**BMB 400**
**Molecular Biology of the Gene**
**Exam 4**
**December 13, 1999**

This examination has 25 questions worth a total of 100 points. All are multiple choice, and count 4 points each. **Pick only one option per question.** Please answer these on the enclosed answer sheet. Pertinent information may be found on the last sheet. BE SURE TO WRITE YOUR NAME, STUDENT NUMBER **AND TEST FORM** ON THE ANSWER SHEET AND **ENCODE** YOUR NUMBER!

## *THIS IS FORM A.*

PLEASE TURN IN YOUR ANSWER SHEET. You may keep the exam for review.

Questions 1-4 discuss the lac operon, which is involved in the metabolic breakdown of lactose. It is regulated by a CAP binding site centered at -62, promoter that includes -35 and -10 consensus sequences, an operator sequence centered at +11, allolactose, and the synthetic inducer IPTG.

1. Which of the following statements about the lac repressor is true?

   A. Lac repressor decreases the affinity of RNA polymerase for the promoter in the presence of IPTG.
   B. Lac repressor binds to the operator in the presence of IPTG.
   C. Lac repressor decreases the rate of formation of a closed complex
   D. Lac repressor decreases the rate of transition from a closed to an open complex when bound to the operator sequence.
   E. When bound to allolactose, lac repressor completely blocks binding of RNA polymerase to the promoter.

2. Which of the following statements about CAP is FALSE?

   A. CAP-cAMP interacts witho the alpha subunit of RNA polymerase in the presence of cAMP.
   B. CAP-cAMP regulates expression of several operons in addition to the lac operon.

C. When bound to its site, CAP-cAMP decreases the $K_B$ for RNA polymerase-promoter interactions.
D. On some promoters, CAP-cAMP increases isomerization from a closed to an open complex.
E. An altered CAP AR1 domain can still interact with the alpha subunit of RNA polymerase on a class II promoter.

3. If one removes the operator sequence, but leaves the promoter and CAP binding sites intact, which of the following would be observed?

A. Expression will be constitutive, and unaffected by either cAMP or allolactose.
B. RNA polymerase will associate at the same rate in either the presence or absence of cAMP, but open complexes will form more rapidly if allolactose is present.
C. RNA polymerase will associate at the same rate in either the presence or absence of allolactose, but open complexes will form more rapidly if cAMP is present.
D. Expression will be inducible by cAMP, but the rate of open complex formation will be the same whether cAMP is present or not.
E. Expression will be repressed and uninducibleby cAMP

4. If one removes the CAP binding site, leaving the promoter and operator intact, which of the following will one observe?

A. Expression will be unaffected by either IPTG or cAMP.
B. RNA polymerase will associate at the same rate as in question 3 (removal of operator)
C. Open complex formation will occur at the same rate as question 3 (removal of operator)
D. Expression will be inducible by cAMP but not IPTG.
E. RNA polymerase will associate at the same rate in either the presence or absence of IPTG, but open complexes will form more rapidly if IPTG is present.

5. Which of the following will lead to "normal"regulation of the trp operon, in which termination occurs at high concentrations of trp but not low?

A. mutations in region 1 that prevent it from binding to region 2
B. mutations in region 2 that prevent it from binding to region 3, but not 1
C. mutations in region 3 that prevent it from binding to region 4, but not 2

D. mutations in region 2 that prevent it from binding either region 1 or 3
E. mutations in region 3 that prevent it from binding either region 2 or 4

