment. This restriction endonuclease cleaves the circular DNA into 6 fragments, named A, B, C, D, E, and F in a clockwise orientation around the genome. The following results were obtained; a plus (+) means the fragment was radioactively labeled, and a minus (-) means it was not labeled.

Frag.	Time of labeling (min)					
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
A	-	+	+	+	+	+
B	+	+	+	+	+	+
C	-	-	-	-	-	+
D	-	-	-	-	+	+
E	-	-	-	+	+	+
F	-	-	+	+	+	+

25. What restriction fragment has the origin and which has the terminus of replication?

- |    | <u>origin</u> | <u>terminus</u> |
|----|---------------|-----------------|
| a. | B             | E               |
| b. | B             | C               |
| c. | C             | B               |
| d. | A             | F               |

26. Replication of this viral DNA

- is unidirectional, clockwise.
- is unidirectional, counterclockwise.
- is bi-directional.
- uses multiple origins.

27. Okazaki fragments

- are short (1000 to 2000 nucleotide) fragments synthesized discontinuously on the lagging strand during replication.
- are short (1000 to 2000 nucleotide) fragments synthesized discontinuously on the leading strand during replication.
- were discovered as short DNA fragments when replicating DNA was fractionated on native (nondenaturing) sucrose gradients.
- are composed completely of deoxyribonucleotides.

28. Chain growth catalyzed by DNA polymerase

- occurs by the incorporation of dNDPs which are added as dNMPs with the liberation of phosphate.
- is irreversible.
- is reversible by a pyrophosphorolysis reaction.
- occurs by the addition of nucleotides to the 5' end of the growing chain.

29. Which of the following sets of proteins is responsible for mismatch repair in *E. coli*?

- a. UvrA, UvrB, UvrC, and UvrD
- b. UmuC and UmuD
- c. RuvA, RuvB and RuvC
- d. MutH, MutL and MutS

30. Which of the following sets of proteins is involved in nucleotide excision repair in *E. coli*?

- a. UvrA, UvrB, UvrC, and UvrD
- b. UmuC and UmuD
- c. RuvA, RuvB and RuvC
- d. MutH, MutL and MutS

31. Why do models for recombination include the generation of heteroduplexes in the products? Choose the CORRECT answer.

- a. RuvA and RuvB catalyze branch migration, which enlarges the region of heteroduplex.
- b. Information stored at HML or HMR can be transferred to the MAT locus to change the mating type of *S. cerevisiae*, in a unidirectional transfer of genetic information.
- c. The energetic expense of making a double strand break and reunion with another molecule is too high.
- d. Examination of interallelic recombination during spore formation in heterozygous *Ascomycetes* (and other fungi) occasionally show a 3:5 ratio of the spores from each allele of the heterozygote, instead of the expected 4:4 ratio.

32. If a negatively supercoiled, circular DNA molecule shifts from B form to Z form DNA over a region of 120 bp without opening and closing the DNA, what will be the change in W (writhing number)?

- a.  $W = -12$
- b.  $W = +12$
- c.  $W = +22$
- d.  $W = -22$

33. Which of the following is a ribonucleoside?

- a. guanine
- b. guanylic acid
- c. GMP
- d. guanosine

34. An autonomously replicating, circular DNA molecule that carries no essential function but confers a beneficial function (such as antibiotic resistance) to its "host" bacterium is called a

- a. transducing phage
- b. chromosome
- c. plasmid
- d. prophage

35. Denatured genomic DNA from a ciliate will renature as a single homogeneous component with a  $Cot_{1/2}$  of 500. A bacterial DNA standard whose size is  $4 \times 10^6$  bp, renatures with a  $Cot_{1/2}$  of 10 under identical conditions. What is the size of the ciliate genome?

- a.  $8 \times 10^4$  bp
- b.  $2 \times 10^8$  bp
- c.  $2 \times 10^9$  bp
- d.  $8 \times 10^6$  bp

36. Which of the following is an indicator of introns in a gene?

- a. The nuclear RNA from the gene is the same size as the mRNA.
- b. The map of restriction cleavage sites is identical for both the gene and a DNA copy of the mRNA (cDNA).
- c. The gene is present in multiple copies in the genome.
- d. R-loops formed between the mature mRNA and the gene show discontinuities between the mRNA and the DNA, such as loops of DNA not in hybrid with the mRNA.

37. Which statement about nucleosomes is true?

- a. A single nucleosome contains about 300 bp of DNA.
- b. Micrococcal nuclease cuts preferentially in the DNA wrapped around the core histones, not in the spacer (or linker) between the nucleosomal cores.
- c. Nucleosomal cores contain two copies of each of the four histones H2A, H2B, H3 and H4.
- d. The path of the DNA duplex around the histones in the core is a right-handed toroidal turn.

