Genetics/Microbiology 411
Problem set (ANSWERS AT END)

For exam 1: Questions 1-3, 5, 7, 9-12
For exam 2: Questions 4, 6, 8, 13

1. The central region of a protein with a single lysine residue has the sequence .. Phe-Leu-Tyr-Ala-Lys-Gly-Glu. A mutation is found that inactivates the protein and causes it to terminate with Phe-Leu-Tyr-Ala. The mutation is suppressed (i.e., the protein has activity) when the mutant gene is present in bacteria producing an amber suppressor transfer RNA that inserts lysine residues at amber stop codons.

a. Which of the two lysine codons (check codon table ; ;) is used in the wild-type (i.e., nonmutant) protein?

b. The mutation is also suppressed when the mutant gene is present in any of five different bacterial strains that produce amber suppressor transfer RNA molecules that insert serine, tyrosine, leucine, tryptophan or glycine at amber codons. How can you explain this result?

2. a. Which of the codons can mutate to amber codons by A:T-> T:A transversions?

b. Given the following RNA sequence, which base pairs (in the corresponding DNA) can mutate to give amber codons? (Below each codon write "none" or indicate the single DNA base pair change that would produce the amber codon.

AUG GGG UUA AUU UAU CCG GAC CAA GGC GCA CAG ...

3. Imagine that you have isolated mutant cells with altered ribosomes that make fewer translational errors than wild-type. These cells are found to grow much more slowly than wild-type cells under all nutritional conditions. Why might the mutant cells be slow growing?

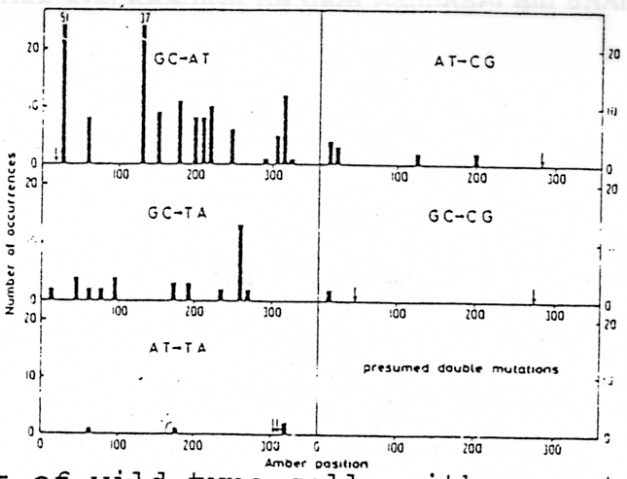
4. <u>E. coli</u> cells mutant in the gene for a protein involved in the SOS response ($RecA^-$) are not efficiently mutagenized by a variety of mutagenic treatments (such as irradiation with ultraviolet light) that are highly mutagenic to wild-type (i.e., $RecA^+$) cells. Explain.

5. Diagram two mechanisms for the spontaneous generation of deletion mutations.

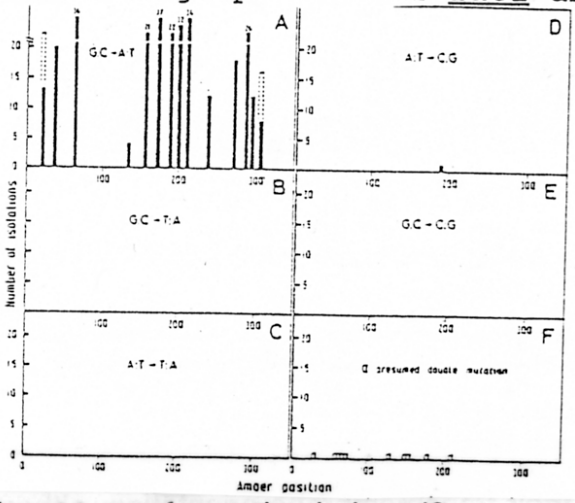
6. Predict how the following E. coil mutants compare to wild-type cells in their frequency of spontaneous mutation, frequency of mutation after treatment with ultraviolet (UV) light, and survival frequency after treatment with ultraviolet light. Remember that the cell often has multiple repair systems that repair the same type of DNA damage because no single system is 100% efficient. (For each mutant below, write "same", "increased" or "decreased" in the appropriate space. Answers for some of the mutants are shown. Disregard polymerization errors occuring during repair processes such as excision repair)

	Spontaneous mutation	**UV-induced mutation**	**Survival after UV treatment**
photoreactivation$^-$	same	increased	decreased
uracil N-glycosylase$^-$	increased	same	same
DNA polymerase proofreading$^-$			
mismatch repair$^-$			
overactive dam methylase (methylates DNA more rapidly than usual)			
excision repair$^-$	same		
error-prone repair$^-$			
RecA$^-$	decreased (slightly)		

7. A diagram showing the pattern of spontaneous lacI amber mutations is shown below.



a. Treatment of wild-type cells with a particular mutagen leads to the following spectrum of lacI amber mutations.

