Biochemistry/ MCB 400 Instructor: Hardison

Final Examination

Fall 1994

December 16, 1994

This examination has 39 questions for a total of 200 points. The first 37 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 two questions require you to write answers on the exam. We will grade the last series of questions and encode those values on the answer sheet, which will then be machine graded.

PLEASE TURN IN YOUR WHOLE EXAM AND ANSWER SHEET. Put your name and student number below. The exams can be picked up outside 206 Althouse Lab after they are graded. Good luck!

Name: ____

Student Number: ________________________________

<u>List of Restriction Endonucleases and their Cleavage Sites:</u> A ' means that the nuclease cuts between these 2 nucleotides to generate a 3' hydroxyl and a 5' phosphate.

Enzyme	Site	Enzyme	Site
*Alu*I	AG'CT	*Not*I	GC'GGCCGC
*Bam*HI	G'GATCC	*Pst*I	CTGCA'G
*Bgl*II	A'GATCT	*Pvu*II	CAG'CTG
*Eco*RI	G'AATTC	*Sal*I	G'TCGAC
*Hae*III	GG'CC	*Sau*3AI	'GATC
*Hha*I	GCG'C	*Sma*I	CCC'GGG
*Hinc*II	GTY'RAC	*Spe*I	A'CTAGT
*Hind*III	A'AGCTT	*Taq*I	T'CGA
*Hinf*I	G'ANTC	*Xba*I	T'CTAGA
*Hpa*II	C'CGG	*Xho*I	C'TCGAG
*Kpn*I	GGTAC'C	*Xma*I	C'CCGGG
*Mbo*I	'GATC		

N = A,G,C or T S = G or C
R = A or G W = A or T
Y = C or T

The Genetic Code

1st	Position in Codon: 2nd = U		2nd = C		2nd = A		2nd = G		3rd
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.

<u>Binding sites for transcription factors</u>

Transcription factor	DNA binding site	Transcription factor	DNA binding site
Sp1	GGGCGG	NF B	GGAAAGTCCCC
GATA1	WGATAR	CREB	TGATGTCA
AP1	TGASTCA	POU	GGAA
Oct1	ATTTGCAT	MyoD	CAGCTG
CBP	CCAAT	Myc	CACGTG
CACBP	CACCC	TBP	TATAAAA

1. Of the following genes or loci in bacteriophage , which can give rise to mutants that can be suppressed by amber suppressors?

a. *cI*, o_R, *cro*
b. *cII*, o_R, *cro*
c. *cI*, *S, cro*
d. *cI*, o_R, *N*

2. The mutants cI^- and cII^- produce no lysogens, so they make clear plaques. If they are coinfected 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.

3. 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 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.

4. The CAP protein activates transcription from the *lac* promoter by

a. catalyzing the formation of the inducer allolactose.
b. catalyzing the formation of cAMP when the intracellular [glucose] is low.
c. forming a complex with cAMP, binding to DNA, bending the DNA and interacting positively with RNA polymerase.
d. forming a complex with cAMP and binding to RNA polymerase to facilitate formation of an open complex at the promoter.

5. Which of the following events listed as (1) through (4) occur in order for transcription to terminate prematurely at the *trp* attenuator?

> (1) The leader peptide (*trpL*) is translated completely.
> (2) Translation of the leader peptide (*trpL*) stalls at trp codons.
> (3) A duplex forms between regions 2 and 3 in the RNA.
> (4) A duplex forms between regions 3 and 4 in the RNA.

a. (1) and (4)
b. (1) and (3)
c. (2) and (4)
d. (2) and (3)

6. Delayed early transcription of the *int* gene from the p_L promoter of   extends past the terminator for *int*, into a region that leads to degradation of the RNA from the 3' end, leading to a loss of *int* RNA. Why does this not happen with transcription from p_{int}?

a. The *cIII* gene product enhances termination at the terminator for *int*.
b. The *cIII* gene product blocks the action of the host RNases.
c. No *nut* site is downstream of p_{int}, so the   N protein does not antiterminate.
d. The   Q and N proteins antagonize for function at p_{int},.