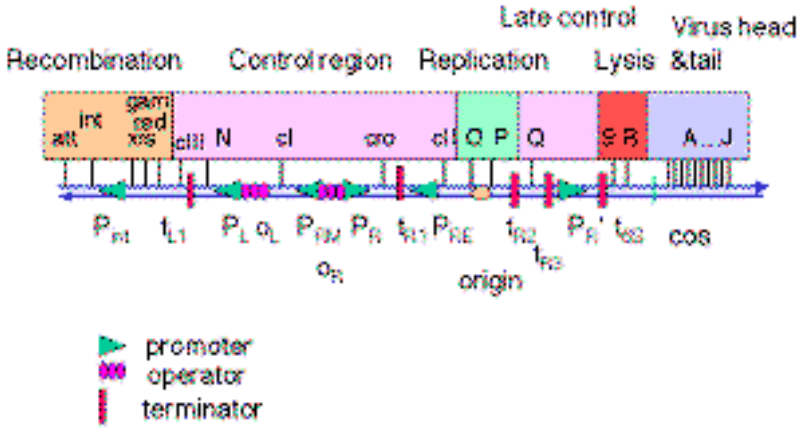
6. Of the answers above (question 5), which will likely result in termination, whether trp is present or not?

A. B and D
B. A and C
C. C and E
D. A and B
E. D and E

7. Placement of 10 more trp codons upstream of the naturally occurring trp operons in the trp leader RNA will :

A. Cause termination of transcription in both the presence and absence of trp.
B. Prevent termination of transcription only at high concentrations of trp
C. Prevent termination of transcription at both low and high concentrations of trp.
D. Facilitate binding of region 2 to region 3 of the trp leader RNA.

Questions 8-12. Following infection, bacteriophage lambda may utilize either a lytic or lysogenic program. The lambda genome is shown below to aid you in answering the following questions.




8. Which of the following would decrease the chances of a lytic infection occurring?

A. mutations that inactivate $P_{RE}$

B. mutations that place a translation stop codon at the beginning of the cII protein
C. mutations in the $P_{R'}$ promoter
D. elimination of the $t_{6S}$ termination site
E. mutations that prevent cI from binding to $O_{R2}$

9. Which of the following is the last to become activated if the bacteriophage follows a lysogenic program?

A. $P_{RE}$
B. $P_{R'}$
C. $P_{R}$
D. $P_{L}$
E. $P_{RM}$

10. Mutations in the C terminal domain of lambda repressor that eliminate dimer interactions are most likely to

A. increase the frequency of lysogeny
B. increase the levels of Cro protein
C. completely prevent the binding of RNA polymerase to $O_{R3}$
D. stimulate transcription from $P_{RM}$
E. increase the $K_B$ for binding of Cro to $O_{R3}$

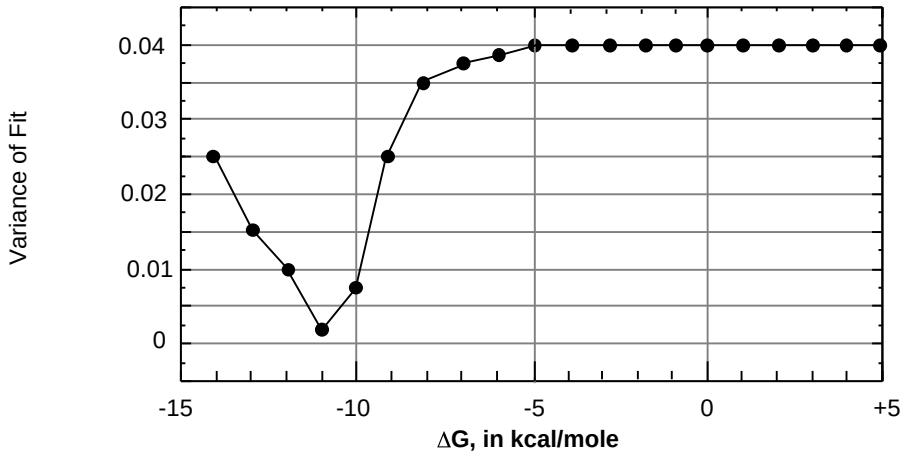
11. Bacteria that become lysogenic for bacteriophage lambda are immune to subsequent lytic infection. Which of the following is essential to this behavior?

