

# **Chapter 18**

# **Gene Mutation and DNA repair**

## **18.1 Effects of Mutations on Gene Structure and Function**

- ❑ Definition of point mutation
- ❑ How mutations within the coding sequence of a gene may alter a polypeptide's structure and function
- ❑ How mutations within noncoding sequences may alter gene function
- ❑ Intragenic and intergenic suppressors
- ❑ How changes in chromosome structure may affect gene expression
- ❑ Germ-line versus somatic mutations.

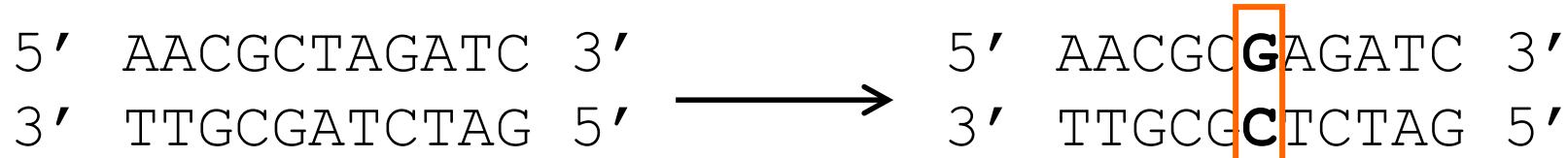
- The term **mutation** refers to a heritable change in the genetic material
- Mutations
  - provide allelic variations
  - Are the foundation for evolutionary change
  - Can be detrimental if they result in an allele that functions more poorly than the original
- Since mutations can be quite harmful, organisms have developed ways to repair damaged DNA

- Mutations can occur at the chromosomal or gene level
- **Chromosomal changes** in structure or number
  - Generally detected by karyotype, affect more than one gene
    - Changes in chromosome structure
      - Inversions
      - Translocations
      - Duplications
      - Deletions
    - Changes in chromosome number
      - Too few or too many - aneuploidy

- **Gene mutation**
  - Not detectable by karyotype, usually affects one gene
    - DNA changes that
      - Change from one nucleotide to another
      - Delete nucleotides
      - Insert nucleotides

# Gene Mutations Change the DNA Sequence

- A **point mutation** is a change in a single base pair
  - It involves a **base substitution**



- A **transition** is a change of a pyrimidine (C, T) to another pyrimidine or a purine (A, G) to another purine
- A **transversion** is a change of a pyrimidine to a purine or vice versa
- Transitions are more common than transversions

# Mutations in the Coding Sequence

- **Silent mutations** do not alter the amino acid sequence
  - Due to the degeneracy of the genetic code
- **Missense mutations** do alter the amino acid sequence
  - Example: Sickle-cell anemia
    - Refer to Figure 18.1 to see how one amino acid substitution changes blood cell morphology
  - Some may not affect function – **neutral mutation**
- **Nonsense mutations** change a codon to a stop codon
  - Produces a truncated polypeptide

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Normal red blood cells

10 μm



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Sickled red blood cells

10 μm

### (a) Micrographs of red blood cells

NORMAL : NH<sub>2</sub> – VALINE – HISTIDINE – LEUCINE – THREONINE – PROLINE – GLUTAMIC ACID – GLUTAMIC ACID...

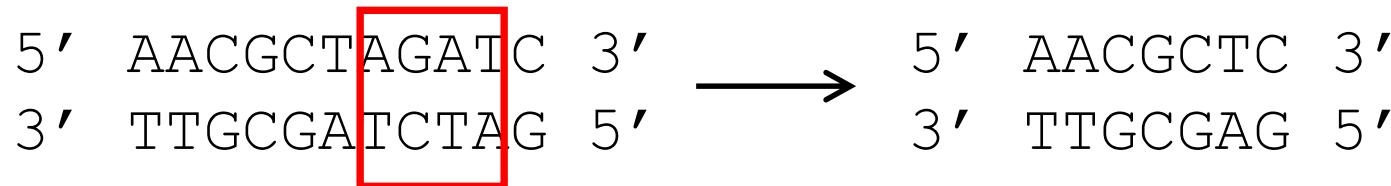
SICKLE CELL : NH<sub>2</sub> – VALINE – HISTIDINE – LEUCINE – THREONINE – PROLINE – VALINE – GLUTAMIC ACID...