7. Which of the following describes the phenomenon of zygotic induction in *E. coli*? Induction in this context means the production of   plaques.

a. Infection of an Hfr strain with   will lead to production of   plaques.
b. Conjugation between male and female   lysogens leads to induction.
c. Mating between an Hfr strain and a female   lysogen leads to induction.
d. Mating between an Hfr strain that is a   lysogen and a non-lysogenic female leads to induction.

8. Which of the following have been recognized as targets for transcriptional activators?

a. TBP, TAFII 40, TAFII 110 and TFIIB
b. TBP, TAFII 40, TFIIF and TFIIB
c. TFIIE, TFIIF, TFIIH and TFIIB
d. TFIIA, TAFII 40, TAFII 110 and TFIIB

The following six phrases refer to particular protein domains commonly found in transcription factors; these domains are involved in DNA binding or dimerization. Match these statements with the domains listed in questions 9 through 11. Choose the response with the most correct choices.

(1) Has two amphipathic helices separated by a loop of variable length.
(2) Half the structure is 2 sheets, and the other half is an helix.
(3) Has three -helices, two of which are perpendicular to the third.
(4) Two thiol side chains and two imidazole nitrogens from a tetrahedral coordination complex with a Zn^{++} ion.
(5) Has one -helix interacting with the edges of base pairs in the major groove.
(6) Has a long helix with a leucine (or sometimes valine) every 2 turns.

9. Helix-loop-helix proteins.

a. (1) and (6)
b. (3)
c. (2) and (4)
d. (1)

10. Homeodomain.

a. (3) and (6)
b. (3) and (5)
c. (2) and (4)
d. (2) and (5)

11. Cys_2His_2 Zn finger

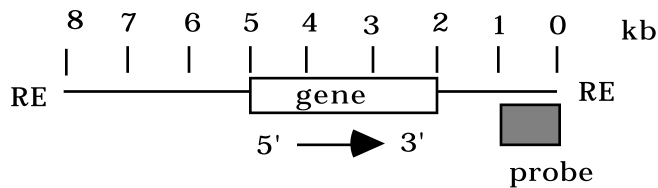
a. (3) and (4)
b. (1) and (5)
c. (2) and (4)
d. (2) and (6)

12. Flies carrying a chromosomal inversion that places the wild-type w^+ gene close to the centromeric region have mostly white eyes (showing loss of function of w^+) but with patches of wild-type red color. This results from

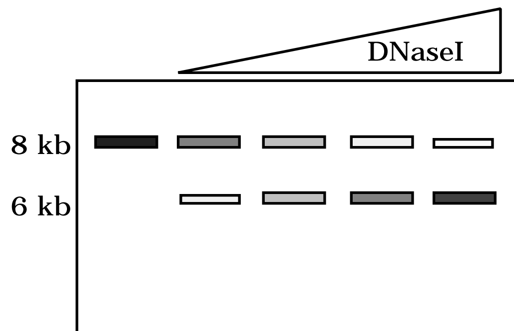
a. clonal dispersion of an activator of the w^+ gene.
b. overexpression of a repressor of the w^+ gene.
c. position effect variegation, due to clonal differences in the extent of heterochromatin from the centromere.
d. transposition of the w^+ gene in different clones of eye cells.

Use the following information to answer questions 13 and 14.

DNase hypersensitive sites around a gene were mapped by treating nuclei from cells that express that gene with increasing amounts of DNaseI. The partially digested DNA was isolated, cut to completion with a restriction enzyme, and analyzed by Southern blot-hybridization using a radioactive probe that is located 3' to the gene. Cleavage of genomic DNA with the restriction enzyme generates an 8 kb fragment that contains the gene, and the probe for the blot hybridization is located at the right end of the fragment (left to right defined as the direction of transcription of the gene). The results of this indirect end-labeling assay shows a gradual fade-out of the 8 kb fragment with increasing [DNaseI], and the appearance of a new band at 6 kb with DNaseI treatment.



