

Biochemistry and Molecular Biology 400
Final Examination
Fall 2001
December 11, 2001

Instructor: Hardison

This examination has 40 questions for a total of 200 points. **All** are multiple choice and are worth 5 points each. Please answer the questions on the enclosed answer sheet. **BE SURE TO ENCODE YOUR STUDENT NUMBER AND TEST FORM ON THE ANSWER SHEET!**

THIS IS FORM A, and has answers.

Useful information and equations are at the end. You may wish to tear these off and use them throughout the exam.

PLEASE TURN IN ONLY THE ANSWER SHEET; you may keep the exam.

Some questions request you to choose all the correct statements from a list. Partial credit is given for choosing **some** of the correct answers, **maximal** credit is given for choosing **all** the correct statements, but **no** credit is given for a choice that includes **any incorrect** statements. **Pick only one option per question.**

1. Which of the following statements about regulation of the *lac* operon in *E. coli* are correct?

- [1] A repressor negatively regulates the operon when the inducer is absent.
- [2] The *lac* repressor is not bound to any DNA sequence when inducer is bound to repressor.
- [3] The *lac* repressor binds with high affinity to the operator.
- [4] The *lac* repressor bound to its operator decreases the rate of the closed to open transition of the RNA polymerase-promoter complex.

Correct choices are:

- a. 1 b. 1, 3 c. 1, 2, 3 d. 2, 3 e. 1, 3, 4
- e is correct, 2 pts for a, 3 pts for b**

2. Which statements about positive regulation of the *lac* operon in *E. coli* are correct?

- [1] Binding of the complex cAMP-CAP stimulates transcription of the operon.
- [2] cAMP-CAP interacts with the α subunit of RNA polymerase.
- [3] RNA polymerase binds more strongly to the *lac* promoter in the presence of cAMP-CAP.
- [4] The α -subunit of RNA polymerase binds to a specific DNA sequence upstream of the -35 box of the *lac* operon to stimulate transcription.

Correct choices are:

- a. 1 b. 2 c. 1, 4 d. 1, 2, 3 e. 1, 2, 3, 4
- d is correct, 2 pts for a or b**

3. Which of the following is **NOT** necessary for antitermination during lytic infection by bacteriophage λ ?

- a. N protein
 - b. *E. coli* NusA protein
 - c. *E. coli* NusG protein
 - d. cIII protein
 - e. a *nut* site
- d is correct.**

For the next four questions, consider the following hypothetical data on repression of an operon for cysteine synthesis in a bacterium. In this bacterium, cysteine is synthesized from serine in two steps, one catalyzed by serine acetyl transferase (SAT) and the other by cysteine synthetase (CS). The genes encoding SAT and CS are induced when the concentration of cysteine (Cys) is low but they are expressed at a low level when the concentration of Cys is high. Four genes or loci, *cysA*, *cysB*, *cysC*, and *cysD*, affecting the activity or regulation of these enzymes were studied in a series of haploid and diploid strains. In the following table, wild-type alleles of the genes or loci are indicated by a + under the letter of the *cys* gene or locus and mutant alleles are indicated by a - under the letter. The activities of the two enzymes, SAT and CS, were assayed after growth in the absence or presence of cysteine. The units of enzyme activity are 100 = induced activity of the wild-type enzyme (in the absence of cysteine), 1 = low activity of the wild-type enzyme (in the presence of cysteine), and 0 = no measurable activity. In the diploid analysis, one copy of each operon is present in each cell.

Strain number	<i>cys</i> A B C D	SAT		CS	
		- Cys	+ Cys	- Cys	+ Cys
1	+ + + +	100	1	100	1
2	- + + +	0	0	100	1
3	+ - + +	1	1	1	1
4	+ + - +	1	1	1	1
5	+ + + -	100	1	0	0
Diploid	A B C D/A B C D				
6	- + + +/+ + + -	100	1	100	1
7	+ - + +/- + + +	1	1	101	2
8	+ + - +/- + + +	100	1	200	2
9	- - + +/+ + - -	100	1	1	1

4. Which genes encode the enzymes? Choose the option with the correct gene under each enzyme.

- | | | | |
|----------------|-------------|----------------|-------------|
| <u>SAT</u> | <u>CS</u> | <u>SAT</u> | <u>CS</u> |
| a. <i>cysA</i> | <i>cysB</i> | d. <i>cysB</i> | <i>cysC</i> |
| b. <i>cysC</i> | <i>cysD</i> | e. <i>cysA</i> | <i>cysD</i> |
| c. <i>cysA</i> | <i>cysC</i> | | |

e is correct

5. What does the analysis of haploid strains tell you about regulation of this operon?
- It is under positive regulation, since mutation of regulatory loci leads to an absence of induction in low Cys.
 - It is under negative regulation, since mutation of regulatory loci leads to constitutive high-level expression.
 - It is under positive regulation, since mutation of regulatory loci leads to constitutive high-level expression.
 - It is under negative regulation, since mutation of regulatory loci leads to an absence of induction in low Cys.

a is correct.

