16

Mutation, Repair, and Recombination

WORKING WITH THE FIGURES

1. In Figure 16-3a, what is the consequence of the new 5′ splice site on the open reading frame? In 16-3b, how big could the intron be to maintain the reading frame (let’s say between 75 and 100 bp)?

Answer: A mutation that generates a new 5′ splice site within an existing exon will result in the loss of information from the open reading frame, since some of the exon will be removed with the splicing of the intron. In addition, because the number of nucleotides deleted is not a multiple of 3 (64 nt) a frameshift mutation will result. In part b of the figure, the retained intron will maintain the reading frame as long as the length in nucleotides is divisible by three (ex. 99 bp) and it does not contain any stop codons in the same frame as exon 1.

2. Using Figure 16-4 as an example, compare the migration of RNA and protein for the wild-type gene and the mutation shown in Figure 16-3b. Assume that the retained intron maintains the reading frame.

Answer: The RNA produced by the mutation in Figure 16-3b will be longer than the mature wild-type RNA and thus will not migrate as far in the gel. The same will be true of the protein (mutant protein will be larger than wild-type protein) if the reading frame is maintained and no stop codons are present in the sequence.

3. In the Ames test shown in Figure 16-17, what is the reason for adding the liver extract to each sample?

Answer: Sometimes compounds only become mutagenic when processed by the enzymes in the vertebrate liver. Therefore, although exposure to these compounds in humans may cause cancer, they would not give a positive result in a bacterial mutation assay.
4. Based on the mode of action of aflatoxin (Figure 16-16), propose a scenario that explains its response in the Ames test (Figure 16-18).

   Answer: Aflatoxin B₁ becomes covalently attached to guanine residues in the DNA. The addition of the aflatoxin adduct destabilizes the N-glycosidic bond, leaving an abasic site. The replicative polymerases cannot synthesize DNA across from an abasic site, requiring the use of a bypass polymerase. In the absence of complimentary base-pairing information, the polymerase will frequently incorporate the wrong nucleotide opposite the abasic site. This will result in daughter cells with base substitutions, as observed in the Ames test.

5. In Figure 16-22, point out the mutant protein(s) in patients with Cockayne syndrome. What protein(s) is/are mutant in patients with XP? How are these different mutations thought to account for the different disease symptoms?

   Answer: The proteins CSA or CSB are mutant in Cockayne syndrome, while the XPB and XPD proteins are defective in patients with Xeroderma pigmentosum. The defective proteins in Cockayne syndrome cannot recognize RNA polymerase complexes stalled by DNA damage. This failure may trigger apoptosis. Defects observed in Cockayne syndrome may be the result of both apoptosis and a failure to repair lesions, while XP defects may result primarily from a failure to repair DNA.

6. The MutH protein nicks the newly synthesized strand (Figure 16-23). How does it “know” which strand this is?

   Answer: The MutH protein nicks the unmethylated strand at hemimethylated GATC sequences. The transient delay in the methylation of the newly synthesized strand at these sequences therefore serves as a signal for repair.

7. What features of the bypass polymerase make it ideal for its role in translesion synthesis, shown in Figure 16-24?

   Answer: The active sites of these polymerases can accommodate bulky adducts that the replicative polymerases cannot, and they lack the 3′-5′ exonuclease “proofreading” activity. In addition, they are distributive polymerases, adding only a few nucleotides at a time before falling off of the substrate, limiting the amount of DNA synthesized by these error prone enzymes.

**BASIC PROBLEMS**

8. Consider the following wild-type and mutant sequences:

   Wild-type   ....CTTGCAAGCGAATC....
Mutant ....CTGCTAGCGAATC....

The substitution shown seems to have created a stop codon. What further information do you need to be confident that it has done so?

Answer: You need to know the reading frame of the possible message.

9. What type of mutation is depicted by the following sequences (shown as mRNA)?

Wild type ....5′ AAUCCUUACGGA 3′....
Mutant ....5′ AAUCCUACGGA 3′....

Answer: The mutant has a deletion of one base, and this will result in a frameshift (−1) mutation.

10. Can a missense mutation of proline to histidine be made with a G·C → A·T transition-causing mutagen? What about a proline-to-serine missense mutation?