### (b) A comparison of the amino acid sequence between normal β-globin and sickle-cell β-globin

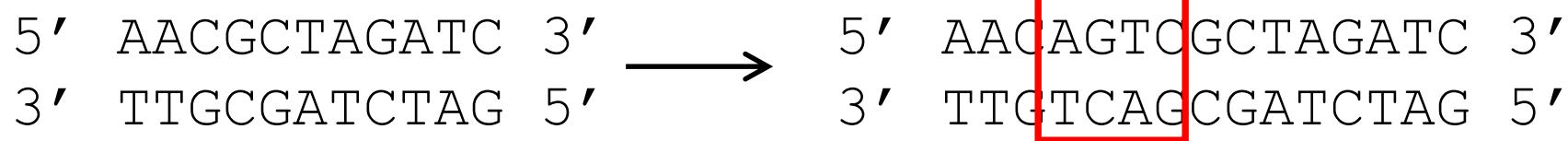
Figure 18.1



- Mutations may also involve the addition or deletion of short sequences of DNA
- Deletion



- Insertion



- Insertions and deletions in the coding regions for proteins can insert new amino acids or cause a shift in the reading frame
  - **Frameshift mutations** involve the addition or deletion of nucleotides in multiples of one or two but not three (the size of one codon)
    - This shifts the reading frame so that a completely different amino acid sequence occurs downstream from the mutation



**TABLE 18.1****Consequences of Point Mutations Within the Coding Sequence**

| Type of Change | Mutation in the DNA | Example*  | Amino Acids Altered | Likely Effect on Protein Function |
|----------------|---------------------|---|---------------------|-----------------------------------|
| None           | None                | 5'-A-T-G-A-C-C-G-A-C-C-C-G-A-A-A-G-G-G-A-C-C-3'<br>Met - Thr - Asp - Pro - Lys - Gly - Thr -<br><br>↓   | None                | None                              |
| Silent         | Base substitution   | 5'-A-T-G-A-C-C-G-A-C-C-C-G-A-A-A-G-G-G-A-C-C-3'<br>Met - Thr - Asp - Pro - Lys - Gly - Thr -<br><br>↓   | None                | None                              |
| Missense       | Base substitution   | 5'-A-T-G-C-C-C-G-A-C-C-C-G-A-A-A-G-G-G-A-C-C-3'<br>Met - Pro - Asp - Pro - Lys - Gly - Thr -<br><br>↓   | One                 | Neutral or inhibitory             |
| Nonsense       | Base substitution   | 5'-A-T-G-A-C-C-G-A-C-C-C-G-T-A-A-A-G-G-G-A-C-C-3'<br>Met - Thr - Asp - Pro - STOP!<br><br>↓             | Many                | Inhibitory                        |
| Frameshift     | Addition/deletion   | 5'-A-T-G-A-C-C-G-A-C-G-C-C-G-A-A-A-G-G-G-A-C-C-3'<br>Met - Thr - Asp - Ala - Glu - Arg - Asp -<br><br>↓ | Many                | Inhibitory                        |

\*DNA sequence in the coding strand. Note that this sequence is the same as the mRNA sequence except that the RNA contains uracil (U) instead of thymine (T). The 3-base codons are shown in alternating black and red colors. Mutations are shown in green.



# Gene Mutations in Noncoding Sequences

- These mutations can still affect gene expression
  - Promoter
    - **Up promoter mutations** increase transcription
    - **Down promoter mutations** decrease transcription
  - Splice junctions in eukaryotes
  - 5' and 3' UTR – alter stability of RNA, translation
  - Regulatory element/operator site – disrupt proper regulation of gene expression

**TABLE 18.2**

**Possible Consequences of Gene Mutations Outside of the Coding Sequence**

| Sequence                         | Effect of Mutation   |
|----------------------------------|--|
| Promoter                         | May increase or decrease the rate of transcription                       |
| Regulatory element/operator site | May disrupt the ability of the gene to be properly regulated             |
| 5'-UTR/3'-UTR                    | May alter the ability of mRNA to be translated; may alter mRNA stability |
| Splice recognition sequence      | May alter the ability of pre-mRNA to be properly spliced                 |

# Gene Mutations and Their Effects on Genotype and Phenotype

- **Wild-type** is the relatively prevalent genotype
  - Rarely, a gene with multiple alleles may have two or more wild types
- A forward mutation changes the wild-type genotype into some new variation
- A **reverse mutation** or **reversion** changes a mutant allele back to the wild-type