6. Which gene or locus shows *cis* dominance, i.e. the particular allele that is in *cis* to the reporter gene is dominant?

- cysA*
- cysB*
- cysC*
- cysD*

e. none of the genes

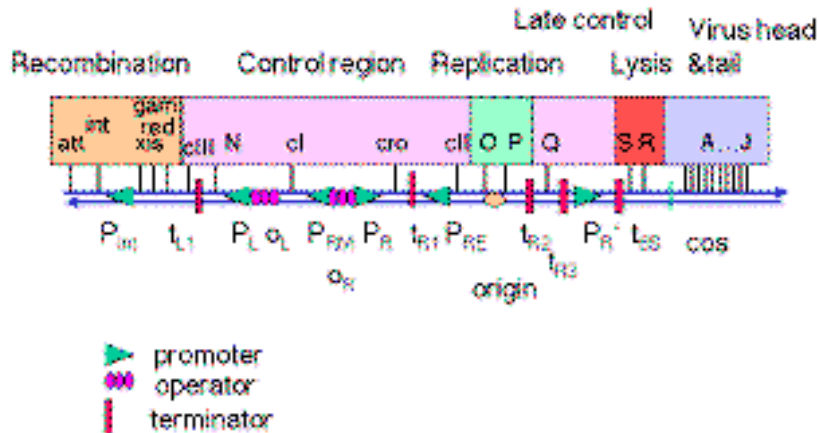
b is correct

7. Which statements about the regulation of the *cys* operon in this bacterium are supported by the data presented?

- The gene *cysD* encodes an activator that binds to the operator sequence *cysB* in the presence of cysteine but not in the absence of cysteine.
- The gene *cysB* encodes an activator that binds to the operator sequence *cysC* in the presence of cysteine but not in the absence of cysteine.
- The gene *cysC* encodes a repressor that binds to the operator sequence *cysB* in the presence of cysteine but dissociates in the absence of cysteine.
- The gene *cysC* encodes an activator that binds to the operator sequence *cysB* in the absence of cysteine but dissociates in the presence of cysteine.
- The gene *cysA* encodes an activator that binds to the operator sequence *cysD* in the absence of cysteine but dissociates in the presence of cysteine.

d is correct, 3 points for c. Option c accounts for higher level expression in the absence of cysteine, but the phenotype of *cysC*- under this model would be constitutive high level expression, which is not seen (i.e. this operon is under positive regulation).

The next 2 questions concern bacteriophage λ . A condensed map of the virus is shown below.



8. Which of the following would increase the chance of lysogeny occurring?

- Mutations that increase transcription from P_{RE} .
- Mutations that inactivate P_{int} .
- Mutations that eliminate the t_{OS} termination site.
- Mutations that decrease the affinity of the *cI* repressor for O_{RI} .

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

e is correct

9. Two mutant strains of bacteriophage λ , cI^- and cII^- , produce no lysogens, and thus they make clear plaques individually when they infect *E. coli*. If they are coinfecting into *E. coli* (i.e. cells are infected with both cI^- and cII^-), which of the following will result?

- a. No lysogens will be formed.

- b. Lysogens will be formed, with cI^- as the prophage.
- c. Lysogens will be formed, with cII^- as the prophage.
- d. Lysogens will be formed, some with cI^- as the prophage and some with cII^- as the prophage.

c is correct, 2 pts for d

10. Which of the following events occur when *E. coli* is starved for the amino acid tryptophan?

- [1] The ribosome translates the leader peptide completely (to the UGA stop codon).
- [2] A G+C rich stem-loop structure forms in the nascent RNA (regions 3 and 4) at the attenuator site.
- [3] A step-loop structure forms in the nascent RNA (regions 2 and 3) that precludes formation of the G+C rich stem-loop at the attenuator site.
- [4] Transcription reads through the attenuator into *trp* *EDCBA*.

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

d is correct, 2 pts for c.

11. Which of the following mutations in the leader of the *E. coli trp* operon will result in transcriptional termination at the attenuator, regardless of whether the concentration of tryptophan is high or low?

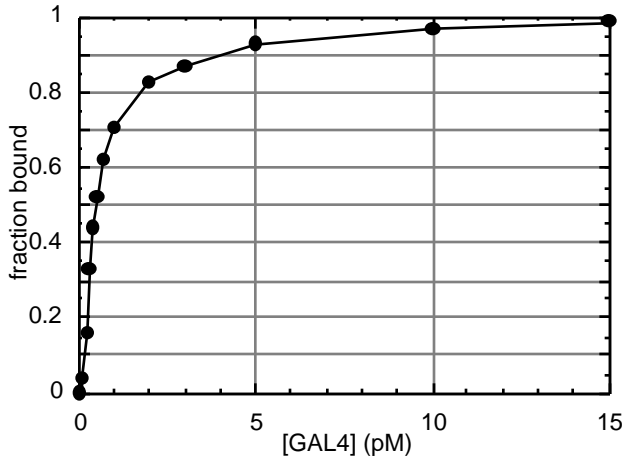
- [1] Mutations in region 1 that prevent it from binding to region 2.
- [2] Mutations in region 2 that prevent it from binding to region 3 but not region 1.
- [3] Mutations in region 3 that prevent it from binding to region 4, but not region 2.
- [4] Mutations in region 3 that prevent it from binding to either region 2 or region 4.

