

1. (10 pts) You isolate new mutator strains of *E. coli* by screening for mutants showing an increased rate of  $lacI^+ \rightarrow lacI^-$  mutations in single colonies using the following procedure. A culture of wild-type (=nonmutator)  $lacI^+$  bacteria is first treated with 5-bromodeoxyuridine (5-BU). Cells are plated and allowed to grow into single colonies on a medium on which  $lacI^-$  secondary colonies can be easily detected. Colonies showing a large number of  $lacI^-$  secondary colonies (papillae) are mutator strains, and may show increases in different types of mutations. Which of the following base pair changes could such mutator strains increase in frequency? Assume that all possible base pairing errors may occur during replication and potentially lead to mutations. (Circle the appropriate choice or choices.)

- a. A:T  $\rightarrow$  G:C changes
- b. A:T  $\rightarrow$  T:A changes
- c. G:C  $\rightarrow$  A:T changes
- d. G:C  $\rightarrow$  C:G changes
- e. G:C  $\rightarrow$  T:A changes

2. (13 pts) Imagine that you have isolated several different mutants which increase the transposition frequency of a newly discovered IS element. Some of the mutations alter the sequence of the IS element; others fall outside the element in the chromosome. Based on how transposition is regulated for other IS elements, name two possible mutations which could lead to the enhanced transposition, one altering the IS element sequence, the other not. (< 10 words each)

1. IS element mutation:

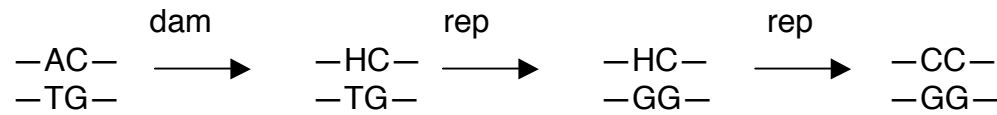
- **Mutation that increase efficiency of transposase promoter.**
- **Frame shift mutation that correct transposase reading frame.**
- **Mutation that give rise to transposase that is insensitive to protease**
- **Mutation in dam methylation site.**
- **Elimination of hairpin loop to present RBS.**

2. Non-IS element (chromosomal) mutation:

- **dam<sup>-</sup>**
- **protease<sup>-</sup>**
- **mutation in sigma factor to preferentially transcribe transposase gene**

3. (12 pts). Imagine a spontaneous DNA damage event that converts A residues into a derivative (HA) which sometimes (rarely) base-pairs with G rather than T. The cell has three repair functions (MutA, MutB and MutC) to help limit mutation due to HA, as shown in the figure at the bottom. Diagram the series of events leading to the most common increased mutation for each strain listed below. The events leading to increased A:T → C:G mutations in *mutC*<sup>-</sup> mutants are shown (“H”=HA). Show only the DNA which becomes mutated, use the fewest number of steps possible, and label the steps (“dam”=damage, “rep”=replication, “MutA”=MutA function, “MutB”=MutB function and “MutC”=MutC function). Remember that no repair function is 100% efficient.