Answer: Proline can be coded for by CCN (N stands for any nucleotide) and histidine can be coded for by CAU or CAC. For a mutation to change a proline codon to a histidine codon requires a transversion (C to A) at the middle position. Therefore, a transition-causing mutagen cannot cause this change. Serine can be coded for by UCN. A change from C to U at the first position (a transition) would cause this missense mutation and would be possible with this mutagen.

11. By base-pair substitution, what are all the synonymous changes that can be made starting with the codon CGG?

Answer: Assuming single base-pair substitutions, then CGG can be changed to CGU, CGA, CGC, or AGG and still would code for arginine.

12. a. What are all the transversions that can be made starting with the codon CGG?
   b. Which of these transversions will be missense? Can you be sure?

Answer:
   a. and b. By transversion, CGG (arginine) can be become AGG (arginine), GGG (glycine), CCG (proline), CUG (leucine), CGC (arginine), or CGU (arginine).
13. **a.** Acridine orange is an effective mutagen for producing null alleles by mutation. Why does it produce null alleles?
   
b. A certain acridine-like compound generates only single insertions. A mutation induced with this compound is treated with the same compound, and some revertants are produced. How is this outcome possible?

Answer:
   
a. Acridine orange causes frameshift mutations and frameshift mutations often result in null alleles.

b. A +1 frameshift mutation can be reverted by two further single insertions so that the reading frame is re-established.

14. **Defend the statement “Cancer is a genetic disease.”**

   Answer: The following is a list of observations that argue “cancer is a genetic disease:”

   1. Certain cancers are inherited as highly penetrant simple Mendelian traits.
   2. Most carcinogenic agents are also mutagenic.
   3. Various oncogenes have been isolated from tumor viruses.
   4. A number of genes that lead to the susceptibility to particular types of cancer have been mapped, isolated, and studied.
   5. Dominant oncogenes have been isolated from tumor cells.
   6. Certain cancers are highly correlated to specific chromosomal rearrangements. (See Chapter 17 of the companion text.)

15. **Give an example of a DNA-repair defect that leads to cancer.**

   Answer: XP (xeroderma pigmentosum) patients lack nucleotide excision repair and are highly prone to developing pigmented skin cancers. Individuals with HNPCC (hereditary nonpolyposis colorectal cancer) are prone to colorectal cancer due to a loss of the mismatch repair system. Individuals homozygous for mutations in *BRCA1* or *BRCA2* (breast cancer predisposition genes 1 and 2) are prone to breast cancer due to the loss of repair of double-stranded breaks.
16. In mismatch repair in *E. coli*, only a mismatch in the newly synthesized strand is corrected. How is *E. coli* able to recognize the newly synthesized strand? Why does this ability make biological sense?

Answer: DNA in *E. coli* is methylated. To distinguish the old template strand from the newly synthesized strand, the mismatch repair mechanism takes advantage of a delay in the methylation of the new strand. This makes sense as replication errors produce mismatches only on the newly synthesized strand, so the mismatch repair system replaces the “wrong” base on that strand.

17. A mutational lesion results in a sequence containing a mismatched base pair:

\[
\begin{align*}
5' & \text{AGCTGCCTT} \ 3' \\
3' & \text{ACGATGGAA} \ 5'
\end{align*}
\]

Codon

If mismatch repair occurs in either direction, which amino acids could be found at this site?

Answer: The mismatched “T” would be corrected to C and the resulting ACG, after transcription, would be 5’ UGC 3’ and code for cysteine. Or, if the other strand was corrected, ATG would be transcribed to 5’ UAC 3’ and code for tyrosine.

18. Under what circumstances could nonhomologous end joining be said to be error prone?

Answer: NHEJ (nonhomologous end-joining) is error prone as some sequence may be lost in the repair process. The consequences of imperfect repair may be far less harmful than leaving the lesion unrepaired. Presumably this repair pathway evolved because, unless repaired, the broken ends can degrade further, leading to loss of more genetic information. Also, these lesions can initiate potentially harmful chromosomal rearrangements that could lead to cell death.

19. Why are many chemicals that test positive by the Ames test also classified as carcinogens?

Answer: There is a very strong correlation between mutagens and carcinogens. As discussed in Problem 14, cancer is a genetic disease. Therefore, any chemical classified as a mutagen by the Ames test should also be considered a carcinogen.
20. The Spo11 protein is conserved in eukaryotes. Do you think it is also conserved in bacterial species? Justify your answer.