Correct choice(s) are:

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

a is correct.

For the next two questions, let's imagine that you mixed increasing amounts of GAL4 with a constant amount of a labeled duplex oligonucleotide containing the specific binding site for GAL4, called UAS_G, and measured the fraction of the labeled DNA bound to GAL4 as a function of [GAL4]. You obtained the following data. The X-axis is the concentration of GAL4 in pM (1 pM = 1 x 10⁻¹² M).



The equation that describes this curve is

$$\frac{[DP]}{[D]_{total}} = \frac{K_B[P]}{1 + K_B[P]}$$

where [DP] = the concentration of UAS_G DNA bound by the protein GAL4,
 [D]_{total} = the concentration of total DNA, i.e. [D]_{free} + [DP],
 [P] = the concentration of the protein GAL4, and
 K_B = the equilibrium constant for GAL4 binding to its specific site on DNA.

12. When half of the labeled DNA is bound to GAL4, what is the relationship between [P] and K_B?

- a. $[P] = \frac{K_B}{1 + K_B}$
- b. $[P] = \frac{[D]_{total}}{2K_B}$
- c. $[P] = K_B$
- d. $[P] = \frac{1}{K_B}$

d is correct

13. What is the measured value of K_B?

- a. 15 x 10⁻¹² M
 - b. 1.5 x 10⁻⁹ M
 - c. 2 x 10¹² M⁻¹
 - d. 2 x 10⁹ M⁻¹
- c is correct**

14. Imagine that you measured the amount of a purified protein, called FIN3, bound to a specific sequence in DNA, using increasing amounts of FIN3 with a constant amount of a labeled duplex oligonucleotide containing the binding site. The measured fraction of DNA bound by FIN3 as a function of [FIN3], was analyzed by nonlinear, least squares regression analysis at a wide range of possible values for ΔG for the binding reaction. The fits of the curve using $\Delta G = -8$ kcal/mole had the least error; other values, such as $\Delta G = -10$ kcal/mole had a higher error. What is the value of the equilibrium binding constant, K_B ?

a. 1×10^{-8} M

b. 4.79×10^3 M⁻¹

c. 7.74×10^5 M⁻¹

d. 2.3×10^7 M⁻¹

e. 1×10^{10} M⁻¹

c. is correct (7.74×10^5 M⁻¹; $\Delta G = -RT \ln K_B$)

15. Consider infection of a bacterium by a phage that can be negatively regulated by a repressor (R) binding with high affinity to an operator (O), as described by the equations below. The concentration of total DNA (nonspecific binding sites) in this bacterium is 7×10^{-3} M.



$$K_s = \frac{[RO]}{[R][O]} = 10^{11} \text{ M}^{-1} \quad (\text{eqn 2})$$

$$K_{ns} = \frac{[RD_{ns}]}{[R][D_{ns}]} = 10^5 \text{ M}^{-1} \quad (\text{eqn 3})$$

where

R = Repressor dimer

O = Operator site

D_{ns} = a nonspecific binding site in the genomic DNA

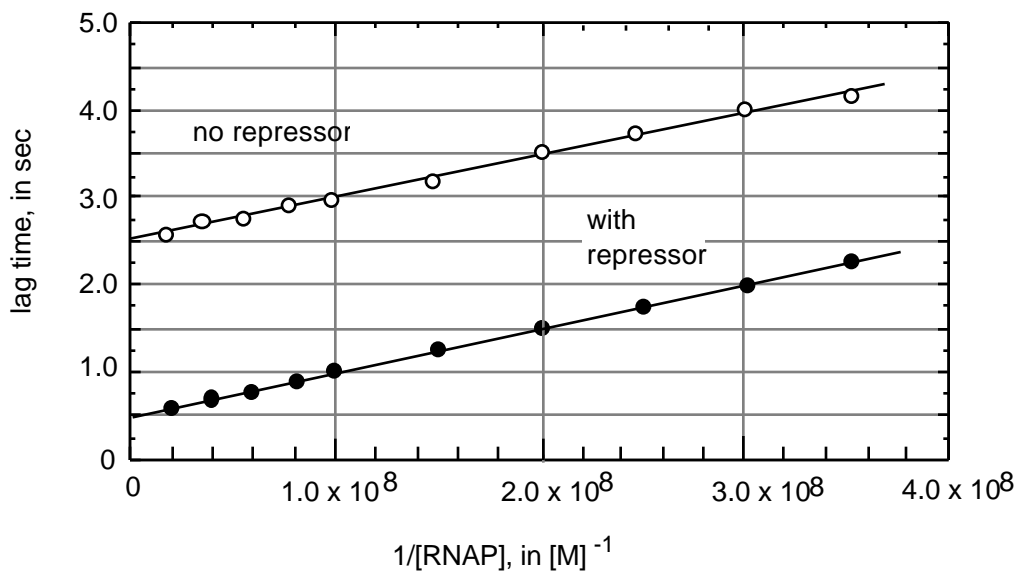
At the beginning of the infection, when there is only one phage DNA molecule in the cell, the molar concentration of the operator, i.e. [O], is 1.7×10^{-9} M. For this [O], how many molecules of the repressor are needed per cell to occupy 90% of the operator sites? This is equivalent to $\frac{[RO]}{[O]} = 9$. First calculate the [R] needed to achieve this, and then express this in molecules of repressor per cell. Avagadro measured about 6.02×10^{23} molecules per mole, and consider the volume of a bacterial cell to be 1×10^{-15} L.

