

## Chapter Eighteen: Gene Mutations and DNA Repair

### COMPREHENSION QUESTIONS

#### Section 18.1

- \*1. What is the difference between somatic mutations and germ-line mutations?  
*Germ-line mutations are changes in the DNA of germ (reproductive) cells and may be passed to offspring. Somatic mutations are changes in the DNA of an organism's somatic tissue cells and cannot be passed to offspring.*
- \*2. What is the difference between a transition and a transversion? Which type of base substitution is usually more common?  
*Transition mutations are base substitutions in which one purine (A or G) is changed to the other purine, or a pyrimidine (T or C) is changed to the other pyrimidine. Transversions are base substitutions in which a purine is changed to a pyrimidine or vice versa. Although transversions would seem to be statistically favored because there are eight possible transversions and only four possible transitions, about twice as many transition mutations are actually observed in the human genome.*
- \*3. Briefly describe expanding trinucleotide repeats. How do they account for the phenomenon of anticipation?  
*Expanding trinucleotide repeats occur when DNA insertion mutations result in an increase in the number of copies of a trinucleotide repeat sequence. Such an increase in the number of copies of a trinucleotide sequence may occur by errors in replication or unequal recombination. Within a given family, a particular type of trinucleotide repeat may increase in number from generation to subsequent generation, increasing the severity of the mutation in a process called anticipation.*
4. What is the difference between a missense mutation and a nonsense mutation? A silent mutation and a neutral mutation?  
*A base substitution that changes the sequence and the meaning of a mRNA codon, resulting in a different amino acid being inserted into a protein, is called a missense mutation. Nonsense mutations occur when a mutation replaces a sense codon with a stop (or nonsense) codon.*  
*A nucleotide substitution that changes the sequence of a mRNA codon, but not the meaning is called a silent mutation. In neutral mutations, the sequence and the meaning of a mRNA codon are changed. However, the amino acid substitution has little or no effect on protein function.*
5. Briefly describe two different ways that intragenic suppressors may reverse the effects of mutations.  
*Intragenic suppression is the result of second mutations within a gene that restore a wild-type phenotype. The suppressor mutations are located at different sites within the gene from the original mutation. One type of suppressor mutation restores the*

*original phenotype by reverting the meaning of a previously mutated codon to that of the original codon. The suppressor mutation occurs at a different position than the first mutation, which is still present within the codon. Intragenic suppression may also occur at two different locations within the same protein. If two regions of a protein interact, a mutation in one of these regions could disrupt that interaction. The suppressor mutation in the other region would restore the interaction. Finally, a frameshift mutation due to an insertion or deletion could be suppressed by a second insertion or deletion that restores the proper reading frame.*

- \*6. How do intergenic suppressors work?  
*Intergenic suppressor mutations restore the wild-type phenotype. However, they do not revert the original mutation. The suppression is the result of a mutation in a different gene reducing the phenotypic effect of the original mutation. For example, because many proteins interact with other proteins, the original mutation may have altered one protein in a way that disrupts a protein-protein interaction, while the second mutation alters the second protein to restore the interaction. A second type of intergenic suppression occurs when a mutation within an anticodon region of a tRNA molecule allows the tRNA anticodon to pair with the codon containing the original mutation. This results in the substitution of a functional amino acid in the protein.*

## Section 18.2

- \*7. What is the cause of errors in DNA replication?  
*Two types of events have been proposed that could lead to DNA replication errors: mispairing due to tautomeric shifts in nucleotides and mispairing through wobble or flexibility of the DNA molecule. Current evidence suggests that mispairing through wobble caused by flexibility in the DNA helix is the most likely cause.*
8. How do insertions and deletions arise?  
*Strand slippage that occurs during DNA replication and unequal crossover events due to misalignment at repetitive sequences have been shown to cause deletions and additions of nucleotides to DNA molecules. Strand slippage results from the formation of small loops on either the template or the newly synthesized strand. If the loop forms on the template strand, then a deletion occurs. Loops formed on the newly synthesized strand result in insertions. If, during crossing over, a misalignment of the two strands at repetitive sequence occurs, then the resolution of the cross over will result in one DNA molecule containing an insertion and the other molecule containing a deletion.*
- \*9. How do base analogs lead to mutations?  
*Base analogs have structures similar to the nucleotides and if present, may be incorporated into the DNA during replication. Many analogs have an increased tendency for mispairing, which can lead to mutations. DNA replication is required for the base analog-induced mutations to be incorporated into the DNA.*