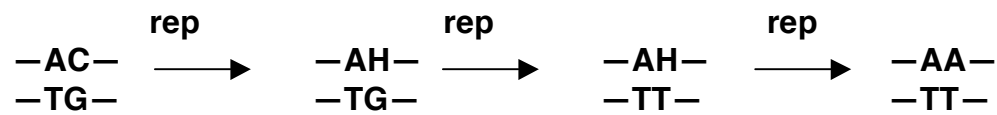
*mutC*<sup>-</sup>



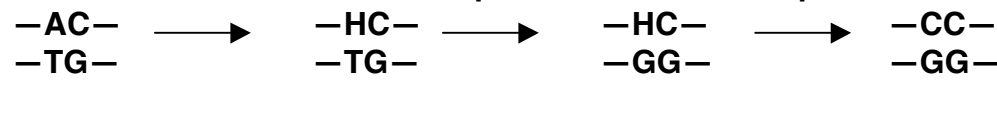
a. *mutA*<sup>-</sup>



b. *mutB*<sup>-</sup>



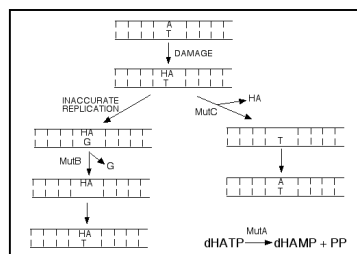
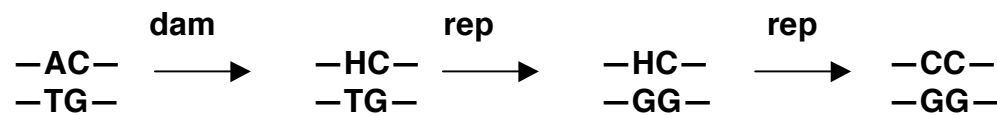
or:



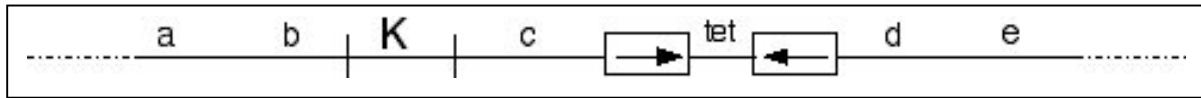
c. *mutA*<sup>-</sup> *mutB*<sup>-</sup>



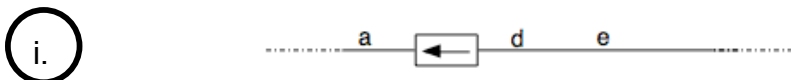
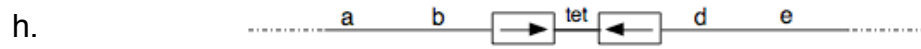
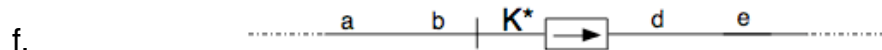
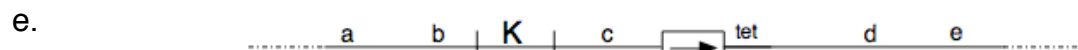
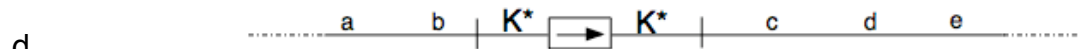
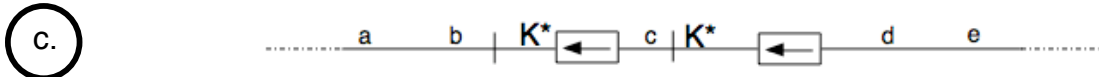
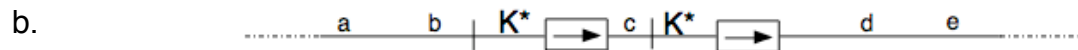
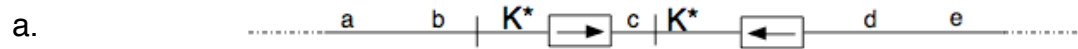
d. *mutB*<sup>-</sup> *mutC*<sup>-</sup>



4. (18 pts) Imagine a region of the *E. coli* genome carrying a gene K as shown in the diagram below. Near the gene is situated a Tn10 insertion that does not interfere with gene K function in any way. ("a"- "e" represent different sites in the DNA; "tet" represents the tetracycline resistance gene.)



The rate of spontaneous mutational inactivation of gene K (i.e., the rate of  $K^+ \rightarrow K^-$  mutation) is high due to genetic events involving the nearby Tn10. Based on your knowledge of Tn10, which of the following structures would you predict to be associated with  $K^-$  mutants? Circle the letters corresponding to the correct structures. Assume only one genetic event occurs per mutation. "K\*" represents **part** of the K gene. (Assume that cells are growing and thus may contain two or more chromosomes.)



5. (15 pts) You determine the spontaneous frequency of rifampicin resistant mutants for a series of strains, as indicated below. (Assume all mutations completely eliminate the activity of the corresponding protein).