a. 4 molecules per cell

b. 39 molecules per cell

- c. 100 molecules per cell
 - d. 4000 molecules per cell
 - e. Cannot be calculated from the information given.
- b is correct**

For the next two problems, consider a hypothetical eubacterial operon in which the operator extends from -5 to $+15$, where $+1$ is the start site for transcription. Measurement of the lag time before production of abortive transcripts as a function of the inverse of the RNA polymerase concentration ($1/[\text{RNAP}]$) gave the results shown below. The **open** circles are the results of the assay in the **absence** of repressor, and the **closed** circles are the results in the **presence** of repressor bound to the operator.



16. What is the value of the forward rate constant (k_f) for closed to open complex formation under the two different conditions?

- | <u>k_f, no repressor</u> | <u>k_f with repressor</u> |
|---------------------------------------|--|
| a. 2.0 per sec | 0.4 per sec |
| b. 2.5 per sec | 0.5 per sec |
| c. 2.0 per sec | 2.0 per sec |
| d. 0.5 per sec | 0.4 per sec |
| e. 0.4 per sec | 2.0 per sec |

e is correct, 0.4 per sec without repressor, 2 per sec with repressor; the y-intercept is $1/k_f$

17. From these data, how does the equilibrium constant (K_B) for binding of the RNA polymerase to the promoter change in the presence or absence of repressor?

- a. K_B is decreased 50-fold in the presence of repressor.
- b. K_B is decreased 5-fold in the presence of repressor.
- c. K_B is decreased 2-fold in the presence of repressor.
- d. K_B is the same in the absence and presence of repressor.
- e. K_B is increased 10-fold in the presence of repressor.

b is correct.

18. Which statement(s) about acetylation of histone N-terminal tails are true?

- [1] Specific serines are acetylated in the histone N-terminal tails.
- [2] Acetylation of the histone N-terminal tails is associated with an open chromatin structure.
- [3] The enzymes that carry out histone acetylation on nucleosomes are single polypeptides.
- [4] The acetyl groups can be removed by histone deacetylases.

a. 1 b. 1, 2 c. 2, 4 d. 1, 2, 3, 4 e. 2

c is correct, 3 pts for e.

19. What evidence supports a role of histone acetyl transferases in gene activation?

- [1] Increasing histone acetylation can turn on some genes.
- [2] Immunoprecipitation of DNA cross-linked to chromatin with antibodies against acetylated histones enriches for actively transcribed genes.
- [3] The protein Gcn5p is a histone acetyl transferase involved in regulation of many genes in yeast.
- [4] The proteins P300 and CBP are histone acetyl transferases that interact with many transcription factors and are needed for activation by these factors.

a. 1 b. 1, 2 c. 1, 3, 4 d. 1, 2, 3, 4 e. 2, 3

d is correct, 4 for c, 3 for b or e, 2 for a.

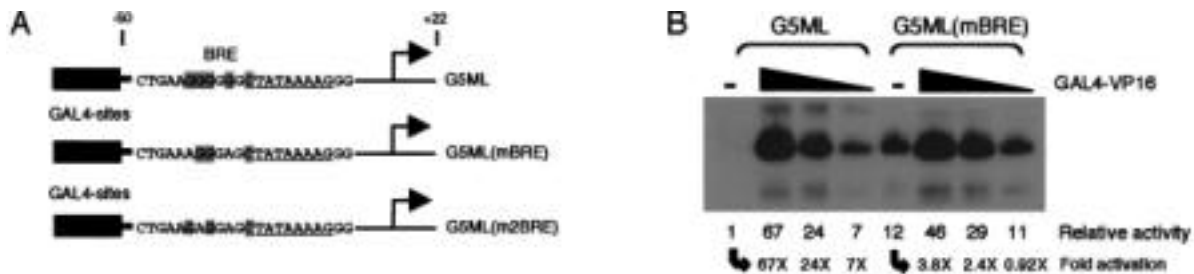
20. Which statements about the SWI/SNF complex and its reactions are true?