10. How do alkylating agents, nitrous acid, and hydroxylamine produce mutations?  
*Alkylating agents donate alkyl groups (either methyl or ethyl) to the nucleotide bases. The addition of the alkyl group results in mispairing of the alkylated base and typically leads to transition mutations. Nitrous acid treatment results in the deamination of cytosine, producing uracil, which pairs with adenine. During the next round of replication, a CG to AT transition will occur. The deamination of guanine by nitrous acid produces xanthine. Xanthine can pair with either cytosine or thymine. If paired with thymine, then a CG to TA transition can occur. Hydroxylamine works by adding a hydroxyl group to cytosine, producing hydroxylaminocytosine. The hydroxylaminocytosine has an increased tendency to undergo tautomeric shifts, which allow pairings with adenine, resulting in GC to AT transitions.*
11. What types of mutations are produced by ionizing and UV radiation?  
*Ionizing radiation promotes the formation of radicals and reactive ions that result in the breakage of phosphodiester linkages within the DNA molecule. Single- and double-strand breaks can occur. Double-strand breaks are difficult to repair accurately and may result in the deletion of genetic information. UV radiation promotes the formation of pyrimidine dimers between adjacent pyrimidines in a DNA strand. Inefficient repair of the dimers by error-prone DNA repair systems results in an increased mutation rate.*
- \*12. What is the SOS system and how does it lead to an increase in mutations?  
*The SOS system is an error-prone DNA repair system consisting of at least 25 genes. Induction of the SOS system results in a bypass of damaged DNA regions, which allows for DNA replication across the damaged regions. However, the bypass of damaged DNA results in a less accurate replication process, and thus more mutations will occur.*

### Section 18.3

13. What is the purpose of the Ames test? How are *his<sup>-</sup>* bacteria used in this test?  
*The Ames test allows for rapid and inexpensive detection of potentially carcinogenic compounds using bacteria. The majority of carcinogenic compounds result in damage to DNA and are mutagens. The reversion of *his<sup>-</sup>* bacteria to *his<sup>+</sup>* is used to detect the mutagenic potential of the compound being tested.*

### Section 18.4

- \*14. List at least three different types of DNA repair and briefly explain how each is carried out.  
*(1) Mismatch repair. Replication errors that are the result of base-pair mismatches are repaired. Mismatch-repair enzymes recognize distortions in the DNA structure due to mispairing and detect the newly synthesized strand by the lack of methylation on the new strand. The bulge is excised and DNA polymerase and DNA ligase fill in the gap.*

- (2) *Direct repair.* DNA damage is repaired by directly changing the damaged nucleotide back to its original structure.
- (3) *Base-excision repair.* The damaged base is excised, and then the entire nucleotide is replaced.
- (4) *Nucleotide-excision repair.* Repair enzymes recognize distortions of the DNA double-helix. Damaged regions are excised by enzymes, which cut phosphodiester bonds on either side of the damaged region. The gap generated by the excision step is filled in by DNA polymerase.
15. What features do mismatch repair, base-excision repair, and nucleotide-excision repair have in common?  
*Mismatch repair, base excision repair, and nucleotide-excision repair all result in the removal of nucleotides from DNA. All repair mechanisms that excise nucleotides share a common four-step pathway:*
- (1) *DNA damage is detected.*
- (2) *The damage is excised by DNA repair endonucleases.*
- (3) *Following excision, DNA polymerase adds nucleotides to the free 3' OH group, using the remaining strand as a template*
- (4) *Ligation of nicks in the sugar phosphate backbone is performed by DNA ligase.*

## APPLICATION QUESTIONS AND PROBLEMS

### Section 18.1

- \*16. A codon that specifies the amino acid Gly undergoes a single-base substitution to become a nonsense mutation. In accord with the genetic code given in Figure 15.10, is this mutation a transition or a transversion? At which position of the codon does the mutation occur?  
*By examining the four codons that encode for Gly, GGU, GGC, GGA, and GGG, and the three nonsense codons, UGA, UAA, and UAG, we can determine that only one of the Gly codons, GGA, could be mutated to a nonsense codon by the single substitution of a U for a G at the first position:*
- GGA → UGA**
- Because uracil is a pyrimidine and guanine is a purine, the mutation is a transversion.*
- \*17. a. If a single transition occurs in a codon that specifies Phe, what amino acids could be specified by the mutated sequence?  
*Two codons can encode for Phe, UUU, and UUC. A single transition could occur at each of the positions of the codon resulting in different meanings.*

<i>Original codon</i>	<i>Mutated codon (amino acid encoded)</i>
<i>UUU</i>	<i>CUU (Leu), UCU (Ser), UUC (Phe)</i>
<i>UUC</i>	<i>CUC (Ser), UCU (Ser), UUU (Ser)</i>

- b. If a single transversion occurs in a codon that specifies Phe, what amino acids could be specified by the mutated sequence?

<i>Original codon</i>	<i>Mutated codon (amino acid encoded)</i>
<i>UUU</i>	<i>AUU (Ile), UAU (Tyr), UUA (Leu), GUU (Val), UGU (Cys), UUG (Leu)</i>
<i>UUC</i>	<i>AUC (Ile), UAC (Tyr), UUA (Leu), GUC (Val), UGC (Cys), UUG (Leu)</i>

- c. If a single transition occurs in a codon that specifies Leu, what amino acids could be specified by the mutated sequence?