Result of the indirect end-label assay:



13. Where is the DNase I hypersensitive site?

a. 8 kb from the right end of the restriction fragment.
b. 6 kb from the right end of the restriction fragment.
c. 5 kb from the right end of the restriction fragment.
d. 2 kb from the right end of the restriction fragment.

14. If the start site for transcription is 5 kb from the right end of the restriction fragment, which of the following are likely possibilities for the function of the region mapped by the DNase hypersensitive site?

a. An enhancer located 1 kb 5' to the start site of transcription.
b. A promoter adjacent to the start site of transcription.
c. A locus control region located 6 kb 5' to the start site of transcription.
b. An enhancer located 1 kb 3' to the gene.

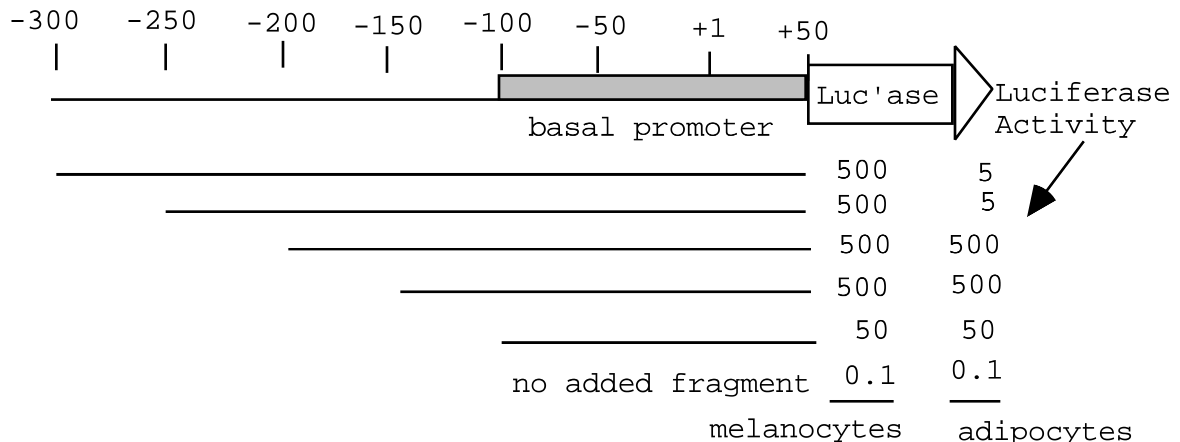
15. A switch in mating type from **a** to in homothallic strains of *Saccharomyces cerevisiae* requires the HO endonuclease to cleave between the Y and Z regions at which locus?

a. *HML*α
b. *HMR***a**
c. *MAT*α
d. *MAT***a**

Use the following information for questions 16 to 22.

The *agouti* gene in mice controls the amount and distribution of pigments within coat hairs. Some mutations of this gene also lead to adult-onset obesity, a mild diabetes-like syndrome, tumor susceptibility and recessive embryonic lethality. The gene encodes a predicted protein of 131 amino acids that has the structural features of a secreted protein, but no striking homology to other known proteins has been recognized. This protein is likely to be a regulator of melanin pigment synthesis, and it may also be a more general metabolic regulator.

Let's suppose that you are investigating the regulation of the *agouti* gene, and have the capacity to transfect a melanocyte cell line, which transcribes the wild-type *agouti* gene, and an adipocyte cell line, which transcribes the wild-type *agouti* gene only at a very low level. Further, you already know that the basal promoter is in a DNA segment located between -100 and +50. You make progressive 5' deletions of a fragment that includes -300 to +50, link it to a luciferase reporter gene, and transfect the constructs into melanocyte and adipocyte cells, with the following results.



16. What do you conclude about the region between -250 and -200?

a. It has no effect on transcription in either cell line.
b. It has a positive effect on transcription in melanocytes.
c. It has a negative effect on transcription in melanocytes.
d. It has a positive effect on transcription in adipocytes.
e. It has a negative effect on transcription in adipocytes.