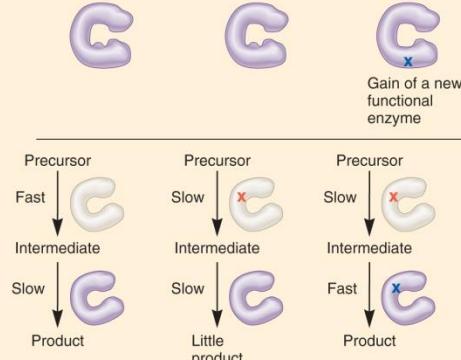
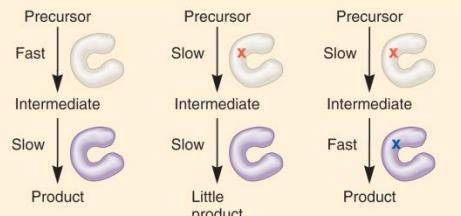
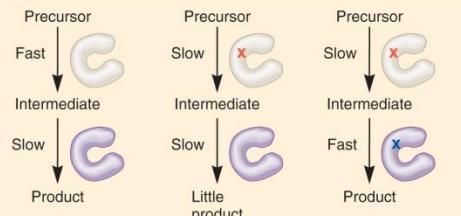
- A **neutral mutation** does not alter protein function
- A **deleterious mutation** lowers the chance of survival and reproduction
- A **beneficial mutation** enhances the survival or reproductive success
- Occasionally, whether a mutation is beneficial or deleterious will depend on environmental conditions
  - Example: Sickle cell allele
    - Heterozygotes have increased survival in the presence of malaria

- A **conditional mutation** is one that affects the phenotype only under specific conditions
  - Example: **Temperature-sensitive (ts) mutants**
    - Used by geneticists to study gene function
    - Ex: *E. coli* with a ts mutation may grow below 38°C but not above 40°C

# Suppressor Mutations

- **Suppressor** – a second mutation that affects the phenotypic expression of a first mutation
  - Different than a reversion – it occurs in a second site
- **Intragenic suppressor** – The second mutation is in the same gene as the first
- **Intergenic suppressor** – The second mutation is in a different gene than the first
  - Second protein may take on the role of the first
  - Proteins may act in the same cellular pathway
  - Polypeptides may be subunits of the same multimeric protein

**TABLE 18.3**  
Examples of Suppressor Mutations

| Type                             | No Mutation  | First Mutation  | Second Mutation  | Description  |
|----------------------------------|--|---|--|--|
| Intragenic                       | <br>Transport can occur                                 | <br>Transport inhibited  | <br>Transport can occur                                | A first mutation disrupts normal protein function, and a suppressor mutation affecting the same protein restores function. In this example, the first mutation inhibits lactose transport function, and the second mutation restores it.   |
| Intergenic<br>Redundant function | <br>Functional enzyme                                   | <br>Nonfunctional enzyme                                      |   | A first mutation inhibits the function of a protein, and a second mutation alters a different protein to carry out that function. In this example, the proteins function as enzymes.   |
| Common pathway                   | <br>Precursor<br>Fast ↓ Intermediate<br>Slow ↓ Product | <br>Precursor<br>Slow ↓ Intermediate<br>Slow ↓ Little product | <br>Precursor<br>Slow ↓ Intermediate<br>Fast ↓ Product | Two or more different proteins may be involved in a common pathway. A mutation that causes a defect in one protein may be compensated for by a mutation that alters the function of a different protein in the same pathway.   |
| Multimeric protein               | <br>Active  | <br>Inactive   | <br>Active   | A mutation in a gene encoding one protein subunit that inhibits function may be suppressed by a mutation in a gene that encodes a different subunit. The double mutant has restored function.  |
| Transcription factor             | <br>Normal function                                   | <br>Loss of function   |    | A first mutation causes loss of function of a particular protein. A second mutation may alter a transcription factor and cause it to activate the expression of another gene. This other gene encodes a protein that can compensate for the loss of function caused by the first mutation. |