<i>Original codon</i>	<i>Mutated codon (amino acid encoded)</i>
<i>CUU</i>	<i>UUU (Phe), CCU (Pro), CUC (Leu)</i>
<i>CUC</i>	<i>UUC (Phe), CCC (Pro), CUG (Leu)</i>
<i>CUA</i>	<i>UUA (Leu), CCA (Pro), CUG (Leu)</i>
<i>CUG</i>	<i>UUG (Leu), CCG (Pro), CUA (Leu)</i>
<i>UUG</i>	<i>CUG (Leu), UCG (Ser), UUA (Ser)</i>
<i>UUA</i>	<i>CUA (Leu), UCG (Ser), UUG (Leu)</i>

- d. If a single transversion occurs in a codon that specifies Leu, what amino acids could be specified by the mutated sequence?

<i>Original codon</i>	<i>Mutated codon (amino acid encoded)</i>
<i>UUA</i>	<i>AUA (Met), UAA (Stop), UUU (Phe), GUA (Val), UGA (Stop), UUC (Phe)</i>
<i>UUG</i>	<i>AUG (Met), UAG (Stop), UUU (Phe), GUG (Val), UGG (Trp), UUC (Phe)</i>
<i>CUU</i>	<i>GUU (Val), CGU (Arg), CUG (Leu), AUU (Ile),</i>
<i>CUC</i>	<i>AUC (Ile), CAC (His), CUA (Leu), GUC (Val), CGC (Arg), CUG (Leu)</i>
<i>CUA</i>	<i>AUA (Ile), CAA (Gln), CUC (Leu), GUA (Val), CGA (Arg), CUG (Leu)</i>
<i>CUG</i>	<i>AUG (Met), CAG (Gln), CUC (Leu), GUG (Val), CGG (Arg), CUU (Leu)</i>

18. Hemoglobin is a complex protein that contains four polypeptide chains. The normal hemoglobin found in adults—called adult hemoglobin—consists of two  $\alpha$  and two  $\beta$  polypeptide chains, which are encoded by different loci. Sickle-cell hemoglobin, which causes sickle-cell anemia, arises from a mutation in the  $\beta$  chain of adult hemoglobin. Adult hemoglobin and sickle-cell hemoglobin differ in a single amino acid: the sixth amino acid from one end in adult hemoglobin is glutamic acid, whereas sickle-cell hemoglobin has valine at this position. After consulting the genetic code provided in Figure 15.10, indicate the type and location of the mutation that gave rise to sickle-cell anemia.

There are two possible codons for glutamic acid, GAA and GAG. Single-base substitutions at the second position in both codons can produce codons that encode valine:

GAA-----> GUA (Val)

GAG-----> GUG (Val)

Both substitutions are transversions. However, in the gene encoding the  $\beta$  chain of hemoglobin, the GAG codon is the wild-type codon and the mutated GUG codon results in the sickle-cell phenotype.

- \*19. The following nucleotide sequence is found on the template strand of DNA. First, determine the amino acids of the protein encoded by this sequence by using the genetic code provided in Figure 15.10. Then, give the altered amino acid sequence of the protein that will be found in each of the following mutations.

Sequence of DNA template: 3'-TAC TGG CCG TTA GTT GAT ATA ACT-5'

Nucleotide number  $\rightarrow$  1 24

mRNA sequence: 5'-AUG ACC GGC AAU CAA CUA UAU UGA-3'

amino acid sequence: Amino-Met Thr Gly Asn Gln Leu Tyr Stop-Carboxyl

- a. Mutant 1: A transition at nucleotide 11

The transition results in the substitution of Ser for Asn.

original sequence: 3'-TAC TGG CCG TTA GTT GAT ATA ACT-5'

mutated sequence: 3'-TAC TGG CCG TCA GTT GAT ATA ACT-5'

mRNA sequence: 5'-AUG ACC GGC AGU CAA CUA UAU UGA-3'

amino acids: Amino-Met Thr Gly **Ser** Gln Leu Tyr Stop-Carboxyl

- b. Mutant 2: A transition at nucleotide 13

The transition results in the formation of a UAA nonsense codon.

original sequence: 3'-TAC TGG CCG TTA GTT GAT ATA ACT-5'

mutated sequence: 3'-TAC TGG CCG TTA ATT GAT ATA ACT-5'

mRNA sequence: 5'-AUG ACC GGC AAU UAA CUA UAU UGA-3'

amino acid sequence: Amino-Met Thr Gly Asn **STOP**-Carboxyl

- c. Mutant 3: A one-nucleotide deletion at nucleotide 7

The one-nucleotide deletion results in a frameshift mutation.

original sequence: 3'-TAC TGG CCG TTA GTT GAT ATA ACT-5'

mutated sequence: 3'-TAC TGG **CGT TAG TTG ATA TAA CT**-5'

mRNA sequence: 5'-AUG ACC **GCA GUC AAC UAU AUU GA**-3'

amino acids: Amino-Met Thr **Ala Ile Asn Tyr Ile** -Carboxyl

- d. Mutant 4: A T  $\rightarrow$  A transversion at nucleotide 15

The transversion results in the substitution of His for Gln in the protein.