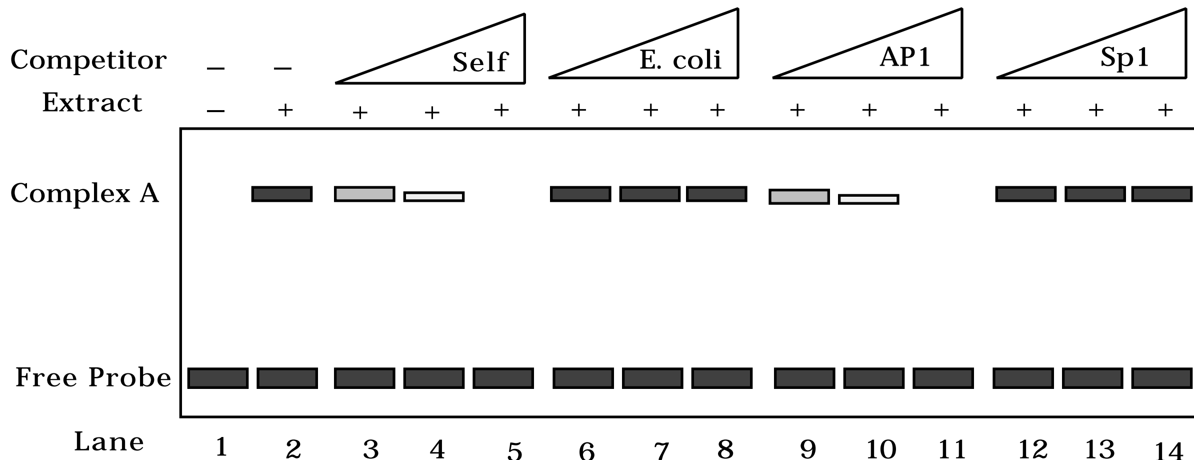
17. What do you conclude about the region between -200 and -150?

a. It has no effect on transcription in either cell line.
b. It has a positive effect on transcription in melanocytes.
c. It has a negative effect on transcription in melanocytes.
d. It has a positive effect on transcription in adipocytes.
e. It has a negative effect on transcription in adipocytes.

18. What do you conclude about the region between -150 and -100?
a. It has no effect on transcription in either cell line.
b. It has a positive effect on transcription in both cell lines.
c. It has a negative effect on transcription in both cell lines.
d. It has a positive effect on transcription only in adipocytes.
e. It has a negative effect on transcription only in melanocytes.

You also investigate the binding of nuclear proteins to these DNA segments located upstream of the *agouti* gene. Extracts containing nuclear proteins from melanocytes were tested for the ability to bind to the fragments delineated in the deletion series above.

The fragment from -150 to -100 was used as the labeled probe in a mobility shift assay. The mobility of the free probe is shown in lane 1, and the pattern after binding to melanocyte nuclear extract is shown in lane 2. Lanes 3-14 show the mobility shifts after addition of the competitors to the binding reaction; the triangle above the lanes indicates that an increasing amount of competitor is used in successive lanes. "Self" is the same -150 to -100 fragment that is used as a probe, but it is unlabeled and present in an excess over the labeled probe (lanes 3-5). A completely different DNA (sheared E. coli DNA) was used as a nonspecific competitor (lanes 6-8). Two different duplex oligonucleotides, one containing the binding site for AP1 (lanes 9-11) and the other containing the binding site for Sp1 (lanes 12-14) were also tested. Thinner, less densely filled boxes denote bands of less intensity than the darker, thicker bands. Use these results to answer questions 19 and 20.