A. cI repressor
B. Cro protein
C. the Q antiterminator
D. $t_{6S}$
E. $P_L$

12. Which of the following is NOT necessary for antitermination in a bacteriophage lambda lytic infection?

A. N protein
B. E. coli Nus A
C. E. coli Rho protein
D. $t_{L1}$
E. Q protein

Questions 13-17. You discover a new DNA binding protein (TT1) in which 4 His residues are associated in a tetrahedral configuration around a Zn ion. You also discover that binding of cAMP alters the affinity of TT1 for its binding site. You call the motif a Zn thumb, and set out to determine how well it binds to DNA in the absence of cAMP. It's binding site is TTAAGGCC. After labelling an oligonucleotide containing the binding site, you hybridize a constant amount of the labelled probe to increasing amounts of the TT1 protein. You measure the fractional occupancy of the DNA (the fraction of DNA bound by TT1 as a function of total TT1) at each concentration. You then carry out a non-linear least squares analysis to determine the DG with the lowest error. You obtain the data shown below.



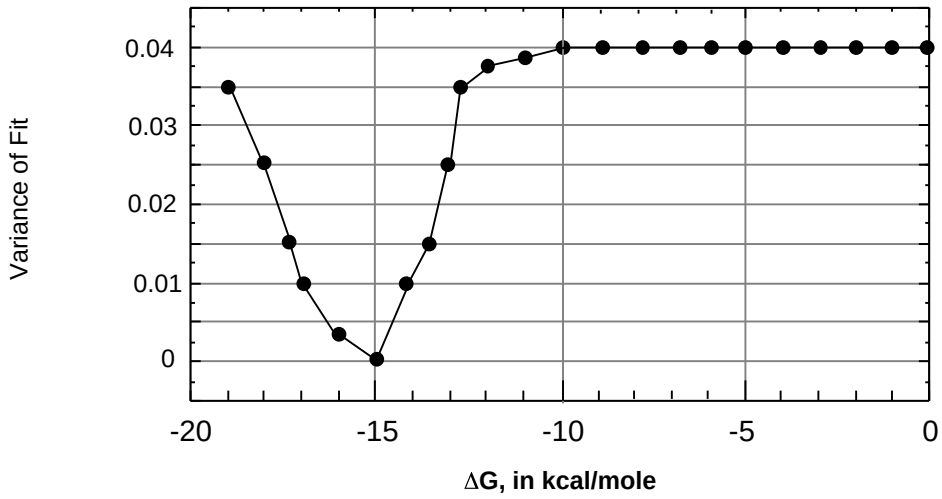

13. What is the value of ΔG that is most accurate ?

A. -14 kcal/mole
B. -11 kcal/mole
C. -9 kcal/mole
D. -5 kcal/mole
E. +11 kcal/mole

14. What is the most accurate measure of the binding constant, $K_B$, for binding of TT1 to this oligonucleotide?

A. $K_B = 1.25 \times 10^{-15}$ M
B. $K_B = 10^{-11}$ M
C. $K_B = 10^{-11}$ $M^{-1}$
D. $K_B = 1.25 \times 10^{8}$ $M^{-1}$
E. $K_B = 2.5 \times 10^{-9}$ $M^{-1}$

15. You carry out similar analyses to determine how well it binds to the same site in the presence of cAMP. You obtain the following data. Does TT1 bind to its recognition sequence better or worse in the presence of cAMP?




A. Better
B. Worse

16. You then discover that TT1 also binds nonspecifically to DNA, with a $K_{NS}$ of $1 \times 10^4$ in either the presence or absence of cAMP. You determine that there are 100 molecules of TT1 per cell, 1 binding site per genome and a genome size of $5 \times 10^6$. In the presence of cAMP, what is the approximate ratio of free TT1 binding sites to sites bound by TT1?