38. Regions of chromatin that are hypersensitive to DNase I

- a. frequently contain regulatory sites, such as promoters and enhancers.
- b. are in heterochromatin.
- c. are composed of DNA whose sequence is preferentially cleaved by DNase I in protein-free DNA.
- d. contain a high concentration of RNA primers not excised during replication.

39. Which structures are required for segregation of chromosomes at meiosis and mitosis?

- a. Telomeres
- b. Centromeres
- c. Polytene bands
- d. Origins of replication

40. Which of the following reactions is carried out by an RNA molecule, either in catalytic or stoichiometric amounts?

- (1) splicing of the intron of pre-rRNA in *Tetrahymena*
- (2) cleavage by RNase P
- (3) RNA editing
- (4) synthesis of telomeres by telomerase
- (5) elongation by DNA polymerase III

Correct choices are

- a. 1, 2
- b. 3, 4
- c. 1, 2, 3, 4
- d. 1, 2, 3, 4, 5

41. Which of the following catalyzes the synthesis of RNA primers on the lagging strand during replication of *E. coli* DNA?

- a. DnaG
- b. DnaA
- c. DnaB
- d. PriA

42. Which enzyme is responsible for transcribing the genes for rRNA in most eukaryotes?

- a. RNA polymerase
- b. RNA polymerase I
- c. RNA polymerase II
- d. RNA polymerase III

43. Which of the following general transcription factors do **NOT** contain the TATA-binding protein (TBP)?

- a. TFIID
- b. TFIIB
- c. TFIIIC
- d. SL1

44. (28 points total) Consider a hypothetical operon responsible for synthesis of the porphyrin ring (the heterocyclic ring that is a precursor to heme, cytochromes and chlorophyll). Four genes or loci, *porA*, *porB*, *porC*, and *porD*, that affect the activity or regulation of the biosynthetic enzymes were studied in a series of haploid and diploid strains. In the the following table, wild type alleles of the genes or loci are indicated by a + under the letter of the *por* gene or locus and mutant alleles are indicated by a - under the letter. The activities of two enzymes involved in porphyrin biosynthesis,  $\delta$ -aminolevulinic acid synthetase and  $\delta$ -aminolevulinic acid dehydrase (referred to in the table as ALA synthetase and ALA dehydrase), were assayed in the presence or absence of heme (one product of the pathway). The units of enzyme activity are 100 = non-repressed activity of the wild-type enzyme, 1 = repressed activity of the wild-type enzyme (in the presence of heme), and 0 = no measurable activity. In the diploid analysis, one copy of each operon is present in each cell.

Strain number	<i>por</i>				<u>ALA synthetase</u>		<u>ALA dehydrase</u>		
	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>- heme</u>	<u>+ heme</u>	<u>-heme</u>	<u>+heme</u>	
Haploid									
1	+	+	+	+	100	1	100	1	
2	-	+	+	+	100	100	100	100	
3	+	-	+	+	0	0	100	1	
4	+	+	-	+	100	1	0	0	
5	+	+	+	-	100	100	100	100	
Diploid	<u>A</u>	<u>B</u>	<u>C</u>	<u>D/A</u>	<u>B</u>	<u>C</u>	<u>D</u>		
6	+	-	+	+/+	+	-	+	100	1
7	-	+	+	+/+	+	-	+	200	100
8	+	+	+	-/+	+	-	+	200	1
9	-	+	-	+/+	-	+	-	100	100

Use these data to answer the questions on the next page.

Name: \_\_\_\_\_ Student number: \_\_\_\_\_

44a. (10 pts) Describe the phenotypes of the following the strains with respect to ALA synthetase and ALA dehydrase activities. A single word will suffice for each phenotype.

	<u>Strain</u>	<u>ALA synthetase</u>	<u>ALA dehydrase</u>
2		_____	_____
3		_____	_____
4		_____	_____
5		_____	_____
6		_____	_____

44b. (8 pts) What is the relationship (dominant or recessive) between wild-type and mutant alleles of the four genes, and which strain demonstrates this? Please answer in a sentence with the syntax in this example: "Strain 20 is repressible, which shows that mutant *grk1* is dominant to wild type."

*porA* Strain \_\_\_ is \_\_\_\_\_, which shows that \_\_\_\_\_ *porA* is \_\_\_\_\_.

*porB* Strain \_\_\_ is \_\_\_\_\_, which shows that \_\_\_\_\_ *porB* is \_\_\_\_\_.

*porC* Strain \_\_\_ is \_\_\_\_\_, which shows that \_\_\_\_\_ *porC* is \_\_\_\_\_.

*porD* Strain \_\_\_ is \_\_\_\_\_, which shows that \_\_\_\_\_ *porD* is \_\_\_\_\_.

44c. (8 pts.) What is the role of each of the genes in activity or regulation of porphyrin biosynthesis? Brief phrases will suffice.

*porA*

*porB*

*porC*

*porD*

44d. (2 pts) Is this operon under positive or negative control?