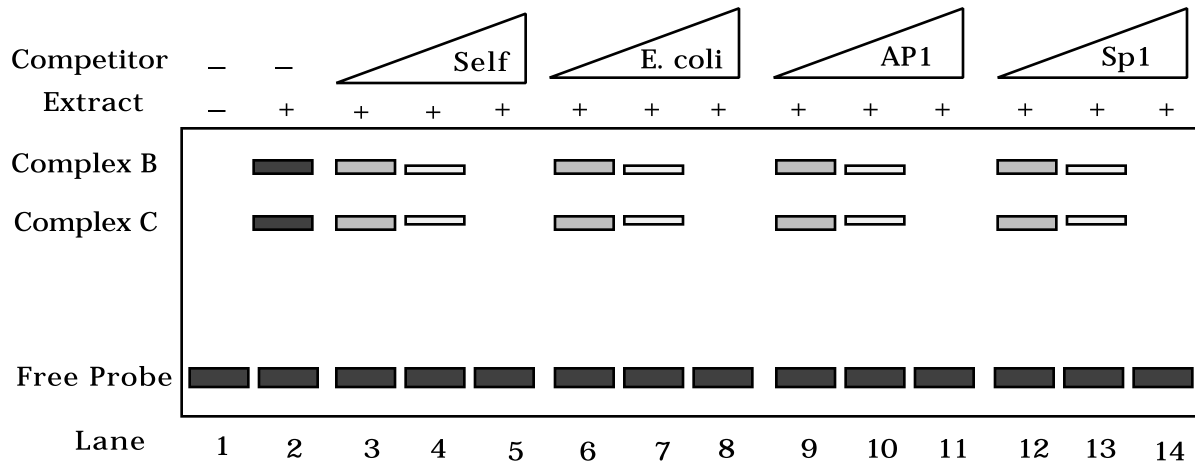
19. What do you conclude from these data?

a. Some nuclear protein from melanocytes binds specifically to the -150 to -100 segment.
b. The binding to the -150 to -100 segment is not specific for a particular sequence.
c. An E. coli protein binds to the -150 to -100 segment.
d. The protein Sp1 binds to the -150 to -100 segment.

20. What sequence within the -150 to -100 segment might you expect to be bound in melanocyte nuclei? Use the table on page 2 of the test.

a. GGGGCGGGG
b. TGATAG
c. ATTTGCAT
d. TGAGTCA

21. The fragment from -200 to -150 was also used as a labeled probe in a mobility shift assay similar to that described for the -150 to -100 segment, as shown below.



What do you conclude from these data?

a. Some nuclear protein from melanocytes binds specifically to the -200 to -150 segment.
b. The binding to the -200 to -150 segment is not specific for a particular sequence.
c. An E. coli protein binds specifically to the -200 to -150 segment.
d. The protein Sp1 binds specifically to the -200 to -150 segment.

22. Some mutant alleles of the *agouti* gene are expressed ectopically (i.e. in the wrong tissue). Just using the information on the 5' deletions above, what region is a likely candidate for the position of a loss-of-function mutation that leads to ectopic expression in adipose tissue?

a. -300 to -250
b. -250 to -200
c. -200 to -150
d. -150 to -100
e. -100 to -50

For questions 23 - 25, use the "universal" genetic code on page 2 of the exam.

23. If translation begins at the first three nucleotides in the following RNA sequence, what oligopeptide would be made using it as the mRNA?

5' AUGGUUGCUUCACGAAUC 3'

a. Met-Trp-Gly-Val-Cys-Phe
b. Leu-Ser-Thr-Ser-Leu-Val
c. Met-Val-Ala-Ser-Arg-Ile
d. Ile-Arg-Ser-Thr-Met-Val

24. Which of the following amino acid replacements could occur as a result of a single-base mutation within one codon?

a. Phe -> Lys
b. His -> Glu
c. Lys -> Ala
d. Ala -> Asp

25. A codon for Leu can be converted by a single-base mutation into either Ser, Val, or Met, using a different nucleotide substitution for each amino acid replacement. What is this codon for Leu?

a. UUG
b. CUU
c. CUC
d. CUG
e. UUA

26. Which of the following is required for translocation after peptide bond formation on the bacterial ribosome during elongation?