Answer: Meiotic recombination is initialized when the Spo11 protein makes double-strand cuts in one of the homologous chromosomes. It is highly conserved in eukaryotes, indicating that this mechanism to initiate recombination is also conserved. Bacterial species do not have reciprocal meiotic recombination, so you would not expect this function to be conserved.

21. Differentiate between the elements of the following pairs:
   a. Transitions and transversions
   b. Synonymous and neutral mutations
   c. Missense and nonsense mutations
   d. Frameshift and nonsense mutations

Answer:
   a. A transition mutation is the substitution of a purine for a purine or the substitution of a pyrimidine for a pyrimidine. A transversion mutation is the substitution of a purine for a pyrimidine, or vice versa.

   b. Both are base-pair substitutions. A synonymous mutation is one that does not alter the amino acid sequence of the protein product from the gene, because the new codon codes for the same amino acid as did the nonmutant codon. A neutral mutation results in a different amino acid that is functionally equivalent, and the mutation therefore has no known adaptive significance.

   c. A missense mutation results in a different amino acid in the protein product of the gene. A nonsense mutation causes premature termination of translation, resulting in a shortened protein.

   d. Frameshift mutations arise from addition or deletion of one or more bases in other than multiples of three, thus altering the reading frame for translation. Therefore, the amino acid sequence from the site of the mutation to the end of the protein product of the gene will be altered. Frameshift mutations can and often do result in premature stop codons in the new reading frame, leading to shortened protein products. A nonsense mutation causes premature termination of translation in the original reading frame, resulting in a shortened protein.

22. Describe two spontaneous lesions that can lead to mutations.

Answer: Depurination results in the loss of the adenine or guanine base from the DNA backbone. Because the resulting apurinic site cannot specify a
complementary base, replication is blocked. Under certain conditions, replication proceeds with a near random insertion of a base opposite the apurinic site. In three-fourths of these insertions, a mutation will result.

Deamination of cytosine yields uracil. If left un repaired, the uracil will be paired with adenine during replication, ultimately resulting in a transition mutation.

Deamination of 5-methylcytosine yields thymine and thus frequently leads to C to T transitions.

Oxidatively damaged bases, such as 8-OxodG (8-oxo-7-hydrodeoxyguanosine) can pair with adenine, resulting in a transversion.

Errors during DNA replication can lead to spontaneous indel mutations.

23. What are bypass polymerases? How do they differ from the replicative polymerases? How do their special features facilitate their role in DNA repair?

Answer: Translesion or bypass polymerases are able to replicate past damaged DNA that otherwise would stall replicative polymerases. They differ from replicative polymerases in that they can tolerate large adducts on the bases (as they have much larger active sites that can accommodate damaged bases), they are much more error-prone (as they lack the 3’ to 5’ proofreading function), and they can only add relatively few nucleotides before falling off. Their main function is to unblock the replication fork, not to synthesize long stretches of DNA that could contain many mismatches.

24. In adult cells that have stopped dividing, what types of repair systems are possible?

Answer: There are many repair systems that are available: direct reversal, excision repair, transcription-coupled repair, and non-homologous end-joining.

25. A certain compound that is an analog of the base cytosine can become incorporated into DNA. It normally hydrogen bonds just as cytosine does, but it quite often isomerizes to a form that hydrogen bonds as thymine does. Do you expect this compound to be mutagenic, and, if so, what types of changes might it induce at the DNA level?

Answer: Yes. It will cause CG-to-TA transitions.
26. Two pathways, homologous recombination and nonhomologous end joining (NHEJ), can repair double-strand breaks in DNA. If homologous recombination is an error-free pathway whereas NHEJ is not always error free, why is NHEJ used most of the time in eukaryotes?

Answer: Since cells of higher eukaryotes are usually not replicating their DNA, error-free repair is not possible because there are no undamaged strands or sister chromatids available as templates for new DNA synthesis.

27. Which repair pathway recognizes DNA damage during transcription? What happens if the damage is not repaired?

Answer: DNA damage that stalls transcription is repaired by TC-NER (transcription-coupled nucleotide excision repair). Humans lacking this pathway suffer from Cockayne syndrome. A consequence of this defect is that a cell is much more likely to activate its apoptosis (cell suicide) pathway. Affected individuals are very sensitive to sunlight and have short stature, the appearance of premature aging, and a variety of developmental disorders.