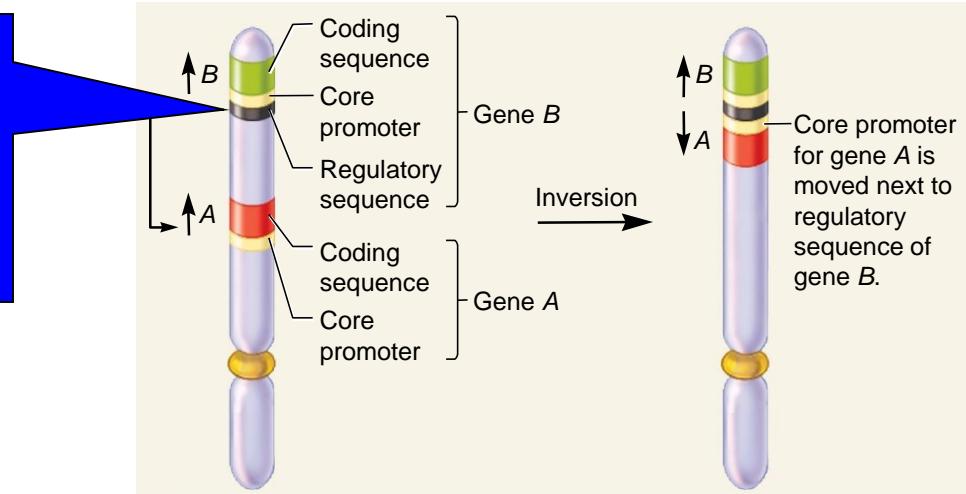
**Table 18.3**

# Changes in Chromosome Structure Can Affect Gene Expression

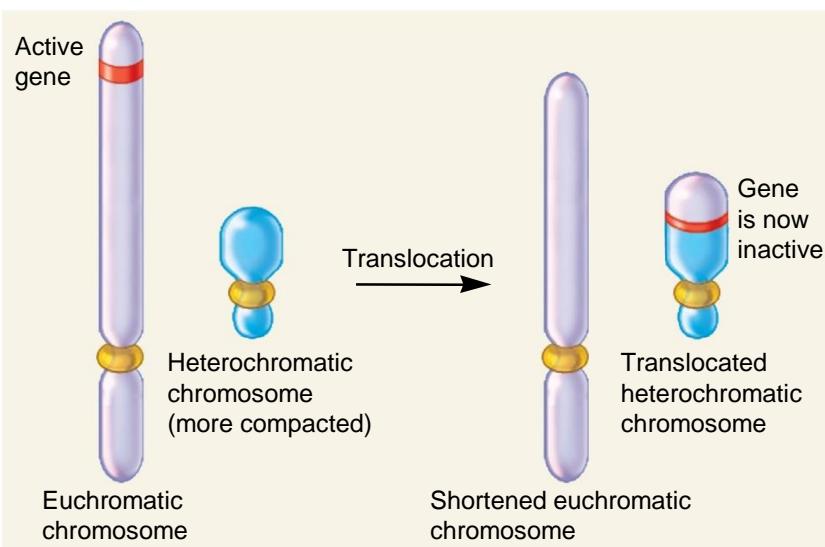
- A chromosomal rearrangement may affect a gene because the **breakpoint** occurred within the gene itself
- Or, a gene may be left intact, but its expression may be altered because of its new location
  - This is called **position effect**

- There are two common reasons for position effects:
  1. Movement to a position near **regulatory sequences**
  2. Movement to a **heterochromatic** region
- Refer to Figure 18.2a and b

Regulatory sequences are often bidirectional



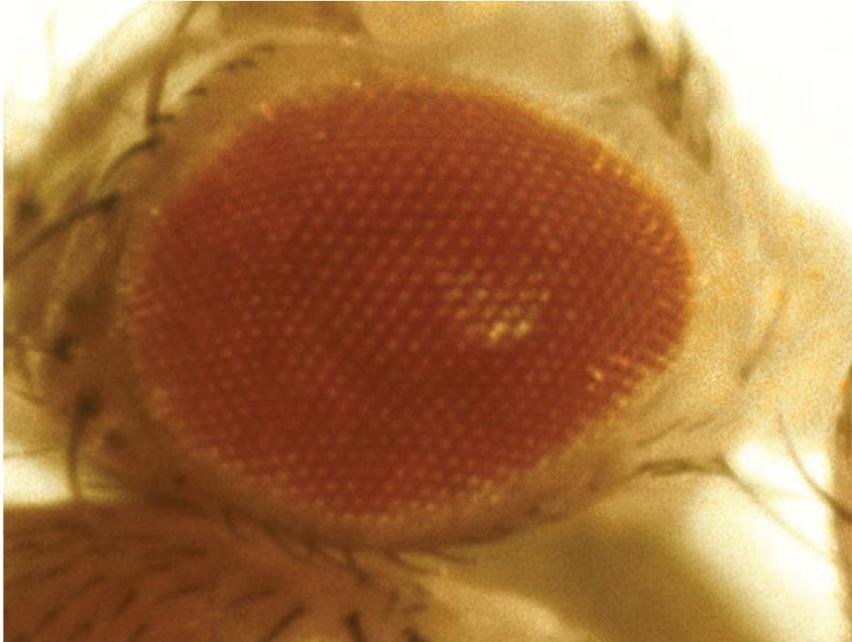
(a) Position effect due to regulatory sequences