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

27. The purified single copy component from genomic DNA of a salamander was denatured and allowed to renature. The kinetics of renaturation showed a single homogeneous component with a $Cot_{1/2}$ of 50,000. A bacterial DNA standard, whose size is 4×10^6 bp, renatures with a $Cot_{1/2}$ of 10 under identical conditions. What is the kinetic complexity of the salamander single copy DNA?

a. 5×10^4 bp
b. 4×10^8 bp
c. 2×10^9 bp
d. 2×10^{10} bp
e. 2×10^{11} bp

Match the following phrases with the RNA splicing event listed in questions 28-30. Pick the choice with the most correct matches.

(1) Uses ATP to form a large spliceosome.
(2) Uses a phosphoester transfer mechanism.
(3) Introns begin with GU and end with AG.
(4) Initiation of the splicing reaction uses the 3' OH of a guanine nucleot(s)ide.
(5) Is self-splicing in vitro.
(6) Initiation of the splicing reaction uses the 2' OH of an internal adenine nucleotide.

28. Splicing of group I introns (which include the precursor to rRNA in *Tetrahymena*.)

a. (1), (2), (6)
b. (1), (4), (5)
c. (2), (4), (5)
d. (3), (5), (6)

29. Splicing of group II introns.

a. (2), (5), (6)
b. (1), (2), (6)
c. (3), (4), (5)
d. (2), (3), (5)

30. Splicing of introns from nuclear pre-mRNA.

a. (2), (3), (6)
b. (1), (2), (3), (6)
c. (2), (3), (4), (5)
d. (2), (4), (5)

For questions 31 to 33, choose the polymerase that will be best for the designated task.

31. Labeling by nick translation, i.e. introducing radioactive nucleotides into nicked, circular duplex DNA, starting with [^{32}P] dNTPs.

a. A thermostable DNA polymerase, such as Taq polymerase.
b. The Klenow fragment of E. coli DNA polymerase I.
c. Intact E. coli DNA polymerase I.
d. E. coli DNA polymerase III holoenzyme.
e. An RNA-dependent DNA polymerase from a retrovirus.

32. Amplification of a defined segment of DNA by the polymerase chain reaction.

a. A thermostable DNA polymerase, such as Taq polymerase.
b. The Klenow fragment of E. coli DNA polymerase I.
c. Intact E. coli DNA polymerase I.
d. E. coli DNA polymerase III holoenzyme.
e. An RNA-dependent DNA polymerase from a retrovirus.

33. Copying RNA into the complementary DNA prior to amplification by the polymerase chain reaction.

a. A thermostable DNA polymerase, such as Taq polymerase.
b. The Klenow fragment of E. coli DNA polymerase I.
c. Intact E. coli DNA polymerase I.
d. E. coli DNA polymerase III holoenzyme.
e. An RNA-dependent DNA polymerase from a retrovirus.

For questions 34 to 36, choose the enzyme(s) that carry out the designated process.

34. Mismatch repair

a. UvrA, UvrB, UvrC, UvrD
b. MutH, MutL, MutS
c. UmuC, UmuD
d. Uracil-N-glycosylase