**CHALLENGING PROBLEMS**

28. a. Why is it impossible to induce nonsense mutations (represented at the mRNA level by the triplets UAG, UAA, and UGA) by treating wild-type strains with mutagens that cause only A·T → G·C transitions in DNA?

b. Hydroxylamine (HA) causes only G·C → A·T transitions in DNA. Will HA produce nonsense mutations in wild-type strains?

c. Will HA treatment revert nonsense mutations?

Answer:

a. Because 5′-UAA-3′ does not contain G or C, a transition to a GC pair in the DNA cannot result in 5′-UAA-3′. 5′-UGA-3′ and 5′-UAG-3′ have the DNA antisense-strand sequence of 3′-ACT-5′ and 3′-ATC-5′, respectively. A transition to either of these stop codons occurs from the nonmutant 3′-ATT-5′. However, a DNA sequence of 3′-ATT-5′ results in an RNA sequence of 5′-UAA-3′, itself a stop codon.

b. Yes. An example is 5′-UGG-3′, which codes for trp, to 5′-UAG-3′.

c. No. In the three stop codons the only base that can be acted upon is G (in UAG, for instance). Replacing the G with an A would result in 5′-UAA-3′, a stop codon.
29. Several auxotrophic point mutants in *Neurospora* are treated with various agents to see if reversion will take place. The following results were obtained (a plus sign indicates reversion; HA causes only G · C → A · T transitions).

<table>
<thead>
<tr>
<th>Mutant</th>
<th>5-BU</th>
<th>HA</th>
<th>Proflavin</th>
<th>Spontaneous reversion</th>
</tr>
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<tbody>
<tr>
<td>1</td>
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</tbody>
</table>

**a.** For each of the five mutants, describe the nature of the original mutation event (not the reversion) at the molecular level. Be as specific as possible.

**b.** For each of the five mutants, name a possible mutagen that could have caused the original mutation event. (Spontaneous mutation is not an acceptable answer.)

**c.** In the reversion experiment for mutant 5, a particularly interesting prototrophic derivative is obtained. When this type is crossed with a standard wild-type strain, the progeny consist of 90 percent prototrophs and 10 percent auxotrophs. Give a full explanation for these results, including a precise reason for the frequencies observed.

**Answer:**

**a. and b.** Mutant 1: most likely a deletion. It could be caused by radiation.

Mutant 2: because proflavin causes either additions or deletions of bases and because spontaneous mutation can result in additions or deletions, the most probable cause was a frameshift mutation by an intercalating agent.

Mutant 3: 5-BU causes transitions, which means that the original mutation was most likely a transition. Because HA causes GC-to-AT transitions and HA cannot revert it, the original must have been a GC-to-AT transition. It could have been caused by base analogs.

Mutant 4: the chemical agents cause transitions or frameshift mutations. Because there is spontaneous reversion only, the original mutation must have been a transversion. X-irradiation or oxidizing agents could have caused the original mutation.

Mutant 5: HA causes transitions from GC-to-AT, as does 5-BU. The original mutation was most likely an AT-to-GC transition, which could be caused by base analogs.
c. The suggestion is a second-site reversion linked to the original mutant by 20 map units and therefore most likely in a second gene. Note that auxotrophs equal half the recombinants.

**Unpacking the Problem**

30. You are using nitrosoguanidine to “revert” mutant nic-2 (nicotinamide-requiring) alleles in *Neurospora*.

You treat cells, plate them on a medium without nicotinamide, and look for prototrophic colonies. You obtain the following results for two mutant alleles. Explain these results at the molecular level, and indicate how you would test your hypotheses.

a. With nic-2 allele 1, you obtain no prototrophs at all.

b. With nic-2 allele 2, you obtain three prototrophic colonies A, B, and C, and you cross each separately with a wild-type strain. From the cross prototroph A × wild type, you obtain 100 progeny, all of which are prototrophic. From the cross prototroph B × wild type, you obtain 100 progeny, of which 78 are prototrophic and 22 are nicotinamide requiring. From the cross prototroph C × wild type, you obtain 1000 progeny, of which 996 are prototrophic and 4 are nicotinamide requiring.

Answer:

a. A lack of revertants suggests either a deletion or an inversion within the gene.

b. To understand these data, recall that half the progeny should come from the wild-type parent.

Prototroph A: because 100 percent of the progeny are prototrophic, a reversion at the original mutant site may have occurred.

Prototroph B: half the progeny are parental prototrophs, and the remaining prototrophs, 28 percent, are the result of the new mutation. Notice that 28 percent is approximately equal to the 22 percent auxotrophs. The suggestion is that an unlinked suppressor mutation occurred, yielding independent assortment with the nic mutant.