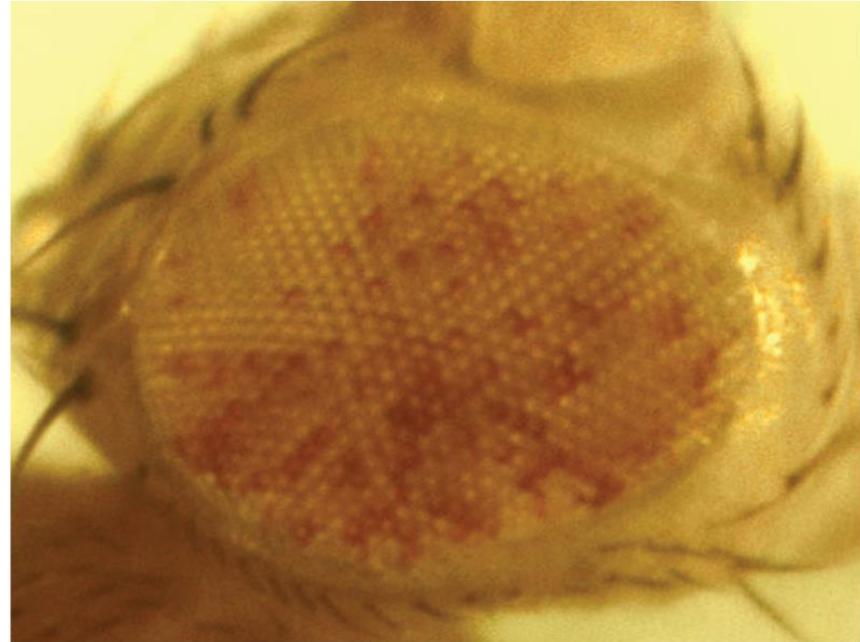
(b) Position effect due to translocation to a heterochromatic chromosome