original sequence: 3'-TAC TGG CCG TTA GTT GAT ATA ACT-5'

mutated sequence: 3'-TAC TGG CCG TTA **GTA** GAT ATA ACT-5'

mRNA sequence: 5'-AUG ACC GGC AAU CAU CUA UAU UGA-3'

amino acids: Amino-Met Thr Gly Asn **His** Leu Tyr Stop-Carboxyl

or

mutated sequence: 3'-TAC TGG CCG TTA **GTG** GAT ATA ACT-5'

mRNA sequence: 5'-AUG ACC GGC AAU CAC CUA UAU UGA-3'

amino acids: Amino-Met Thr Gly Asn **His** Leu Tyr Stop-Carboxyl

- e. Mutant 5: An addition of TGG after nucleotide 6  
*The addition of the three nucleotides results in the addition of Thr to the amino acid sequence of the protein.*  
*original sequence: 3'-TAC TGG CCG TTA GTT GAT ATA ACT-5'*  
*mutated sequence: 3'-TAC TGG TGG CCG TTA GTT GAT ATA ACT-5'*  
*mRNA sequence: 5'-AUG ACC ACC GGC AAU CAA CUA UAU UGA-3'*  
*amino acids: Amino-Met Thr **Thr** Gly Asn Gln Leu Tyr Stop-Carboxyl*
- f. Mutant 6: A transition at nucleotide 9  
*The protein retains the original amino acid sequence.*  
*original sequence: 3'-TAC TGG CCG TTA GTT GAT ATA ACT-5'*  
*mutated sequence: 3'-TAC TGG CCA TTA GTT GAT ATA ACT-5'*  
*mRNA Sequence: 5'-AUG ACC GGU AAU CAA CUA UAU UGA -3'*  
*amino acids: Amino-Met Thr **Gly** Asn Gln Leu Tyr Stop-Carboxyl*

20. A polypeptide has the following amino acid sequence:

Met-Ser-Pro-Arg-Leu-Glu-Gly

The amino acid sequence of this polypeptide was determined in series of mutants listed in parts a. through e. For each mutant, indicate the type of change that occurred in the DNA (single-base substitution, insertion, deletion) and the phenotypic effect of the mutation (nonsense mutation, missense mutation, frameshift, etc.).

- a. Mutant 1: Met-Ser-Ser-Arg-Leu-Glu-Gly  
*A missense mutation has occurred resulting in the substitution of Ser for Pro in the protein. The change is most likely due to a single-base substitution in the Ser codon resulting in the production of a Pro codon. Four of the Ser codons can be changed to Pro codons by a single transition mutation.*

<u>Pro</u>	<u>Ser</u>
CCU	UCU
CCC	UCC
CCA	UCA
CCG	UCG

- b. Mutant 2: Met-Ser-Pro  
*A single-base substitution has occurred in the Arg codon resulting in the formation of a stop codon. Two of the potential codons for Arg can be changed by single substitutions to stop codons. The phenotypic effect is a nonsense mutation.*

<u>Arg</u>	<u>Stop</u>	
CGA	UGA	transition mutation
AGA	UGA	transversion mutation

- c. Mutant 3: Met-Ser-Pro-Asp-Trp-Arg-Asp-Lys  
*The deletion of a single nucleotide at the first position in the Arg codon (most likely CGA) has resulted in a frameshift mutation in which the mRNA is read in a different frame, producing a different amino acid sequence for the protein.*

**d.** Mutant 4: Met-Ser-Pro-Glu-Gly

*A six-base pair deletion has occurred, resulting in the elimination of two amino acids (Arg and Leu) from the protein. The result is a truncated polypeptide chain.*

**e.** Mutant 5: Met-Ser-Pro-Arg-Leu-Leu-Glu-Gly

*The addition or insertion of three nucleotides into the DNA sequence has resulted in the addition of a Leu codon to the polypeptide chain.*

\*21. A gene encodes a protein with the following amino acid sequence:

Met-Trp-His-Arg-Ala-Ser-Phe.

A mutation occurs in the gene. The mutant protein has the following amino acid sequence:

Met-Trp-His-Ser-Ala-Ser-Phe.

An intragenic suppressor restores the amino acid sequence to that of the original protein:

Met-Trp-His-Arg-Ala-Ser-Phe.

Give at least one example of base changes that could produce the original mutation and the intragenic suppressor. (Consult the genetic code in Figure 15.10.)

*Four of the six Arg codons could be mutated by a single-base substitution to produce a Ser codon. However, only two of the Arg codons mutated to form Ser codons could subsequently be mutated at a second position by a single-base substitution to regenerate the Arg codon. In both events, the mutations are transversions.*

<u>Original Arg codon</u>	<u>Ser codon</u>	<u>Restored Arg codon</u>
CGU	AGU	AGG or AGA
CGC	AGC	AGG or AGA

22. A gene encodes a protein with the following amino acid sequence:

Met-Lys-Ser-Pro-Ala-Thr-Pro

A nonsense mutation from a single-base-pair substitution occurs in this gene, resulting in a protein with the amino acid sequence Met-Lys. An intergenic suppressor mutation allows the gene to produce the full-length protein. With the original mutation and the intergenic suppressor present, the gene now produces a protein with the following amino acid sequence:

Met-Lys-Cys-Pro-Ala-Thr-Pro

Give the location and nature of the original mutation and the intergenic suppressor. *The original mutation is located in the Ser codon. Two of the six potential Ser codons (UCA and UCG) can be changed to stop codons by a single-base substitution.*