- [1] It remodels nucleosomes by phosphorylating them.
- [2] It uses ATP hydrolysis to remodel nucleosomes.
- [3] The remodeled nucleosomes have lost some of their histones.
- [4] The products of the reaction are remodeled dimers of nucleosomes.
- [5] The association of DNA with the histones is altered after the SWI/SNF reaction.

a. 2, 5 b. 1, 2 c. 2 d. 1, 4, 5 e. 2, 4, 5

e is correct, 2 pts for c, 3 pts for a; 2, 4, 5 are correct.

For the next 4 problems, consider the following. A recent paper (Evans, Fairley and Roberts, 2001, *Genes & Development* 15:2945-2949) reveals a different role for the general transcription initiation factor, TFIIB. At some promoters, including the major late promoter for adenovirus, TFIIB will bind to a specific site called a BRE (for TFIIB response element) located just 5' to the TATA box. In this paper, the authors examined the effect of mutating the BRE in a hybrid promoter (called G5ML), in which GAL4-binding sites (called UAS_G sites in our text but just GAL4-sites in this paper) are present upstream of the adenovirus late promoter. (See panel A in the figure below; the start site for transcription is marked by the arrow.) Constructs with a mutated BRE are called G5ML(mBRE) and G5ML(m2BRE). The amount of *in vitro* transcription from the G5ML and G5ML(mBRE) promoters was measured using a crude HeLa cell nuclear extract, which contains RNA polymerase and all the general transcription initiation factors (but no GAL4). These results are shown in panel B in the lanes labeled -. The effect of adding increasing amounts of the artificial transcriptional activator GAL4-VP16, which binds to the GAL4-sites, was also examined, as shown in the lanes under the black triangle (the higher the triangle, the more GAL4-VP16). The results of the assay of transcripts by primer extension are shown in the gel in panel B below. The promoter activity relative to that of the basal adenovirus major late promoter is reported as "Relative activity" and the increase in activity in the presence of GAL4-VP16 is reported as "Fold activation." Similar results were obtained for G5ML(m2BRE) as for G5ML(mBRE).



21. What is the effect of TFIIB binding to the BRE with respect to activity in the absence of the GAL4-VP16 activator?

- a. TFIIB binding to the BRE increases the basal level of transcription.
- b. TFIIB binding to the BRE decreases the basal level of transcription
- c. TFIIB binding to the BRE has no effect on the basal level of transcription.
- d. TFIIB binding to the BRE has variable effects on the basal level of transcription.

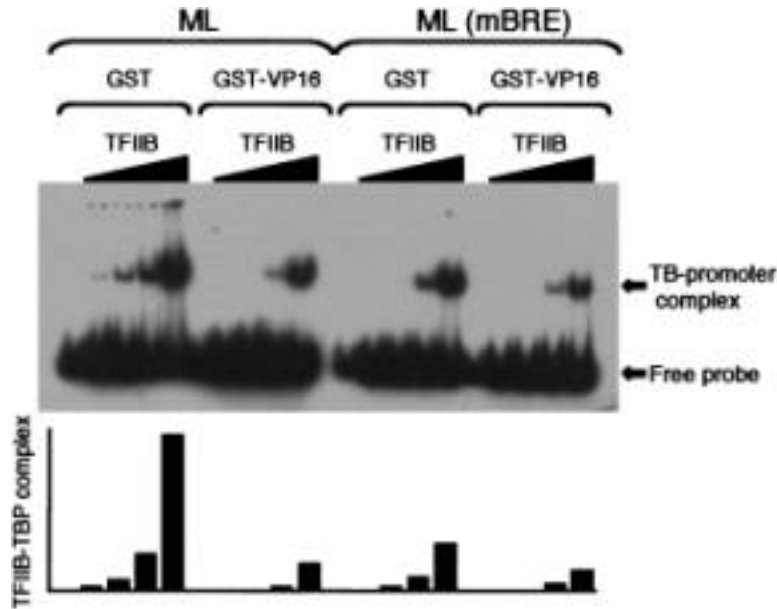
b. is correct.

22. What is the effect of TFIIB binding to the BRE with respect to activity in the presence of the GAL4-VP16 activator?

- a. TFIIB binding to the BRE increases the activation of transcription.
- b. TFIIB binding to the BRE decreases the activation of transcription.
- c. TFIIB binding to the BRE has no effect on the activation of transcription.
- d. TFIIB binding to the BRE decreases the activation of transcription at low concentrations of GAL4-VP16 and increases the activation of transcription at high concentrations of GAL4-VP16.

a. is correct.