Figure 18.2

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**(a) Normal eye**



**(b) Variegated eye**

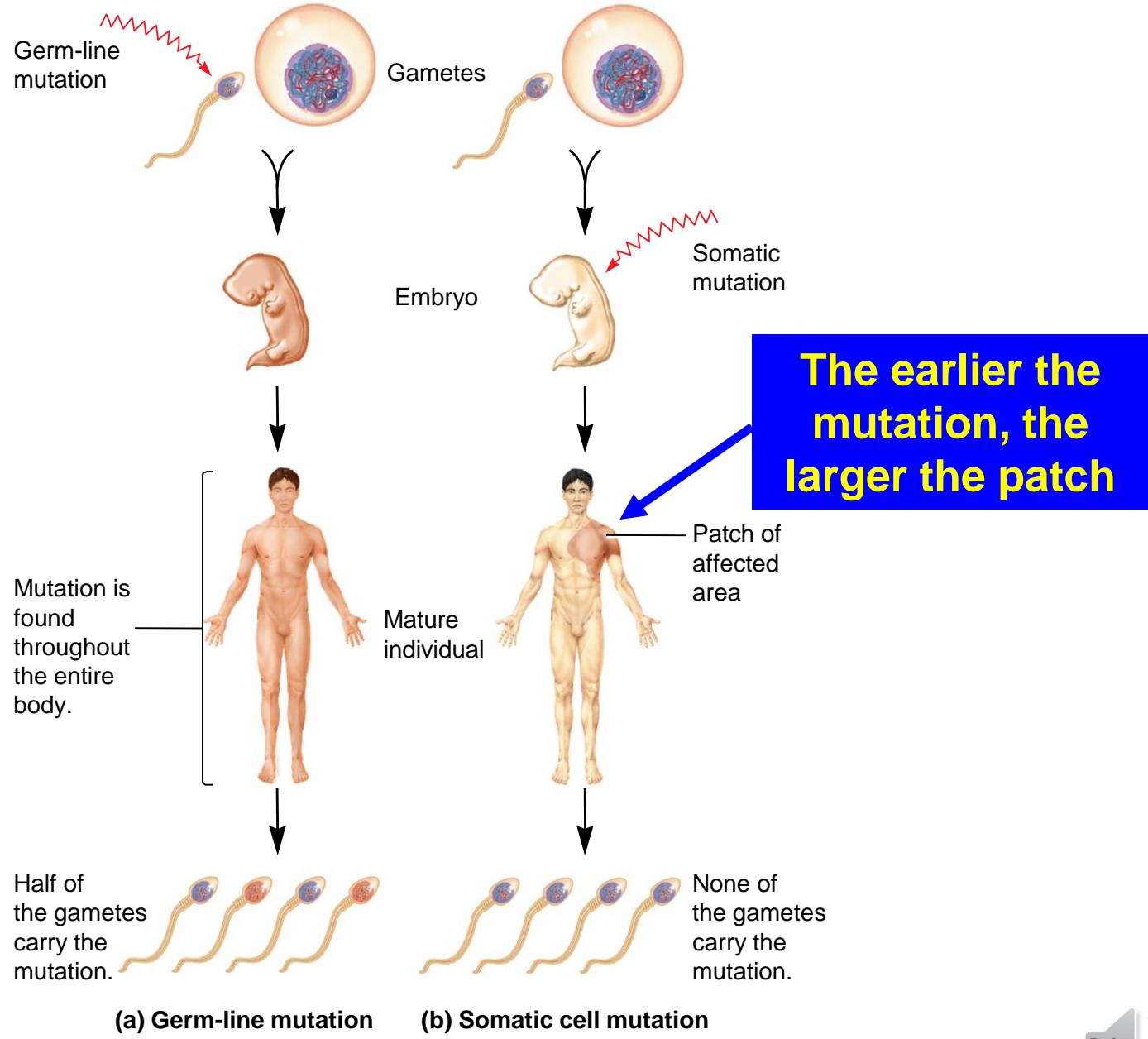
(a-b): © Dr. Jack R. Girton

**Figure 18.3**

# Mutations Can Occur in Germ-Line or Somatic Cells

- Geneticists classify animal cells into two types
  - **Germ-line cells**
    - Cells that give rise to gametes such as eggs and sperm
  - **Somatic cells**
    - All other cells
    - Ex: Muscle, nerve, or skin cells

- **Germ-line mutations** are those that occur directly in a sperm or egg cell, or in one of their precursor cells
  - Mutation may be passed onto future generations
- **Somatic mutations** are those that occur directly in a body cell, or in one of its precursor cells
  - Mutation cannot be passed onto future generations
  - An Individual with somatic cells that are genotypically different from each other is called a **genetic mosaic**
  - Refer to Figure 18.4 and 18.5



**Figure 18.4**



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**Figure 18.5**

## 18.2 Random Nature of Mutations

- The results of the Lederbergs and how they are consistent with the random mutation theory

# Are Mutations Random Events?

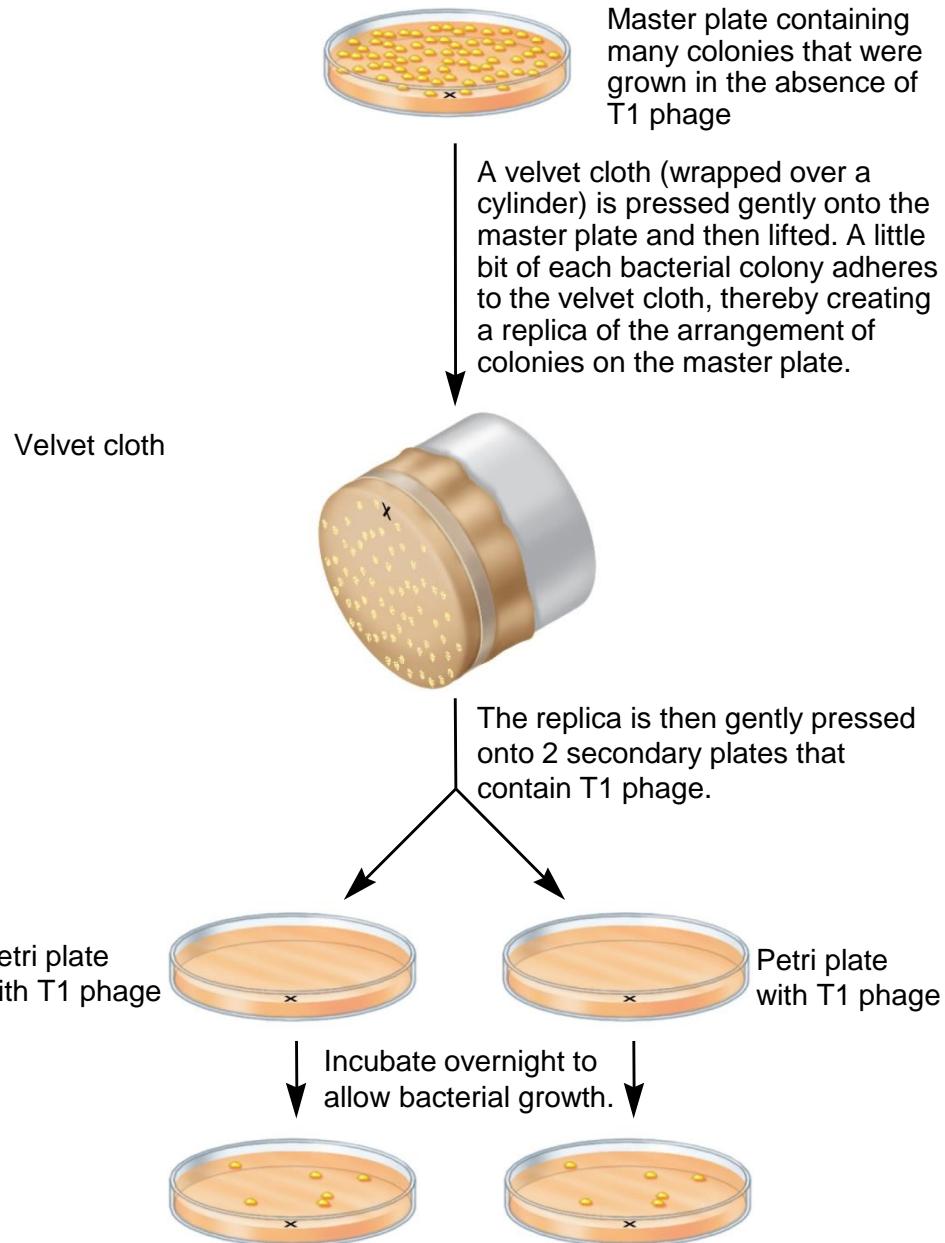
- In the 19th century there were two opposing theories on how mutations arose
  - Jean Baptiste Lamarck
    - Thought physiological events (e.g. use and disuse) determined genetic variation and whether traits are passed along to offspring
  - Charles Darwin
    - Thought genetic variation occurs by chance and natural selection selects for better-adapted organisms