*UCA → UGA (transversion)*

*UCG → UAG (transversion)*

*However, only the UCA codon is likely to be suppressed by Cys-tRNA containing a single-base substitution (a transversion mutation) in the anticodon (5'-ACA-3' to 5'-UCA-3'), allowing for pairing with the UGA nonsense codon and suppression of the nonsense phenotype. The suppression is due to the insertion of Cys for the codon UGA.*

23. XG syndrome is a rare genetic disease that is due to an autosomal dominant gene. A complete census of a small European country reveals that 77,536 babies were born in 2004, of whom 3 had XG syndrome. In the same year, this country had a population of 5,964,321 people, and there were 35 living persons with XG syndrome. What is the mutation rate of XG syndrome in this country?  
*The mutation rate for XG syndrome during the year 2000 can be expressed as the number of mutations per gamete, and because each individual is the product of two gametes, then  $3/(77,536 \cdot 2) = 1.93 \times 10^{-5}$ .*

### Section 18.2

- \*24. Can nonsense mutations be reversed by hydroxylamine? Why or why not?  
*No, hydroxylamine cannot reverse nonsense mutations. Hydroxylamine modifies cytosine-containing nucleotides and can only result in GC to AT transition mutations. In a stop codon, the GC to AT transition will result only in a different stop codon.*

For 5'-UGA-3':      Template DNA:      3'-ACT-5'  
    Coding DNA:      5'-TGA-3'  
 Transition results:      Template DNA:      3'-ATT-5'  
 GC to AT                    Coding DNA:      3'-TAA-5'  
    mRNA codon:      5'-UAA-3'

For 5'-UAG-3':      Template DNA:      3'-ATC-5'  
    Coding DNA:      5'-TAG-3'  
 Transition results:      Template DNA:      3'-ATT-5'  
 In GC to AT                    Coding DNA:      3'-TAA-5'  
    mRNA codon:      5'-UAA-3'

- \*25. The following nucleotide sequence is found in a short stretch of DNA:

5'-ATGT-3'  
 3'-TACA-5'

If this sequence is treated with hydroxylamine, what sequences will result after replication?

*Hydroxylamine adds hydroxyl groups to cytosine, enabling the modified cytosine to occasionally pair with adenine, which ultimately can result in a GC to AT transition. Therefore, only one base pair in the sequence will be affected. Ultimately, after replication, one of the dsDNA molecules will have the transition but not the other dsDNA molecule.*

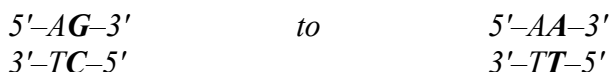
<u>Original sequence</u>	<u>Mutated sequence</u>
5'-ATGT-3'	5'-ATAT-3'
3'-TACA-5'	3'-TATA-5'

26. The following nucleotide sequence is found in a short stretch of DNA:

5'-AG-3'  
 3'-TC-5'

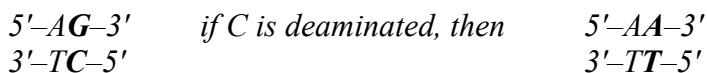
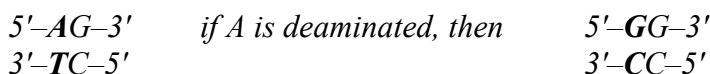
- a. Give all the mutant sequences that may result from spontaneous depurination occurring in this stretch of DNA.

*The strand contains two purines, adenine and guanine. Because repair of depurination typically results in adenine being substituted for the missing purine, only the loss of the guanine by depurination will result in a mutant sequence.*



- b. Give all the mutant sequences that may result from spontaneous deamination occurring in this stretch of DNA.

*Deamination of guanine, cytosine, and adenine can occur. However, the deamination of only cytosine and adenine are likely to result in mutant sequences because the deamination products can form improper base pairs. The deamination of guanine does not pair with thymine but can still form two hydrogen bonds with cytosine, thus no change will occur.*



27. In many eukaryotic organisms, a significant proportion of cytosine bases are naturally methylated to 5-methylcytosine. Through evolutionary time, the proportion of AT base pairs in the DNA of these organisms increases. Can you suggest a possible mechanism by which this increase occurs?  
*Spontaneous deamination of 5-methylcytosine produces thymine. If the subsequent repair of the GT mispairing is repaired incorrectly or, more likely, not repaired at all because the thymine is a normal base, then a GC to AT transition will result. Over time, the incorrect repairs will lead to an increase in the number of AT base pairs.*

### Section 18.3

- \*28. A chemist synthesizes four new chemical compounds in the laboratory and names them PFI1, PFI2, PFI3, and PFI4. He gives the PFI compounds to a geneticist friend and asks her to determine their mutagenic potential. The geneticist finds that all four are highly mutagenic. She also tests the capacity of mutations produced by the PFI compounds to be reversed by other known mutagens and obtains the following results. What conclusions can you make about the nature of the mutations produced by these compounds?

