Winter 2009 Name:

Gene Action Exam 3 (Traxler/Gray)

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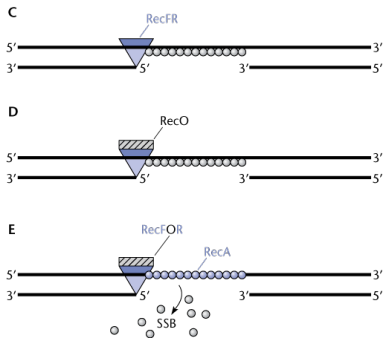
Question #1 (12 pts)

Draw a cartoon of a DNA substrate acted on during RecFOR recombination, with binding sites for RecFOR and RecA indicated. Why are *E. coli* mutants in the RecFOR recombination pathway not deficient in recombining DNA delivered during generalized transduction by phage P1? What is the function of RecA in RecFOR recombination? (do not exceed the space given)

Fig. 10.6 (p 441) ***RecFOR mutants still recombine DNA brought in via P1 generalized transduction because it is RecBCD that process the linear dsDNA fragment that the phage delivers.*** ***RecA coats the ssDNA in the gapped molecule and identifies the target dsDNA where recombination should occur; forms triple-stranded structure with target during recombination***	

Question 2 (12 pts)

Shown below is a table with the consensus sequences for a few different holoenzyme forms of RNA polymerase, with the relevant σ factor involved for each promoter. For each consensus sequence, the least important bases are shown in lowercase letters.

σ factor/use	-35 sequence (upstream)	Spacing	-10 sequence (downstream)
σ^{70}/genes for normal vegetative cell growth	TTgaCa	16-18 bp	TAtaaT
σ^{28}/expression of *E. coli* flagellar genes	TaAa	17 bp	CCgatAT
σ^{Q}/phage Q late lytic infection genes	GGgaCcC	13 bp	TtgGT

You isolated a new dsDNA bacteriophage, which you named "Q." Q relies on the *E. coli* flagellum for infection. Fill in the table below with the sequences expected for each promoter.

For answers below, most important bases are in uppercase.

Gene(s) controlled	-35 sequence	Spacing	-10 sequence
rRNA operons	***TTGACA***	***16-18***	***TATAAT***
Q major capsid genes	***GGGACCC***	***13 bp***	***TTGGT***
σ^{Q} gene	***TTgaCa or TaAa***	***16-18 or 17bp***	***TAtaaT or CCgatAT***
Q minor capsid gene ***(somewhat different than consensus)***	***e.g., GGtaCgC***	***13 bp***	***e.g., TagGT***

Question 3 (20 pts)
Imagine that you have identified a new plasmid from an environmental isolate of *E. coli* that you call pWOW. Features of pWOW include:

- Cells containing pWOW are susceptible to infection by a temperate phage Ax (dsDNA genome of 60kb; Ax contains a gene for ampicillin resistance, Amp^r).
- Cells containing pWOW can conjugate with *E. coli* or *Pseudomonas* strains and can mobilize plasmid RSF1010.
- pWOW has a Tn5 insertion conferring Kanamycin resistance (Kan^r)

You mutagenize cells with pWOW using a chemical mutagen and plate the cells on nutrient plates with Kanamycin. After overnight growth, you screen through colonies and find 100 mutants with the following phenotypes:

Class 1: Ax-resistant (Ax^r); pWOW Tra-deficient (Tra-), no RSF1010 mobilization (RSF1010 Mob-)
Class 2: Ax-sensitive (Ax^s); pWOW Tra-deficient (Tra-), but efficient RSF1010 mobilization (Mob+)
Class 3: Ax^s, but cannot form Ax lysogens (no Amp^r lysogens); pWOW Tra-proficient (Tra+) and RSF1010 Mob+

For your responses below, do not use more than the space provided (not to exceed 30 words/explanation).

Part A B, and C: Provide explanations for each of these mutant classes in the space below.

Class 1 (Ax^r Tra- Mob-):

The most likely mutations are knock-out mutations in the genes for the pilus or Mpf proteins

Class 2 (Ax^s Tra- Mob+):

The most likely mutations are knock-out mutations in the genes for the pWOW Dtr proteins. You can also get mutations in the pWOW oriT.