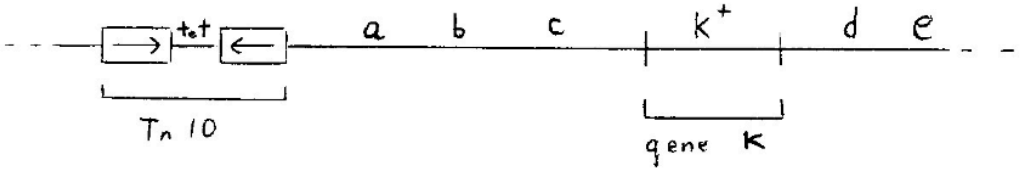


a. What is the dominant type of mutation induced?

b. Name three different mutagens that could lead to this pattern of lacI amber mutations.

c. Treatment of wild-type cells with a second mutagen that increases the frequency of lacI mutations 10-fold leads to a lacI amber mutation spectrum that is identical to that observed without mutagen treatment, and which shows no increase in the frequency of amber mutations. What is the explanation for this apparent paradox?

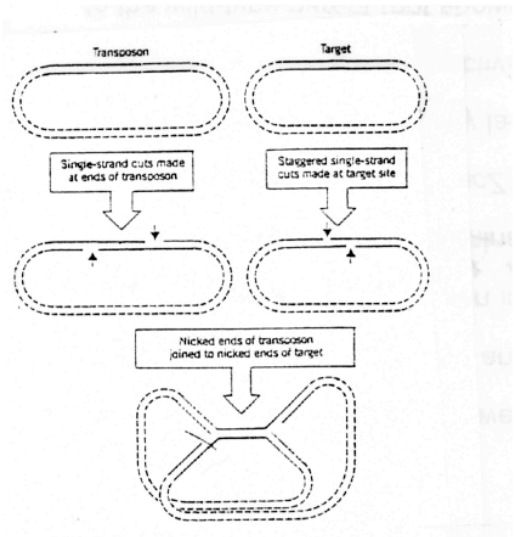
8. You isolate an *E. coli* strain with a Tn*10* insertion near but not in a particular gene, gene K, as shown below. This is the only Tn*10* in the cell.



a. The frequency of $K^+ \rightarrow K^-$ mutations is increased in such cells compared to cells with a Tn*10* insert far from the K^+ gene. Diagram the <u>final structures</u> resulting from two different events which would contribute to the increase in mutation frequency. (Hint: remember that Tn*10* transposes by a conservative mechanism. The Shapiro intermediate structure is shown below for reference.)

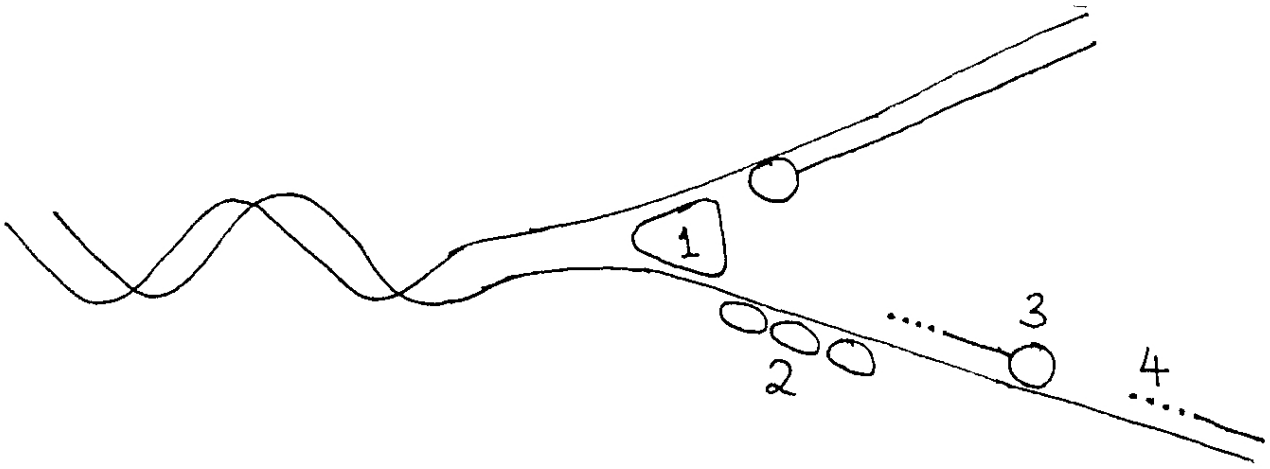
i:

ii:



b. You isolate mutants with an increased frequency of $K^+ \rightarrow K^-$ mutation. This increased frequency depends on the nearby Tn*10* rather than being due to some unrelated mutator mutation. One such mutation eliminates a gene that is nowhere near the Tn*10* and K^+ gene on the chromosome. What gene is rendered inactive? Explain.

9. The following is a schematic diagram ("exploded" view) which represents ongoing DNA synthesis at the replication fork:



a) Label items 1, 2, 3, and 4.
b) Indicate the 3' and 5' ends of the two DNA template molecules being replicated.
c) Indicate the leading strand
d) Indicate the lagging strand
e) What is the function of the molecule depicted as item 2?

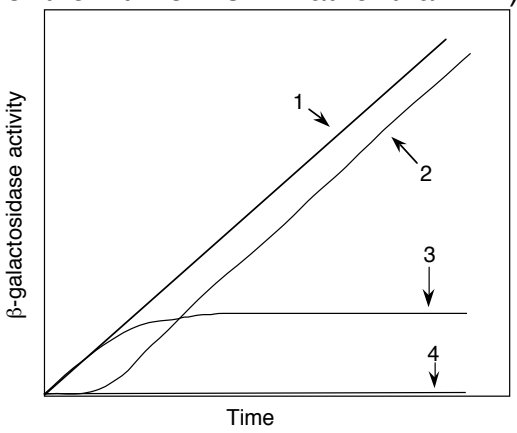
10. Most nonsense mutations in a structural gene for a protein cause a null phenotype.

a. How many of the codons can be converted into nonsense codons by single base pair substitution mutations?

b. How many different amino acids are encoded by the codons that can be changed to a nonsense codon by a single base substitution?

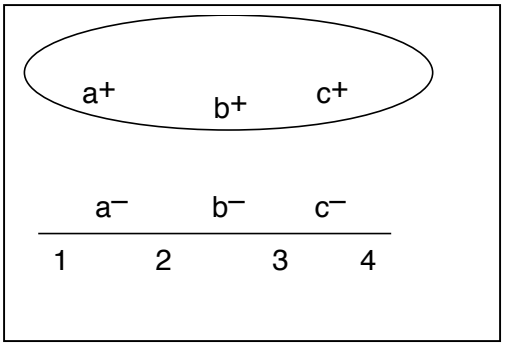
11. The "Pajama" experiment first demonstrated the fact that *E. coli lac* operon regulation follows a negative logic, with a repressor of operon expression inactivated in the presence of lactose. The experiment consisted of measuring β-galactosidase enzymatic activity (a measure of β-galactosidase synthesis) in recipient mutant cells following Hfr conjugal transfer into the cells of a second *lac* region. The analysis utilized a regulatory mutation leading to constitutive expression of beta-galactosidase ($lacI^c$).

Below is a series of lines representing β-galactosidase synthesis in different recipient cells in growth media lacking inducer after conjugation with different Hfr donors. Predict which line would most closely represent the pattern expected for each combination based on the assumption of negative or positive "logic" of regulation. Disregard any beta-galactosidase production in donor cells. (Write one number between 1 and 4 in the "Predicted relationship" column of the table. Three answers are provided.) (Hint: Think about why the predicted relationship for the fifth row is "2" rather than "1".)