Prototroph C: there are 496 “revertant” prototrophs (the other 500 are parental prototrophs) and four auxotrophs. This suggests that a suppressor mutation occurred in a site very close to the original mutation and was infrequently separated from the original mutation by recombination \([100\% \times 4 \times 2)/1000 = 0.8 \text{ m.u.}].\)
31. You are working with a newly discovered mutagen, and you wish to determine the base change that it introduces into DNA. Thus far, you have determined that the mutagen chemically alters a single base in such a way that its base-pairing properties are altered permanently. To determine the specificity of the alteration, you examine the amino acid changes that take place after mutagenesis. A sample of what you find is shown here:

Original: Gln–His–Ile–Glu–Lys
Mutant: Gln–His–Met–Glu–Lys

Original: Ala–Val–Asn–Arg
Mutant: Ala–Val–Ser–Arg

Original: Arg–Ser–Leu
Mutant: Arg–Ser–Leu–Trp–Lys–Thr–Phe

What is the base-change specificity of the mutagen?

Answer: Compare the original amino acid sequences to the mutant ones and list the changes.

original: ile; mutant: met
original: asn; mutant: ser
original: stop; mutant: trp

Now compare the codons that must have been altered by this mutagen.

original: ile AUA; mutant: met AUG
original: asn AAC or AUA; mutant: ser AGC or AGU
original: stop UAG or UGA; mutant: trp UGG

All these mutations can be the result of T to C or A to G transitions in the DNA. The result would be an A to G change in the mRNA that explains all three codon changes. This mutagen, then, might work by altering the base-pairing specificity of T so that it now base pairs with G. Or, the mutagen could alter the pairing specificity of A so that it now pairs with C, which would have the same effect.

32. You now find an additional mutant from the experiment in Problem 31:

Original: Ile–Leu–His–Gln
Mutant: Ile–Pro–His–Gln

Could the base-change specificity in your answer to Problem 31 account for this mutation? Why or why not?
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Answer: Yes. Mutation of the double-stranded DNA sequence by either a T to C transition in the coding strand or an A to G transition in the template strand will result in the CUN to CCN in the mRNA.

Original: leu CUN; Mutant: pro CCN (where N = any base)

33. You are an expert in DNA-repair mechanisms. You receive a sample of a human cell line derived from a woman who has symptoms of xeroderma pigmentosum. You determine that she has a mutation in a gene that has not been previously associated with XP. How is this possible?

Answer: XP is a heterogeneous genetic disorder and is caused by mutations in any one of several genes involved in the process of NER (nucleotide excision repair). As you read in the text about the discovery of yet another protein involved in NHEJ through research on cell line 2BN, it is certainly possible that this new patient has a mutation in as yet an unknown gene that encodes a protein necessary for NER.

34. Ozone (O₃) is an important naturally occurring component in our atmosphere, where it forms a layer that absorbs UV radiation. A hole in the ozone layer was discovered in the 1970s over Antarctica and Australia. The hole appears seasonally and was found to be due to human activity. Specifically, ozone is destroyed by a class of chemicals (called CFCs for chlorofluorocarbons) that are found in refrigerants, air-conditioning systems, and aerosols.

As a scientist working on DNA-repair mechanisms, you discover that there has been a significant increase in skin cancer in the beach communities in Australia. A newspaper reporter friend offers to let you publish a short note (a paragraph) in which you are to describe the possible connection between the ozone hole and the increased skin cancers. On the basis of what you have learned about DNA repair in this chapter, write a paragraph that explains the mechanistic connection.

Answer: Sun worshippers beware! Science has established a clear link between UV exposure and skin cancer, and with the drastic changes occurring in the protective ozone layer, UV levels are rising. UV damages DNA causing our cells to struggle to repair this damage by a number of different mechanisms. While this damage may be so great that the cell dies (the peeling of dead cells after a sunburn), other times the damage is more insidious as it causes permanent changes in the instructions encoded in the DNA itself. These changes are sometimes caused by the cell attempting to repair the damage. In these cases, when the damage prevents the DNA from being “read,” the cell may simply “guess” as to the meaning, changing the instructions. Eventually, the changed instructions may tell a cell to divide when it’s not supposed to, or not die when it is supposed to. These are the very changes that make a cell cancerous.