Strain	Rifampicin resistance (per cell)
Wild-type	$10^{-9}$
DNA polymerase proofreading minus	$10^{-5}$
Mismatch repair minus	$10^{-8}$
DNA polymerase proofreading minus <b>and</b> Mismatch repair minus	$10^{-5}$

a. You (naively) expect the double mutant to exhibit a frequency of Rifampicin resistant mutants of  $10^{-4}$ . Explain why you expect  $10^{-4}$ . (<40 words)

**proofreading<sup>-</sup> :  $10^{-5}/10^{-9} = 10^4$ -fold increase**

**mismatch repair<sup>-</sup> :  $10^{-8}/10^{-9} = 10$ -fold increase**

**Since proofreading and mismatch repair are independent pathways that fix the same errors, you'd expect the mutation frequencies to be multiplicative, i.e.,  $10^4 \times 10 = 10^5$**

**Max: 10pts**

- **2pt – an attempt at answering**
- **2pt – proofreading and MMR both act on replication mismatches**
- **2pt – however, proofreading and MMR are different independent repair systems**
- **4pt – therefore, mutation frequency of double mutant is a multiplicative combination of the frequencies of the single mutants**
  - **$10^4 \times 10^1 \times 10^{-9} = 10^5 \times 10^{-9} = 10^{-4}$  or**
  - **$(10^{-5} \times 10^{-8})/10^{-9} = 10^{-13}/10^{-9} = 10^{-4}$  or**
  - **10-fold more mutations than 10,000-fold is 100,000-fold increase ( $10^{-9} \times 10^5 = 10^{-4}$ ).**

b. How could you rationalize the finding that the observed frequency of Rifampicin resistant mutants is 10-fold lower than your expectation? (<40 words)

**The high number of errors arising from a defective proofreading function overwhelms the mismatch repair pathway; no significant increase results from the defect in mismatch repair.**

6. (18 pts) a. What four repair functions help cells survive DNA damage caused by ultraviolet irradiation?

1. **Excision repair**

② **Photoreactivation**

③ **Error-prone repair**

4. **Recombinational repair**

b. There exist bacterial viruses with single-stranded DNA genomes. When the single-stranded DNA enters a cell, it is copied into double-stranded DNA as a first step in infection. Double-stranded DNA is eventually used to generate single-stranded DNA for packaging into virus particles, which are released from cells. Imagine that such a virus is exposed to UV light prior to infection. Which two of the four repair functions (part a) would you expect to be most important (relative to if the virus had double-stranded DNA) for allowing virus survival after irradiation? Circle the appropriate numbers in the answer to part a above. (Assume that all repair functions are active in cells infected with irradiated single-stranded or double-stranded DNA viruses.)

7. (14 pts) Kendall was seeking to improve upon the method we used in lab for generating Tn/*acZ*/in insertion mutants, and devised the following scheme: He used the same phage lambda vector from our lab to infect and mutagenize an Hfr strain of *E. coli*, then he selected for insertion mutations that had inactivated a gene known to be transferred early (within the first five minutes after mating) by the strain during conjugation. He then used this Tn/*acZ*/in-containing Hfr strain as a vector to introduce the transposon to other cells by combining it with wild-type *E. coli*, "shaking the bed" to interrupt DNA transfer after 8 minutes, then plating to select for chloramphenicol-resistant transconjugants. (His culturing conditions successfully selected against both donor and recipient cells alone.) He was delighted to see that the number of resistant mutants obtained by this procedure was significantly higher than that obtained by the original lambda delivery system, and declared that his new method for transposon mutagenesis was significantly more efficient than the previous one.

What is an alternative explanation for the observed increase in chloramphenicol-resistant mutants? ( $\leq 15$  words)

**They might have arisen by recombination of the transposon-containing locus instead of by transposition. (10 points)**

Briefly explain how could you determine in one (fairly simple) experiment which of the two explanations (yours or Kendall's) was correct. ( $\leq 35$  words)

**Mate the Hfr donor with a recA- recipient strain. (In this case, the recipient can't recombine the donated DNA, but transposition will be unaffected.) If the number of resistant mutants is the same as before, they are transposition mutants; if the numbers decrease, the mutants Kendall found are recombinants. (4 points)**

OR

**Screen Kendall's resistant mutants for activity of the "early" gene (call it "Gene Y"). If they are still wild-type (Y+), they are (random) transposition mutants. If they are mutant (Y-) they simply recombined the donated TnlacZ/in locus. (4 points)**