# Replica Plating and Random Mutations

- Joshua and Esther Lederberg were also interested in the relationship between mutations and the environment
- At that time (1950s), there were two hypotheses
  - **Physiological Adaptation Hypothesis**
    - Selected conditions promote formation of specific mutations allowing the organism to survive
      - Consistent with Lamarck's viewpoint
  - **Random mutation Hypothesis**
    - Environmental factors simply select for the survival of individuals with beneficial mutations
      - Consistent with Darwin's viewpoint

- The Lederbergs developed a technique to distinguish between these two hypotheses
  - **Replica plating**
    - Same bacterial cells can be transferred to two plates at the same time
    - Measure number of *ton<sup>r</sup>* resistant colonies
      - Cells resistant to T1 bacteriophage

- A few *ton<sup>r</sup>* colonies were observed at the same location on both plates
- Indicates that mutations conferring *ton<sup>r</sup>* occurred randomly on the original plate
- The presence of T1 in the secondary plates simply selected for previously occurring *ton<sup>r</sup>* mutants
- This supports the random mutation hypothesis



**Figure 18.6**

- **Random Mutation Theory**
  - Mutations occur in any gene or piece of DNA randomly (although there may be **hot spots**)
  - Do not need selection for the mutation to occur
  - Growth conditions may then select for most adapted organism

# 18.3 Spontaneous Mutations

- ❑ Spontaneous versus induced mutations
- ❑ Examples of spontaneous mutations
- ❑ How mutations arise by depurination, deamination, and tautomeric shifts
- ❑ How reactive oxygen species alter DNA structure and cause mutation
- ❑ The mechanism of trinucleotide repeat expansion

# Causes of Mutation

- Mutations can occur **spontaneously** or be **induced**
- **Spontaneous mutations**
  - Result from abnormalities in cellular/biological processes
    - Example: Errors in DNA replication
- **Induced mutations**
  - Caused by environmental agents
  - Agents known to alter DNA are called **mutagens**
    - These can be chemical or physical agents



**TABLE 18.4**  
**Causes of Mutations**

| Common Causes of Mutations | Description   |
|----------------------------|---|
| <b><i>Spontaneous</i></b>  |   |
| Aberrant recombination     | Abnormal crossing over may cause deletions, duplications, translocations, and inversions (see Chapter 8).   |
| Aberrant segregation       | Abnormal chromosomal segregation may cause aneuploidy or polyploidy (see Chapter 8).  |
| Errors in DNA replication  | A mistake by DNA polymerase may cause a point mutation (see Chapter 13).  |
| Transposable elements      | Transposable elements can insert themselves into the sequence of a gene (see Chapter 19).   |
| Depurination               | On rare occasions, the linkage between purines (i.e., adenine and guanine) and deoxyribose can spontaneously break. If not repaired, it can lead to mutation. |
| Deamination                | Cytosine and 5-methylcytosine can spontaneously deaminate to create uracil or thymine, respectively.  |
| Tautomeric shifts          | Spontaneous changes in base structure can cause mutations if they occur immediately prior to DNA replication.   |
| Toxic metabolic products   | The products of normal metabolic processes, such as reactive oxygen species, may be chemically reactive agents that can alter the structure of DNA.           |
| <b><i>Induced</i></b>      |   |
| Chemical agents            | Chemical substances may cause changes in the structure of DNA.  |
| Physical agents            | Physical phenomena such as UV light and X-rays can damage the DNA.  |