23. The ability of the VP16 activation domain to affect the TFIIB-BRE interaction was examined by electrophoretic mobility shift assays of proteins bound to a radiolabeled major late promoter fragment containing a TATA box and a BRE. These are basically the DNA fragments shown in the figure for the preceding two problems but with no GAL4-sites; both wild-type ML and the major late promoter with mutated BRE, called ML(mBRE) were examined. The labeled DNA was incubated with TBP without and with increasing amounts of TFIIB (shown as triangles of increasing thickness). VP16 was added as a hybrid between a convenient affinity tag (GST) and the VP16, called GST-VP16. GST alone was added for the lanes without VP16. The band representing a complex of the promoter, TBP, and TFIIB is labeled TB-promoter complex. The amount of this complex is plotted underneath the gel image.



Which of the following can be concluded from these data?

- [1] On the major late promoter (ML), VP16 reduces the amount of the TFIIB-TBP-promoter complex.
- [2] On the major late promoter (ML), VP16 increases the amount of the TFIIB-TBP-promoter complex.
- [3] Mutation of the BRE in the major late promoter (ML(mBRE)) reduces the amount of TFIIB-TBP-promoter complex formed.
- [4] VP16 causes a small reduction in the amount of the TFIIB-TBP-promoter complex formed on the major late promoter mutated in the BRE (ML(mBRE)).

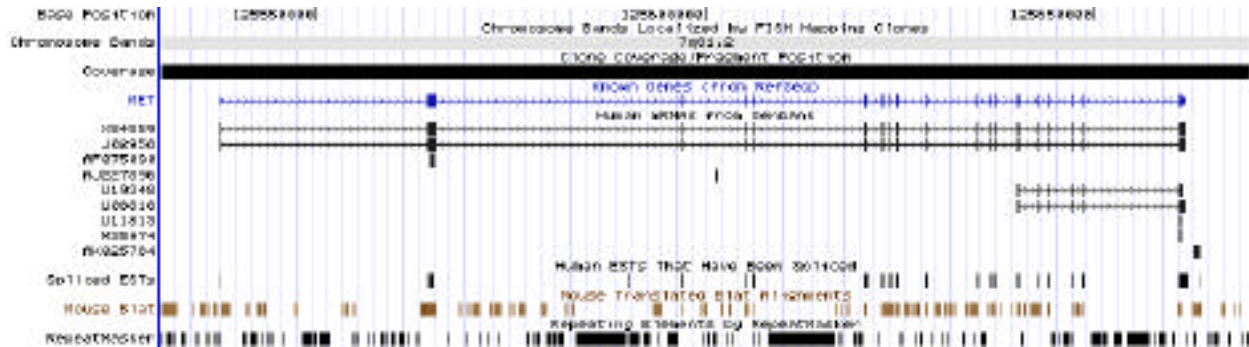
- a. 1 b. 2 c. 1, 3, 4 d. 1, 3 e. 2, 3
c. is correct, 2 pt for a, 3 pt for d, 1, 3, 4 are correct

24. Which conclusion can be drawn from the data in the previous 3 problems?

- a. TFIIB bound to the BRE increases transcription, and synergistically activates transcription in the presence of VP16.
- b. TFIIB bound to the BRE represses basal transcription and blocks activation by VP16.
- c. TFIIB activates transcription by binding to the BRE.
- d. TFIIB competes with TBP for binding to the TATA box.
- e. TFIIB bound to the BRE represses basal transcription, and disruption of the TFIIB-BRE interaction by VP16 results in a strong activation of transcription.

e. is correct.

Much useful information about gene and chromosome structure is accessible via Internet browsers and servers that serve as graphical interfaces to large databases. One that we have used is the Human Genome Browser. A view of the human proto-oncogene *MET*, covering a little over 125 kb of human chromosome 7, is shown below. Use it for the next 3 questions. The DNA strand that is oriented 5' to 3' from left to right, i.e. with increasing numbers on the base position track, is referred to as the + strand on this browser. This is also the nontemplate, or mRNA-synonymous, strand of the *MET* gene. The "Mouse Blat" track shows regions of human that align with mouse genomic DNA sequences, using a rapid alignment program called Blat.



25. How many exons are in *MET*? Use the Known Genes track or the complete mRNAs, which are X54559 and J02958.

- a. 1 b. 4 c. 12 d. 19

d is correct. 19 exons

26. Which of the following statements fit the data for *MET*?

- [1] Introns tend to be larger than exons.
- [2] Exons of human genes tend to be conserved in mouse.
- [3] Only exons are conserved between human and mouse.
- [4] Exons tend to have repeating elements in them.

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

b is correct, 2 pts for a. 1, 2 are correct

27. When you click on the line for U19348, you find that this mRNA is 2016 nucleotides long, and positions 680 to 2016 match with the region of *MET* shown in the figure. Positions 1 to 683 of U19348 match with 100% identity to exons of a gene called *TPR*, located on a different chromosome. The U19348 mRNA was isolated from a cell line from a patient with a chromosomal translocation involving *MET* (i.e. chromosome 7 was broken in *MET* and joined to a different chromosome at *TPR*). What do you conclude about the origin of mRNA U19348?