Regulation	Hfr donor	F^- recipient	Predicted relationship
negative	$lacI^+$ $lacZ^+$	$lacI^c$ $lacZ^-$	3
negative	$lacI^c$ $lacZ^+$	$lacI^c$ $lacZ^-$	
negative	$lacI^c$ $lacZ^-$	$lacI^c$ $lacZ^-$	
negative	$lacI^+$ $lacZ^-$	$lacI^+$ $lacZ^+$	
positive	$lacI^c$ $lacZ^+$	$lacI^+$ $lacZ^-$	2
positive	$lacI^+$ $lacZ^+$	$lacI^+$ $lacZ^+$	
positive	$lacI^+$ $lacZ^+$	$lacI^c$ $lacZ^-$	1
positive	$lacI^c$ $lacZ^-$	$lacI^+$ $lacZ^-$	
positive	$lacI^c$ $lacZ^+$	$lacI^+$ $lacZ^+$	

12. Imagine two double-stranded DNA molecules, one linear and one circular, as shown below. The sequences are closely enough related that recombination can occur anywhere in region a-c. a^+ represents the wild-type and a^- represents a mutation at site a, etc.



a. Draw the structure of the molecule(s) resulting from a single recombination event at site 2:

b. Draw the structure of the molecule(s) resulting from two recombination events, one at site 2 and one at site 3:

c. Draw the structure of the molecule(s) resulting from three recombination events, at sites 1, 3 and 4:

13 . Imagine a substance X that is generated in growing cells and may react with adenine residues in DNA or in nucleotide precursors to generate a compound AX. AX base pairs like A most of the time, but some of the time AX base pairs like C. Cells carry a gene (mutX) that encodes an enzyme that specifically cleaves AX-triphosphate to AX-monophosphate, preventing AX incorporation into DNA during replication.

a. One would naively predict that MutX-minus mutants (lacking all MutX activity) would show increased frequencies of both G:C –>T:A and A:T –> C:G mutations. Below, diagram the steps expected to lead to each of these types of mutation.

i. G:C –>T:A

——GA——
——CT——

ii. A:T –> C:G

——GA——
——CT——

b. When MutX-minus cells are examined, they are found to exhibit a 100-fold increase in G:C –>T:A mutations but almost no increase in A:T –> C:G mutations. By analogy with the way in which the cell limits mutation due to oxidative damage, what is the most likely explanation for the fact that A:T –> C:G mutation frequency is not appreciably increased in MutX-minus cells (≤25 words)?

ANSWERS

1. a. AAG

b. The lysine is in a part of the protein which is tolerant of change, so that many different amino acids can be substituted for lysine to yield an active protein.

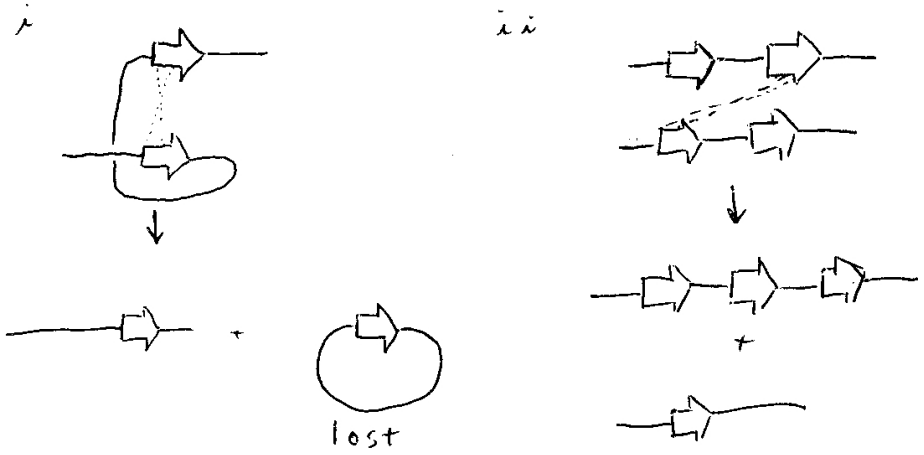
2. a. AAG and TTG

b. AUG GGG UUA AUU UAU CCG GAC CAA GGC GCA CAG .

3. Mutants with overactive proofreading functions will consume excess metabolic energy because more often than normal they will mistakenly remove (after GTP cleavage) appropriate aminoacyl-tRNA molecules.

4. The RecA mutants are unable to induce the SOS response after irradiation. They thus fail to produce the error-prone repair pathway which would normally ("sloppily") replicate opposite pyrimidine dimers in an error-prone manner which can generate mutations. The normal replication machinery in the recA cells cannot replicate over the pyrimidine dimers and cells with such damage die. Thus, the DNA damage kills cells rather than being converted into mutations, so that mutant cells are not recovered and the mutation frequency is lower.