Mutations produced by	Reversed by			
	2-Aminopurine	Nitrous acid	Hydroxylamine	Acridine orange
PFI1		Yes	Yes	Some No
PFI2	No	No	No	No
PFI3		Yes	Yes	No No
PFI4		No	No	No Yes

First, consider the mutagenic actions of the reversion agents: 2-Aminopurine causes GC to AT and AT to GC transitions; nitrous acid causes GC to AT and AT to GC transitions; hydroxylamine causes GC to AT transitions; acridine orange causes single-base insertions or deletions, resulting in frameshift mutations.

PFI1 causes both types of transitions, GC to AT and AT to GC. PFI1 mutations can be reversed by 2-aminopurine, nitrous acid, and occasionally by hydroxylamine, which suggests that it acts as a transition mutagen. The lack of reversion of all PFI1 mutations by hydroxylamine suggests that some of the mutations were caused by GC to AT substitutions because they are not reverted by hydroxylamine.

PFI2 causes transversions, or large deletions, because mutations caused by PFI2 are not reverted by any of the agents.

PFI3 causes GC to AT transitions. Only GC to AT transitions could be reverted by 2-aminopurine and nitrous oxide but not by hydroxylamine.

PFI4 causes single-base insertions or deletions. PFI4 is only reverted by acridine orange, an intercalating agent that only reverts single-base insertions or deletions.

29. Mary Alexander studied the effects of radiation on mutation rates in the sperm of *Drosophila melanogaster*. She irradiated *Drosophila* larvae with either 3000 or 3975 roentgens (r), collected the adult males that developed from irradiated larvae, mated them with unirradiated females, and then counted the number of mutant F<sub>1</sub> flies produced by each male. All mutant flies that appeared were used in subsequent crosses to determine if their mutant phenotypes were genetic. She obtained the following results (M. L. Alexander. 1954. *Genetics* 39:409–428):

Group	Number of offspring	Offspring with a genetic mutation
Control (0 r)	45,504	0
Irradiated (3000 r)	49,512	71
Irradiated (3975 r)	50,159	70

- a. Calculate the mutation rates of the control group and the two groups of irradiated flies.

Because no mutations were detected in the control group, the mutation rate per gamete must be less than  $1/(45,504 \cdot 2)$  or  $1.09 \cdot 10^{-5}$ .

For flies irradiated with 3000 r, the mutation rate per gamete is  $71/(49,512 \cdot 2) = 7.17 \cdot 10^{-4}$ .

*For flies irradiated with 3975 r, the mutation rate per gamete is  $70/(50,159 \cdot 2) = 6.98 \cdot 10^{-4}$ .*

- b.** On the basis of these data, do you think radiation has any effect on mutation? Explain your answer.

*Yes, the radiation significantly increased the mutation rate. Male flies exposed to radiation had higher incidences of offspring with mutations.*

30. A genetics instructor designs a laboratory experiment to study the effects of UV radiation on mutation in bacteria. In the experiment, the students expose bacteria plated on petri plates to UV light for different lengths of time, place the plates in an incubator for 48 hours, and then count number of colonies that appear on each plate. The plates that have received more UV radiation should have more pyrimidine dimers, which block replication; thus, fewer colonies should appear on the plates exposed to UV light for longer periods of time. Before the students carry out the experiment, the instructor warns them that, while the bacteria are in the incubator, the students must not open the incubator door unless the room is darkened. Why should the bacteria not be exposed to light?

*Exposure of DNA to UV light results in the formation of pyrimidine dimers in the DNA molecule. Often the repair of these dimers leads to mutations. Because the SOS repair system is error-prone and leads to an increased accumulation of mutations, UV light produces more mutations in bacteria when the SOS repair system is activated to repair the damage caused by the UV light. However, many species of bacteria have a direct DNA repair system that can repair pyrimidine dimers by breaking the covalent linkages between the pyrimidines that form the dimer. The enzyme that repairs the DNA is called photolyase, and is activated and energized by light. The photolyase is a very efficient repair enzyme and typically makes accurate repairs of the damage. If the bacteria in the UV radiation experiment are exposed to light, then the photolyase will be activated to repair the damage, resulting in fewer mutations in the irradiated bacteria.*

#### Section 18.4

- \*31. A plant breeder wants to isolate mutants in tomatoes that are defective in DNA repair. However, this breeder does not have the expertise or equipment to study enzymes in DNA repair systems. How could the breeder identify tomato plants that are deficient in DNA repair? What are the traits to look for?