A. 0.1
B. 0.25
C. 0.005
D. 10
E. 50

17. Approximately what percentage of the TT1 binding sites are occupied in the absence of cAMP?

F. 5%
G. 25%

H. 0.1%
I. 75%
J. 95%

18. Of the following, which do NOT bind in the major groove of the DNA?

1. The helix-turn-helix -containing lac repressor protein
2. The C2H2 Zn-finger containing protein SP1
3. The GAL4 acidic domain
4. The HMG I(Y) protein that bends DNA in the interferon B-enhancer
5. The glutamine rich region of SP1
6. The bZIP domain of AP1

A. 1 and 2
B. 3 and 6
C. 3, 4 and 5
D. 3, 5 and 6
E. 1, 2 and 6

19. The bZIP domain includes an amphipathic helix. This helix facilitates dimer formation through association of its hydrophobic side to the hydrophobic side of an amphipathic helix of another bZIP molecule.

A. True
B. False

20. Which of the following do not have a regular structure that has been resolved by X-ray crystallography?

1. The leucine zipper amphipathic helix
2. The homeodomain
3. The acidic activation domain
4. The histone 2A and 2B tails
5. The Zn finger

A. 1 and 2
B. 2 and 3
C. 2, 3 and 4
D. 3 and 4
E. 3 and 5

21. The interaction of two histone proteins with each other to form a histone dimer pair occurs via:

A. the amino terminal tails
B. the histone leucine zipper motifs
C. the histone fold motifs
D. the bromodomain motifs
E. the histone helix-loop-helix motifs

22. The nucleosome is much more stable than most protein/DNA complexes because.

A. histone H3 is covalently linked to the phosphate backbone of DNA via a phospho-peptide bridge.
B. the histone proteins interact with the DNA every 10 bp over 147 bp of DNA.
C. the histone proteins are loaded onto DNA in the form of a closed ring with the DNA through the center.
D. the helicase activity of the histones melts DNA forming a stable single stranded bubble.
E. each of the eight histones in the octamer contains a Zn finger which allows cooperative binding.

23. The SAGA complex represents a HAT complex which also functions as a transcription co-activator. The SAGA complex can be recruited to specific promoters in chromatin by direct interactions with

A. the TATA box
B. histones
C. the ADA proteins
D. transcription activators
E. the Spt proteins

24. The SWI/SNF can facilitate the formation of hypersensitive sites in chromatin by

A. catalyzing an alteration in nucleosome structure
B. binding histone and competing them off of DNA
C. acetylating the histone N-terminal tails

D. proteolyzing the histone proteins
E. kinasing the histone leucine zippers

25. Consider a negatively controlled operon with two structural genes (A and B, for enzymes A and B) an operator gene (0) and a regulatory gene (R). In the wild-type haploid strain grown in the absence of inducer, the enzyme activities of A and B are both 1 unit. In the presence of an inducer, the enzyme activities of A and B are both 100 units. Choose the answers that best describes the enzyme activities in the designated strains.

| | | | Uninduced | | Induced | |
|---|---|---|---|---|---|---|
| | | | Enz A | Enz B | Enz A | Enz B |
| 1) | $R^{+}0^{C}A^{+}B^{+}$ | a) | 1 | 1 | 100 | 100 |
| | | b) | 1 | 100 | 100 | 1 |
| | | c) | 50 | 50 | 100 | 100 |
| | | d) | 1 | 1 | 1 | 1 |
| 2) | $R^{-}0^{+}A^{+}B^{+}/R^{+}0^{+}A^{+}B^{+}$ | a) | 2 | 2 | 200 | 200 |
| | | b) | 2 | 101 | 2 | 101 |
| | | c) | 200 | 200 | 200 | 200 |
| | | d) | 1 | 1 | 1 | 1 |

A. d for strains 1 and 2
B. a for strain 1 and b for strain 2
C. c for strain 1 and a for strain 2
D. c for strain 1 and b for strain 2
E. a for strain 1 and c for strain 2