5.



6.

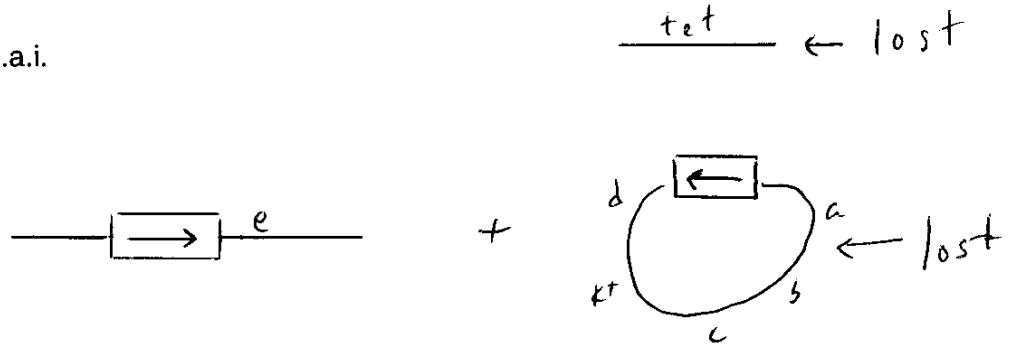
	Spontaneous mutation	UV-induced mutation	Survival after UV treatment
photoreactivation$^-$	same	increased	decreased
uracil N-glycosylase$^-$	increased	same	same
DNA polymerase proofreading$^-$	increased	same	same
mismatch repair$^-$	increased	same	same
overactive dam methylase (methylates DNA more rapidly than usual)	increased	same	same
excision repair$^-$	same	increased	decreased
error-prone repair$^-$	same	decreased	decreased
RecA$^-$	decreased (slightly)	decreased	decreased

7. a. G:C ->A:T

b. nitrous acid
nitrosoguanidine
5 -bromouracil

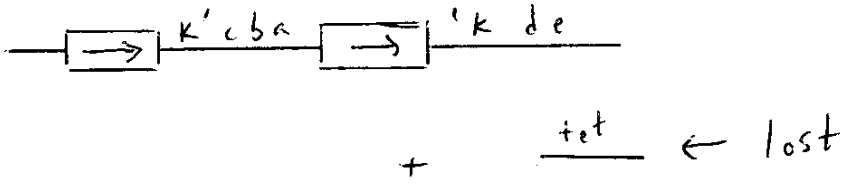
c. The mutagen induces mutations that are not base pair changes (such as deletions or frameshifts) or induces A:T -G:C changes, a type of change that is not detectable in the amber codon spectrum.

8.a.i.



adjacent deletion

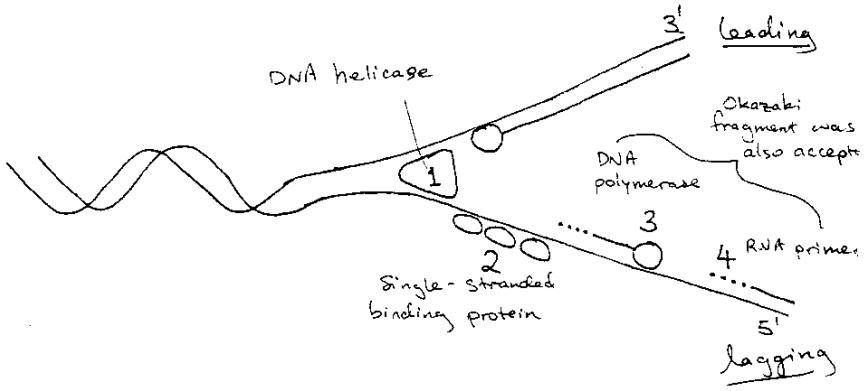
a.ii.



inversion with endpoint in K gene

b. Dam methylase gene. Transposition no longer restricted to period immediately following DNA replication, therefore more of the events inactivating K shown in part a.

9.



9.e. The protein binds to single-stranded DNA and prevents it from reannealing into a duplex (stabilizes the single stranded form) thus maintaining the "substrate" for replication.

replication.