35. Excision repair

a. UvrA, UvrB, UvrC, UvrD
b. MutH, MutL, MutS
c. UmuC, UmuD
d. LexA

36. Assimilation of single strands to form heteroduplex during recombination in *E. coli*.

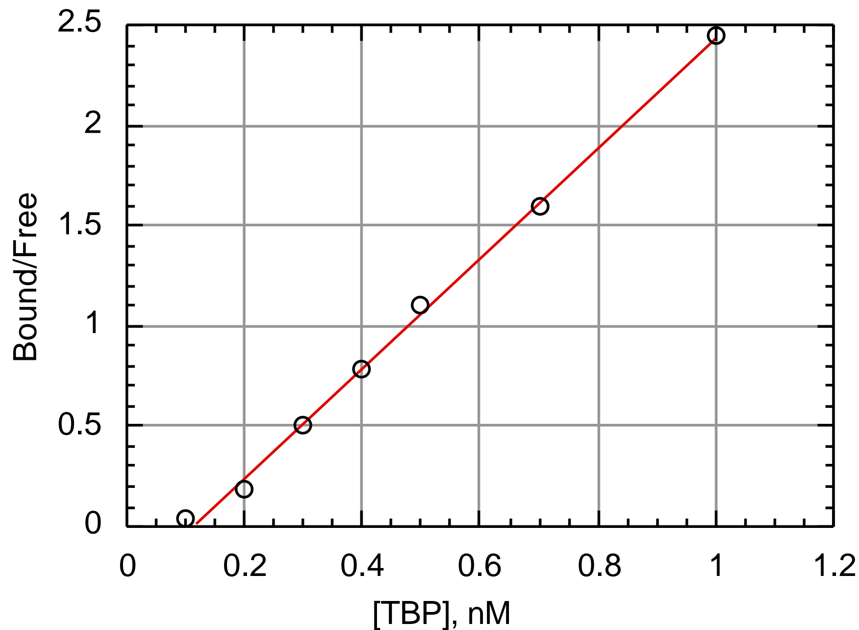
a. RecBCD
b. RecE
c. RecA
d. RuvA and RuvB
e. RuvC

37. The plasmid pBR322 has a gene for resistance to ampicillin and another for resistance to tetracycline. A set of genomic DNA fragments were ligated into the BamHI site, which is within the gene for tetracycline resistance. *E. coli* cells were transformed with the ligation mixture, which contains both the re-ligated parental plasmid with no inserts as well as recombinant plasmids containing new genomic DNA fragments. The recombinant plasmids (with an insert in the BamHI site) will generate which of the following phenotypes in the transformed *E. coli*?

a. Resistant to both tetracycline and ampicillin.
b. Resistant to only ampicillin.
c. Resistant to only tetracycline.
d. Sensitive to both tetracycline and ampicillin.

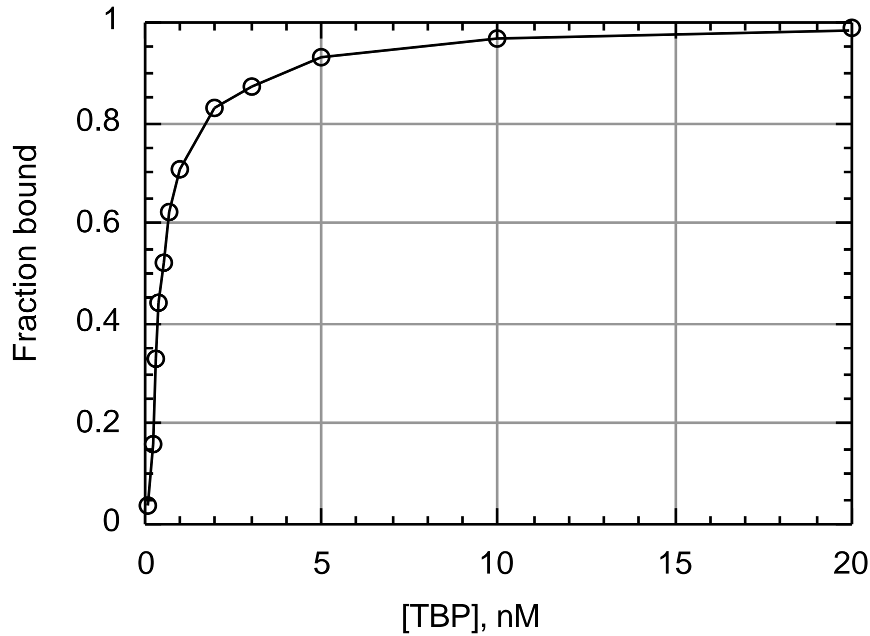
38. (22 pts total)

An undergraduate doing research in Frank Pugh's lab studied the binding of the human TATA-binding protein (TBP) to a labeled duplex oligonucleotide containing the TATA box (TATAAAA) by mobility shift and filter binding assays. Some of his data are plotted below, as the ratio of the bound DNA to free DNA versus the concentration of free TBP. Using the symbols [P] = [free TBP], in nM concentrations, [PD] = [TBP-DNA complex], [D] = [free DNA], the graph shows [PD]/[D] plotted against [P].