- a. It is transcribed from a *TPR-MET* fusion gene.
- b. It is generated by initiating transcription from a cryptic promoter in intron 13 of *MET*.
- c. It is generated by alternative splicing of transcripts of the *MET* gene.
- d. It is generated by *trans*-splicing between *TPR* and *MET*.
- e. The data do not lead to a conclusion.

a is correct, 3 pts for d.

e. The data do not lead to a consistent model.

d is correct.

Use the following list of enzymes or enzyme complexes to answer the next 5 questions.

- [1] UvrABC from *E. coli*
- [2] MutHLS from *E. coli*
- [3] DnaG from *E. coli*
- [4] alpha subunit of DNA polymerase I from *E. coli*
- [5] CPSF, CFI and CFII from mammals
- [6] U2 snRNP from mammals

30. Which of these is needed for cleavage and polyadenylation of mRNA?

- a. 1 b. 1, 3 c. 6 d. 2, 5 e. 5

e is correct, [5] CPSF, CFI and CFII from mammals

31. Which of these is needed for making a primer during DNA replication?

- a. 3 b. 3, 4 c. 1 d. 2, 5 e. 6

a is correct, [3] DnaG from *E. coli*

32. Which of these is needed for mismatch repair?

- a. 3 b. 1 c. 2 d. 5 e. 6

c is correct, [2] MutHLS from *E. coli*

33. Which of these is needed for splicing of mRNA?

- a. 1 b. 3 c. 2 d. 6 e. 5

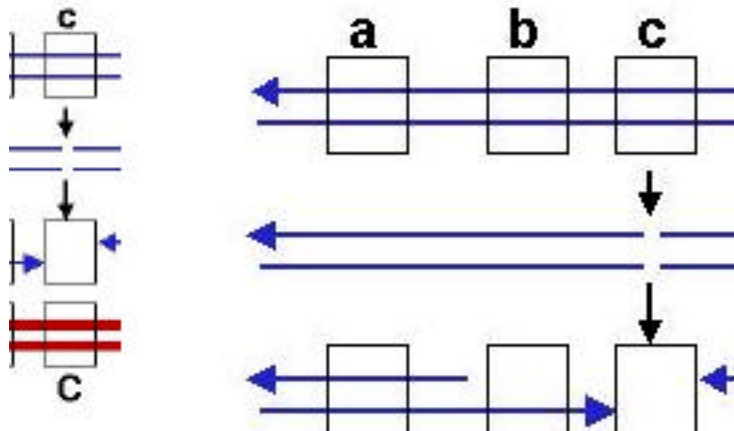
d is correct, [6] U2 snRNP from mammals

34. Which of these catalyze the breaking of phosphodiester bonds in DNA or RNA as part of their mechanism?

- a. 1, 2, 3, 4, 5, 6 b. 1, 2, 5, 6 c. 1, 2, 5 d. 3, 4 e. 4

b is correct, [1, 2, 5, 6], 4 pts for c

35. Recombination between two DNA duplexes by the double-strand break mechanism is initiated as shown below. The parental duplex indicated by thin black lines has recessive alleles for genes A, B, C, D, and E, denoted by the lower case letters. The other parental duplex shown as thick gray lines has dominant alleles, indicated by the upper case letters. The genes are indicated by boxes. The position of the initial double-strand DNA break (in gene C) and the extent of exonuclease cleavage during initiation of recombination are shown.



After strand invasion, repair synthesis and formation of Holliday junctions, what alleles are present for gene C in the intermediate prior to resolution? Assume no branch migration occurred, so that the Holliday junctions are at the points where exonucleolytic activity ended.

- Allele c on one duplex and allele C in the other duplex.
- Allele c on both duplexes.
- Allele C on both duplexes.
- A heteroduplex of c/C on both duplexes.
- A heteroduplex of c/C on one duplex and allele C on the other duplex.

option c is correct.

36. A codon for leucine can be converted to codons for either serine (Ser), valine (Val) or methionine (Met) by a single nucleotide substitution (a different nucleotide substitution for each amino acid replacement). What is the codon for leucine?

- CUG
- CUU
- UUA
- UUG
- CUC

d. is correct

37. Which of the following statements about the translation initiation in *E. coli* is **INCORRECT**?

- The small and large ribosomal subunits join together before mRNA is bound.
- Formylmethionyl-tRNA^{Met} is brought to the small ribosomal subunit in a complex with an initiation factor in a reaction requiring GTP.
- Formylmethionyl-tRNA^{Met} binds to the partial P site on the small ribosomal subunit.
- GTP hydrolysis is needed to remove the initiation factors from the ribosomal subunit.

a. is the correct choice; all the other statements are correct.