*The plant breeder should look for plants that have increased levels of mutations either in their germ-line or somatic tissues. Potentially mutant plants could be exposed to standard mutagens that damage DNA. If they are defective in DNA repair they should have higher rates of mutation. For example tomato plants with defective DNA repair systems should have an increased mutation rate when exposed to high levels of sunlight.*

**CHALLENGE QUESTIONS****Section 18.1**

32. Robert Bost and Richard Cribbs studied a strain of *E. coli* (*araB14*) that possessed a nonsense mutation in the structural gene that encodes L-ribulokinase, an enzyme that allows the bacteria to metabolize the sugar arabinose (R. Bost and R. Cribbs. 1969. *Genetics* 62:1–8). From the *araB14* strain, they isolated some bacteria that possessed mutations that caused them to revert back to the wild type. Genetic analysis of these revertants showed that they possessed two different suppressor mutations. One suppressor mutation (*R1*) was linked to the original mutation in the L-ribulokinase and probably occurred at the same locus. By itself, this mutation allowed the production of L-ribulokinase, but the enzyme was not as effective in metabolizing arabinose as the enzyme encoded by the wild-type allele. The second suppressor mutation (*Su<sup>B</sup>*) was not linked to the original mutation. In conjunction with the *R1* mutation, *Su<sup>B</sup>* allowed the production of L-ribulokinase, but *Su<sup>B</sup>* by itself was not able to suppress the original mutation.
- On the basis of this information, are the *R1* and *Su<sup>B</sup>* mutations intragenic suppressors or intergenic suppressors? Explain your reasoning.  
*R1 is an intragenic suppressor. As the studies indicated, it likely occurred within the same locus as the araB14 mutation. Su<sup>B</sup> is an intergenic suppressor because it was not linked to the original mutation and occurred at a different locus.*
  - Propose an explanation for how *R1* and *Su<sup>B</sup>* restore the ability of *araB14* to metabolize arabinose and why *Su<sup>B</sup>* is able to more fully restore the ability to metabolize arabinose.  
*Potentially, the R1 mutation changed the nonsense codon found in araB14 into a sense codon although not back to the original wild-type codon. The new sense codon in the R1 mutation allows for the insertion of an amino acid and a full-length protein is now synthesized that contains a missense mutation. Su<sup>B</sup> encodes a mutation in a tRNA anticodon that allows for another amino acid to be inserted at the R1 mutant codon restoring more of the L-ribulokinase function.*
33. Achondroplasia is an autosomal dominant disorder characterized by disproportionate short stature—the legs and arms are short compared with the head and trunk. The disorder is due to a base substitution in the gene, located on the short arm of chromosome 4, for fibroblast growth factor receptor 3 (FGFR3).
- Although achondroplasia is clearly inherited as an autosomal dominant trait, more than 80% of the people who have achondroplasia are born to parents with normal stature. This high percentage indicates that most cases are caused by newly arising mutations; these cases (not inherited from an affected parent) are referred to as sporadic. Findings from molecular studies have demonstrated that sporadic cases of achondroplasia are almost always caused by mutations inherited from the father (paternal mutations). In addition, the occurrence of achondroplasia is higher among older fathers; indeed, approximately 50% of children with achondroplasia are born

to fathers older than 35 years of age. There is no association with maternal age. The mutation rate for achondroplasia (about  $4 \cdot 10^{-5}$  mutations per gamete) is high compared with those for other genetic disorders. Explain why most spontaneous mutations for achondroplasia are paternal in origin and why the occurrence of achondroplasia is higher among older fathers.

*In men, sperm cells are produced throughout much of their life. The cells responsible for the sperm production are called spermatogonia. These spermatogonia divide by mitosis to produce more spermatogonia and produce spermatocytes, cells that eventually will divide by meiosis to produce sperm cells. These continued cell divisions by the spermatogonia, particularly to produce more spermatogonia, could lead to an increased chance of mutations within the DNA of the spermatogonia cell. Essentially, the more cell divisions the greater the risk for mutation. Also some DNA sequences may be more susceptible to mutations than others. These locations are called hot spots. Potentially, the base substitution occurs at a hot spot in the FGFR3 gene as more cell divisions occur. In addition, as men age, their exposure to environmental factors over time may increase mutation rates including that of the FGFR3 gene.*

*However, recent data do not support that the increase in mutations is due only to an increase in mutations as a result of spermatogonia mitosis. A second possibility is that the mutation may confer a positive benefit to the spermatogonia or, ultimately, to the sperm cells produced. Potentially, the mutated sperm have a higher survival rate than normal sperm cells leading to an increased risk of fertilization by the FGFR3 mutation containing sperm cells.*

34. Tay–Sachs disease is a severe, autosomal recessive genetic disease that produces deafness, blindness, seizures, and eventually death. The disease results from a defect in the *HEXA* gene, which codes for hexosaminidase A. This enzyme normally degrades GM2 gangliosides. In the absence of hexosaminidase A, GM2 gangliosides accumulate in the brain. Recent molecular studies have shown that the most common mutation causing Tay–Sachs disease is a 4 bp insertion that produces a downstream premature stop codon. Further studies reveal that normal transcription of the *HEXA* gene occurs in individuals with Tay–Sachs disease, but the *HEXA* mRNA is unstable. Propose a mechanism to account for how a premature stop codon could cause mRNA instability.