(a) (11 pts) What is the K_A, i.e. the association constant? Please show your calculation.

This student actually carried out the binding assays to much higher concentrations of TBP, so that the binding site was saturated with protein. These data are easier to see as a plot of fraction of labeled DNA bound as a function of [TBP]. Using the symbols above, this is a plot of [PD]/([PD]+[D]) versus [P]. Note that the data from 0.1 to 1.0 nM TBP are simply a transformation of the data in the first graph.



(b) (3 pts) When [PD] = [D], what is the value for the fraction bound?

(c) (4 pts) What is the significance of the [P] at this value for fraction bound?

(d) (4 pts) What is the K_A according to these data?

39. (30 points total)

A common pathway for catabolism of fatty acids involves their oxidation into two-carbon units (acetylCoA) that can enter the TCA cycle. The first enzyme in this pathway of fatty acid oxidation (FAO) is fatty acyl CoA dehydrogenase, and the last enzyme is thiolase. Imagine that you are studying a bacterium that shows coordinate induction of these two enzymes in response to palmitate (a 16-C fatty acid). Four genes or loci, *faoA*, *faoB*, *faoC*, and *faoD*, that affect 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 *fao* gene or locus and mutant alleles are indicated by a - under the letter. The activities of fatty acyl CoA dehydrogenase (referred to in the table as dehydrogenase) and thiolase were assayed in the presence or absence of the inducer palmitate (abbreviated palm.). The units of enzyme activity are 100 = induced activity of the wild-type enzyme, 1 = repressed activity of the wild-type enzyme, and 0 = no measurable activity. In the diploid analysis, one copy of each operon is present in each cell.

Strain number	*fao*	dehydrogenase		thiolase	
Haploid	A B C D	- palm.	+ palm.	-palm.	+palm.
1	+ + + +	1	100	1	100
2	− + + +	100	100	100	100
3	+ − + +	100	100	100	100
4	+ + − +	1	100	0	0
5	+ + + −	0	0	1	100
Diploid	A B C D/A B C D				
6	+ + − +/+ + + −	1	100	1	100
7	+ − − +/+ + + −	1	100	1	100
8	− + − +/+ + + −	100	100	1	100
9	− + + +/+ + − −	100	100	100	100

a. (10 pts) Describe the phenotypes of the following the strains with respect to fatty acyl CoA dehydrogenase and thiolase activities. A single word will suffice for each phenotype.

Strain	fatty acyl CoA dehydrogenase	thiolase
2	______________________	______________________
3	______________________	______________________
4	______________________	______________________
5	______________________	______________________
6	______________________	______________________

b. (12 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: "Synthesis of the dehydrogenase in strain 20 is repressible, which shows that wild-type *grk1* is dominant to the mutant allele."

faoB : Synthesis of the dehydrogenase in strain ____ is ________________________,

which shows that wild-type *faoB* is ________________________________ to the mutant allele.

faoC Synthesis of thiolase in strain ____ is ________________________, which shows

that wild-type *faoC* is ________________________________ to the mutant allele.

faoD Synthesis of the dehydrogenase in strain ____ is ________________________,

which shows that wild-type *faoD* is ________________________________ to the mutant allele.

faoA Synthesis of the dehydrogenase in strain 9 is ________________________________,

which shows that mutant *faoA* is ________________________________ to the wild-type allele when *faoA*⁻ is in *cis* to the wild-type gene encoding the dehydrogenase.

c. (8 pts.) What is the role of each of the genes in activity or regulation of fatty acid oxidation? Brief phrases will suffice.

faoA

faoB

faoC

faoD

For a review on *agouti*, see Siracusa (1994) Trends in Genetics 10:423-428.