Class 3 (Ax^s Tra+ Mob+, no Ax lysogens):

The most likely mutations are mutations in Ax att site (which could either be on the chromosome or on the plasmid). A mutation that increased the activity of a protease that specifically targeted the Ax lytic expression repressor might do this (but those mutants probably would have other problems).

Part D: Which of these three classes of mutants should be the most abundant among the 100 candidates and why?

Class 1 mutations should be the most abundant since it is the biggest target (lots of Mpf/pilus assembly genes)

Question 4 (12 pts)
Two compounds, "J" and "K", are lethal to cells because they can each be assimilated as part of normal biosynthetic pathways to form an analog of the amino acid "X". This analog form of X ("X*") has the effect of spontaneously terminating translation whenever it's incorporated during protein synthesis. Nothing is known about the amino acid biosynthesis pathway(s) by which these compounds are assimilated to form X*.

Part A) Beth performs an experiment in which she plates *E. coli* on MinGlu + J and MinGlu + K to select for spontaneous mutants that are resistant to <u>either</u> compound. She then screens her mutants for resistance to the <u>other</u> compound. The results of this experiment are shown in the table below:

Cells Plated	Selection	Screening results	
10^8	J resistance (J^r)	100 K^s	200 K^r
10^8	K resistance (K^r)	0 J^s	200 J^r

Assuming 1) that all genes involved are of equal target size and mutability (i.e., there are no mutational "hot spots"), and 2) that all enzymes involved are the product of a single gene, draw the predicted pathway(s) by which J and K are assimilated to form X*. (Show the minimum number of steps supported by the data.)

J → K → → X*

Part B) Imagine instead that the results obtained are those shown in the table below.

Cells Plated	Selection	Screening results	
10^8	J resistance (J^r)	300 K^s	0 K^r
10^8	K resistance (K^r)	200 J^s	0 J^r

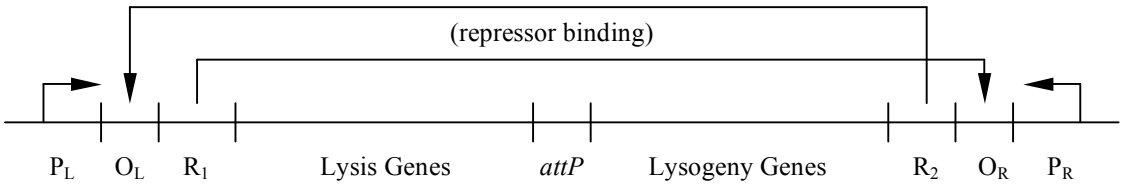
Again, assuming 1) that all genes involved are of equal target size and mutability, and 2) that all enzymes involved are the product of a single gene, draw the predicted pathway(s) by which J and K are assimilated to form X*. (As before, show the minimum number of steps supported by the data.)

J → → → X* ← ← K

Beth then mutagenizes all of her J^r and K^r mutants and plates them on MinGlu + J + K agar. No colonies at all appear following incubation. Based on your answer to part (B), what is the best explanation for why doubly-resistant (J^rK^r) mutants are not obtained in this experiment? (≤ 25 words)

J^rK^r mutants would be defective for both pathways of X biosynthesis, making them auxotrophs for X that can't grow on minimal medium

Question 5 (15 pts) The diagram below represents the genome of a temperate bacteriophage that circularizes upon entry into a host cell, then regulates the decision between lytic and lysogenic growth according to the control circuit shown. (P_L and P_R are promoters, O_L and O_R are operators, and R_1 and R_2 are repressor genes transcribed as part of the operons encoding lytic and lysogenic functions, respectively.) P_L and P_R are equally strong promoters, so the decision between lysogeny and lytic growth is essentially random chance, depending entirely on which repressor, R_1 or R_2, is made first.



The table below shows the results of plaque assays obtained with the wild-type and mutant strains of this phage, each infecting different strains of *E. coli*, as listed.