10. The possible single base substitutions that can produce a nonsense mutation are show in the table below. Note that stop codons are not found within a protein coding region, so although one stop codon could produce another stop codon by a single base substitution, such changes are unlikely to result in a nonsense mutation within a gene. In addition, some codons may produce either an amber or ochre codon by a single base substitution (for example, UAC may mutate to UAG or UAA).

    a. 18 codons
    b. 9 amino acids: Lys, Gln, Glu, Ser, Trp, Leu, Tyr, Arg, and Cys

    Single base substitutions that can yield nonsense mutations

Nonsense mutation	Original codon	Amino Acid
UAG (Amber)	AAG	Lys
	CAG	Gln
	GAG	Glu
	UCG	Ser
	UGG	Trp
	UUG	Leu
	UAA	Stop1
	UAC	Tyr
	UAU	Tyr
UAA (Ochre)	AAA	Lys
	CAA	Gln
	GAA	Glu
	UCA	Ser
	UGA	Stop1
	UUA	Leu
	UAC	Tyr
	UAG	Stop1
	UAU	Tyr
UGA (Opal)	AGA	Arg
	CGA	Arg
	GGA	[illegible] Gly
	UAA	Stop1
	UCA	Ser
	UUA	Leu
	UGC	Ser
	UGG	Trp
	UGU	Cys

11.

Regulation	Hfr donor	F⁻ recipient	Predicted relationship
negative	lacI+ lacZ+	lacIc lacZ–	3
negative	lacIc lacZ+	lacIc lacZ–	1
negative	lacIc lacZ–	lacIc lacZ–	4
negative	lacI+ lacZ–	lacI+ lacZ+	4
positive	lacIc lacZ+	lacI+ lacZ–	2
positive	lacI+ lacZ+	lacI+ lacZ+	4
positive	lacI+ lacZ+	lacIc lacZ–	1
positive	lacIc lacZ–	lacI+ lacZ–	4
positive	lacIc lacZ+	lacI+ lacZ+	2

12.

a.

a^- b^+ c^+ a^+ b^- c^-

b.

a^- b^+ c^-

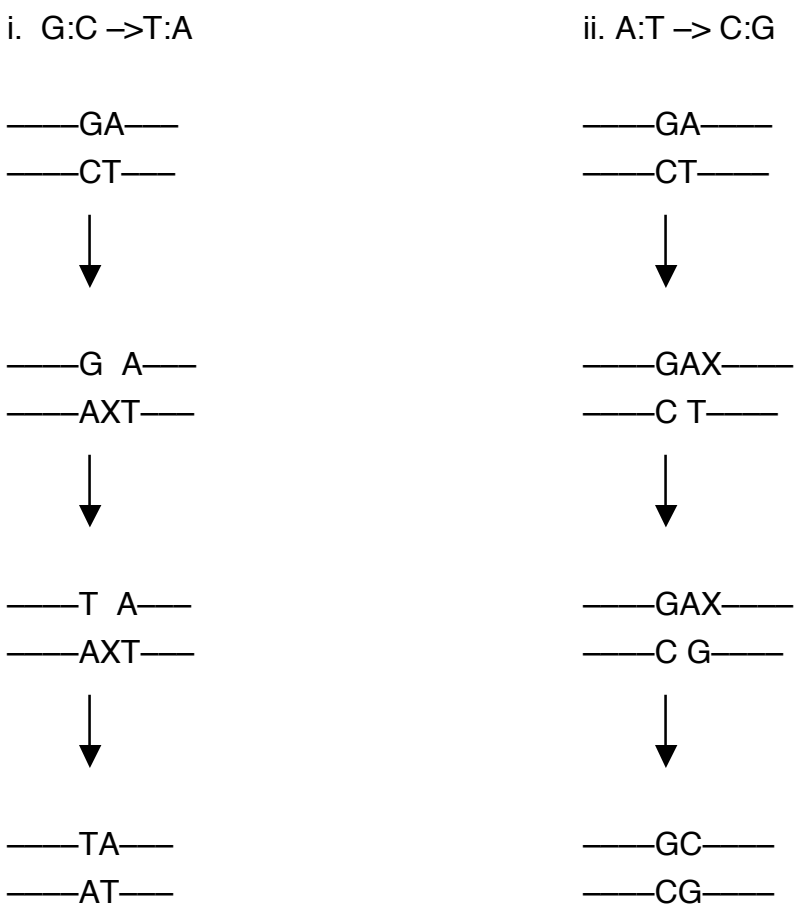
_____ + a^+ b^- c^+

c.

a^+ b^+ c^- a^- b^- c^+

13.

a.



b. There is no MutY-like function to deal with the mismatch after the first round of replication.