38. Which of the following statements about the subunits of *E. coli* RNA polymerase is **INCORRECT**?

- a. Dimerization of the α subunits is the initial step in assembly of the polymerase.
- b. The β subunit confers specificity for a promoter.
- c. The β' subunit blocks abortive initiation of transcription.
- d. The α and β subunits form the catalytic center.

c. is the correct choice; other statements are correct.

39. Iyer et al. (Science 1999, vol. 283:83-87, "The transcriptional program in the response of human fibroblasts to serum") investigated changes in the level of expression of genes in human fibroblasts after they were switched from a non-growing, quiescent state to a state of growth by addition of serum to the culture medium. This treatment releases cells from a block to growth, and they progress synchronously through the cell cycle. DNA complementary to the mRNA from the non-growing cells was labeled with a green fluorescent dye and that from the growing cells was labeled with a red fluorescent dye. About 10,000 genes and ESTs were examined; data for two follow. The normalized ratio of green to red (G/R) and red to green (R/G) fluorescent signals are given in the table. The last column gives the ratios observed for unsynchronized cells growing in culture.

		Hours after addition of serum									
Gene/EST	Signal	0	0.25	0.5	1	2	4	8	16	24	Unsyn
<i>C-FOS</i>	G/R	1.00	0.37	0.12	0.08	0.67	0.63	0.81	0.88	1.13	0.93
	R/G	1.00	2.67	8.14	12.69	1.50	1.58	1.23	1.14	0.89	1.08
EST T51150	G/R	1.00	1.68	13.29	2.25	1.37	1.22	1.64	2.00	1.35	1.87
	R/G	1.00	0.59	0.08	0.44	0.73	0.82	0.61	0.50	0.74	0.54

Expression of which gene and/or EST increases after addition of serum?

- a. *C-FOS* and EST T51150
- b. *C-FOS*
- c. EST T51150
- d. Neither increases
- e. The data do not address the question.

b. *C-FOS* is correct

40. Which of the following statements is **INCORRECT** about Watson-Crick base pairing in DNA?

- a. A purine nucleotide is paired with a pyrimidine nucleotide.
- b. A keto-base nucleotide is paired with an amino-base nucleotide.
- c. A T nucleotide is paired with a C nucleotide.
- d. Three hydrogen bonds connect a G nucleotide with a C nucleotide.

c is the correct option, other statements are correct.

The Genetic Code
Position in Codon

1st	2nd								3rd
	U		C		A		G		
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys	C
	UUA	Leu	UCA	Ser	UAA	Term	UGA	Term	A
	UUG	Leu	UCG	Ser	UAG	Term	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg	C
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg	A
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg	G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser	C
	AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg	A
	AUG*	Met	ACG	Thr	AAG	Lys	AGG	Arg	G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly	C
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly	A
	GUG*	Val	GCG	Ala	GAG	Glu	GGG	Gly	G

* Sometimes used as initiator codons.

Binding equilibria

The equilibrium constant for the binding of a protein (P) to a DNA fragment (D) is K_B .

$$K_B = \frac{[DP]}{[D][P]}$$

The fraction of DNA in a complex with the protein is a function of K_B and $[P]$:

$$\frac{[DP]}{[D]_{total}} = \frac{K_B [P]}{1 + K_B [P]}$$

The specificity of binding of a protein (P) to DNA is described by the following equation. K_S is the equilibrium binding constant to specific DNA sites, or D_S . K_{NS} is the equilibrium binding constant to nonspecific DNA sites, or D_{NS} . Concentrations are moles/L, i.e. molar.

$$\text{specificity} = \frac{K_S}{K_{NS}} = \frac{[PD_S]}{[D_S]} \times \frac{[D_{NS}]}{[P]_{total} - [D_S]_{total}}$$

Relationship between ΔG and K_{eq} , where K_{eq} is an equilibrium constant:

$$\Delta G = -RT \ln K_{eq}$$

$$R = 1.98 \times 10^{-3} \text{ kcal deg}^{-1} \text{ mol}^{-1}$$

$$T = 298^\circ \text{ K}$$

$$RT = 0.59 \text{ kcal/mol}$$

In an abortive transcription assay, the lag time between the mixing of reagents and the optimal rate of abortive transcript production is related to the concentration of RNA polymerase (or [RNAP]) by the following equation. K_B is the equilibrium constant for binding of RNAP to the promoter, and k_f is the forward rate constant for the closed to open transition.

$$\text{lag time} = \frac{1}{K_B k_f} \times \frac{1}{[RNAP]} + \frac{1}{k_f}$$

Supercoiling:

T = Twisting Number

For B form DNA, it is + (# bp/10 bp per twist)

For A form DNA, it is + (# bp/11 bp per twist)

For Z form DNA, it is - (# bp/12 bp per twist)

$$\Delta L = \Delta W + \Delta T$$

Superhelical density = $\sigma = W/T = -0.05$ for natural bacterial DNA