Phage Strain	Growth Phenotype* on *E. coli* Strain: Wild-type (30°)	Wild-type (42°)	Lysogen (30°)	Amber suppressor (30°)	**Possible Mutation (fill in)**
Wild-type	T	T	N	T	⊘
Mutant A	T	N	N	T	***2***
Mutant B	C	T	N	C	***6***
Mutant C	C	C	C	T	***7***
Mutant D	C	C	N	C	***1***
Mutant E	N	N	N	T	***3***
Mutant F	N	N	N	N	***4,5***
Mutant G	C	C	C	C	

* T = turbid plaque, C = clear plaque, N = no plaque

Fill in the last column of the table with the numbers of any specific mutations described in the list below that would give rise to that phenotype. (Your answer should be in terms of <u>single</u> mutations only, <u>not</u> combinations of the different types listed.) **Circle the numbers of any mutations listed below that do NOT appear on the table**. Any cell of the table may also be left blank, if no match is found.

<u>Possible</u> <u>mutations</u>:

1. P_L up-shift (>10-fold enhanced initiation)
2. Heat-sensitive R_1
3. Amber R_1 (nonsense mutation)
4. P_R up- shift (>10-fold enhanced initiation)
5. O_R knockout
6. Cold-sensitive R_2
7. Amber *int* (nonsense mutant of Integrase)
8. Heat-sensitive *xis* (Excisase)

#8 should be circled

Question 6 (14 pts)
The expression of genes needed for growth of *E. coli* on the sugar D-xylose is off when glucose is present in the environment. If glucose is absent and xylose is present, there is abundant transcription of the genes *xylA xylB* (in one operon) and *xylR* (downstream from *xylAB* but transcribed in the same direction from a different promoter). *xylAB* are poorly transcribed in the absence of xylose.

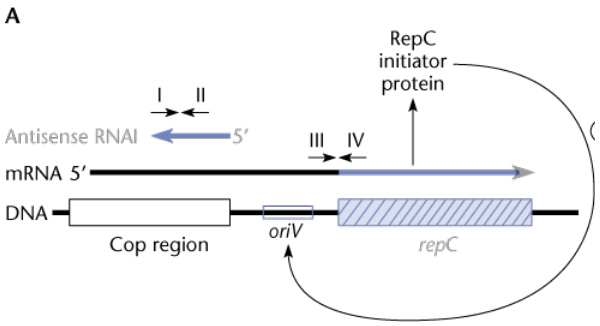
Genetic analysis shows that cells carrying transposon insertions into any of the *xyl* genes cannot grow on minimal xylose agar. $xylAB^+R^+$ strains that have cya^- or crp^- mutations also will not grow on Xylose minimal plates. After uv mutagenesis, you identify 5000 *xyl* mutations of different classes, but only 2 mutants, both in *xylR*, show constitutive *xylAB* expression (high levels of XylAB activity) in the absence of xylose.

Is XylR a positive or negative regulator of *xyl* transcription? Describe how XylR might regulate *xylAB* expression and how cAMP-CRP are likely involved in regulation of the *xylAB* promoter. (Your response should be not more than 50 words; you can include a clearly labeled cartoon.)

XylR is a positive regulator of transcription!

XylR might have a binding site just upstream of the -35 region of the promoter and help the RNA pol holoenzyme bind more efficiently to initiation transcription. By analogy to araBAD promoter, XylR + its inducer (xylose or something derived from it) would bind at this location, and CRP-cAMP would bind upstream of the XylR-binding site. CRP-cAMP would facilitate the binding of the XylR-inducer complex to its specfic binding location (alternatively, CRP-cAMP might make direct contacts with RNA pol holoenzyme-see Fig. 13.4).

Question 7 (15 pts)



pT181 is a 4.5 kb plasmid normally found in *Staphylococcus aureus* with a copy number of 20/cell. Analysis of this plasmid indicated that its rolling circle replication is controlled via antisense RNA I molecule that binds to the mRNA for the RepC protein. Increased amounts of RNA I lead to lower amounts of *repC* mRNA. The region that codes for these different molecules is shown above. Estimate the pT181 copy number of plasmids carrying the mutations of the indicated classes (use a numerical answer).

Mutation	pT181 copy number
Class I: RNA I promoter up mutation (10x more RNA I)	***2***
Class II: Decreased stability for RNA I RNA (10x less RNA I)	***200***
Class III: Increased stability for the RepC protein (same amount of protein made but present 2x longer in cell)	***About 20***
Class IV: *repC* promoter up mutation (10 x more mRNA containing *repC*)	***200***
Class V: Base substitutions in region A of RNA I	***20***