*The stability of the mRNA molecule may be dependent on the translational machinery. If the ribosome terminates prematurely, then the mRNA molecule may become a target of ribonucleases. Essentially, the premature stop codon has marked the HEXA mRNA for destruction in Tay–Sachs individuals.*

*Recent data have shown that the premature stop codon within the HEXA mRNA molecule leads to the rapid destruction of the HEXA mRNA within the cell. The destruction is likely accomplished through a process called nonsense-mediated mRNA decay (NMD). In the current model of NMD function, the NMD pathway is stimulated by the premature termination of protein synthesis at a nonsense codon located 50 or more nucleotides upstream of the final exon-exon junction. A exon junction protein complex (EJC) forms approximately 20 to 24 nucleotides upstream of the final exon-exon junction. The EJC stimulates decay of the mRNA molecule*

*unless it is removed by the ribosome during translation. In the normal HEXA mRNA translation, the first ribosome removes the EJC because the termination codon is located downstream of the EJC. In individuals with Tay Sachs, the premature stop codon is located upstream of the EJC resulting in termination before the first ribosome can remove the EJC. Because the EJC is not removed, stimulation of NMD occurs and the mutated HEXA mRNA is degraded.*

35. Mutations *ochre* and *amber* are two types of nonsense mutations. Before the genetic code was worked out, Sydney Brenner, Anthony O. Stretton, and Samuel Kaplan applied different types of mutagens to bacteriophages in an attempt to determine the bases present in the codons responsible for *amber* and *ochre* mutations. They knew that *ochre* and *amber* mutants were suppressed by different types of mutations, demonstrating that each is a different termination codon. They obtained the following results:
- (1) A single-base substitution could convert an *ochre* mutation into an *amber* mutation.
  - (2) Hydroxylamine induced both *ochre* and *amber* mutations in wild-type phages.
  - (3) 2-Aminopurine caused *ochre* to mutate to *amber*.
  - (4) Hydroxylamine did not cause *ochre* to mutate to *amber*.

These data do not allow the complete nucleotide sequence of the *amber* and *ochre* codons to be worked out, but they do provide some information about the bases found in the nonsense mutations.

- a. What conclusions about the bases found in the codons of *amber* and *ochre* mutations can be made from these observations?

*In considering the data, it is important to remember the mutagenic actions of hydroxylamine and 2-aminopurine. Hydroxylamine produces only GC to AT transition mutations. However, 2-aminopurine can produce both types of transitions, GC to AT and AT to GC.*

*Because hydroxylamine can be used to produce amber and ochre mutations from wild-type phages, then amber and ochre codons must contain uracil and/or adenine. The production of amber mutations from ochre codons by 2-aminopurine but not by hydroxylamine suggests that ochre mutations do contain adenine and uracil, while amber mutations contain guanine and also contain either adenine and/or uracil.*

- b. Of the three nonsense codons (UAA, UAG, UGA), which represents the *ochre* mutation?

*Based on the mutagenesis data, only the UAA codon matches the results for the ochre mutation. It is the only stop codon that does not contain guanine.*

### Section 18.3

36. To determine whether radiation associated with the atomic bombings of Hiroshima and Nagasaki produced recessive germ-line mutations, scientists examined the sex ratio of the children of the survivors of the blasts. Can you explain why an increase in germ-line mutations might be expected to alter the sex ratio?

*Recessive germ-line mutations on the X chromosome have the potential to alter the sex ratios. Female offspring who have a single recessive mutation on the X chromosome will also possess a nonmutated allele on their other X chromosome. Therefore, females will be heterozygous for the pair of alleles. Male offspring, however, have only one X chromosome, and a mutation within an allele on the X chromosome will result in a phenotypic change. If the allele mutated is essential, then the mutation for males will be lethal. Essentially, recessive mutations on the X chromosome in the germ-line could lead to fewer males being born.*

#### **Section 18.4**

37. Trichothiodystrophy is an inherited disorder in humans that is characterized by premature aging, including osteoporosis, osteosclerosis, early graying, infertility, and reduced life span. Recent studies have shown that the mutation that causes this disorder occurs in a gene that encodes a DNA helicase. Propose a mechanism for how a mutation in a DNA helicase might cause premature aging. Be sure to relate the symptoms of the disorder to possible functions of the helicase enzyme.

*The DNA helicase protein (XPD) is involved in DNA repair process, specifically in nucleotide excision repair to displace damaged nucleotides. If the helicase is not functioning properly, then the damaged nucleotides may not be removed, which may result in the accumulation of DNA damage within the chromosomes of these cells. The accumulation of DNA damage in these cells could lead either to apoptosis (programmed cell death) or to cellular senescence followed by cellular death. In either case, the cells affected will not divide and reproduce through cellular division processes. Cells responsible for bone formation, hair color, and fertility if affected by the DNA damage and subsequent lack of repair would ultimately cease dividing. This lack of new cell production would lead to bone weakening and loss, lack of production of new melanocytes needed for hair color, and a lack of production of reproductive cells.*

*Because this DNA helicase is also associated with a transcription factor (TFIIH) needed in transcription by RNA polymerase II, a second possibility may involve its role in transcription leading to a decline in levels of transcription. Current data suggest that the DNA helicase may be involved in transcriptionally coupled DNA repair, where its function may be to remove the stalled RNA polymerase from DNA that has been damaged. The removal of the RNA polymerase would allow for repair of the damaged section. If the RNA polymerase is not removed, it may prevent repair. A decline in transcriptional function due to malfunctioning transcription factors or a lack of repair because of a stalled RNA polymerase could lead to a decline in cellular functions and cellular death as well.*