## Table 18.4

- **Spontaneous mutations** can be caused by
  - Aberrant recombination
  - Aberrant segregation
  - Errors in DNA replication
  - Transposable elements
  - Depurination
  - Deamination
  - Tautomeric shifts
  - Toxic metabolic products

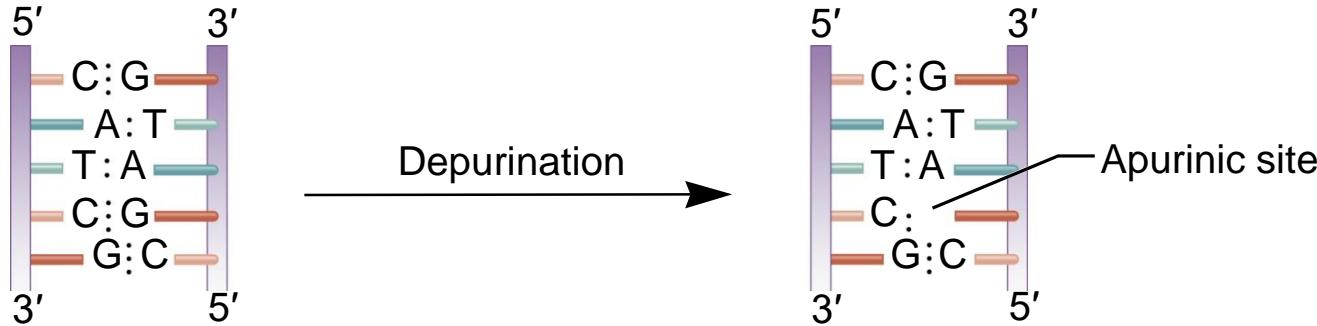
- Spontaneous mutations can arise by three types of chemical changes

**1. Depurination**

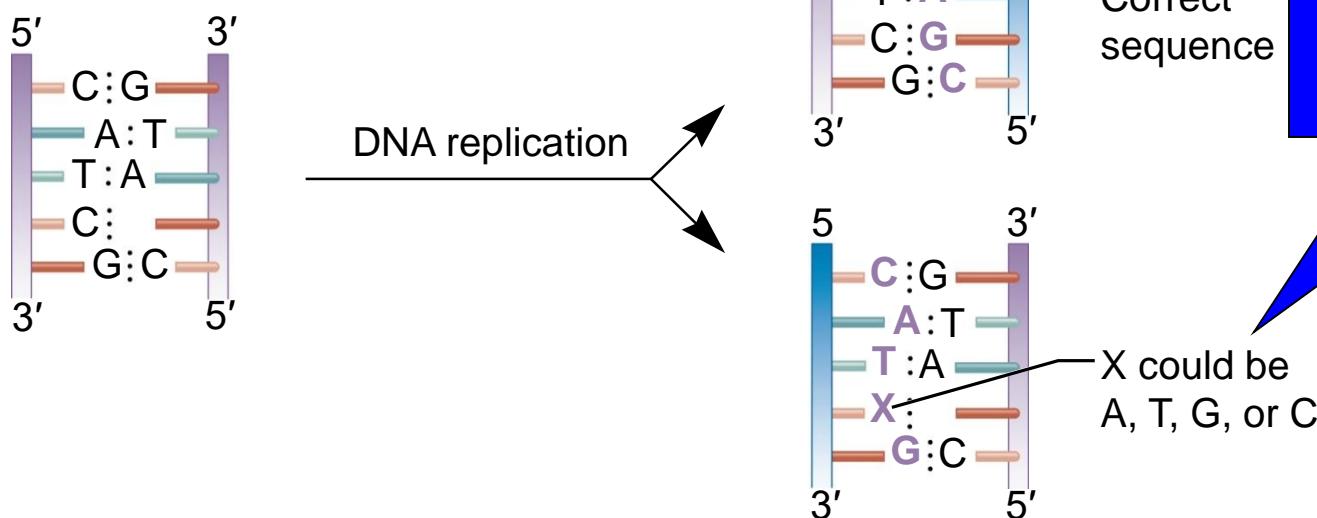
**2. Deamination**

**3. Tautomeric shift**

- **Depurination**
  - The most common type of chemical change
  - Removal of a purine (guanine or adenine) from the DNA
  - Covalent bond between deoxyribose and a purine base is somewhat unstable
    - Occasionally undergoes a spontaneous reaction with water that releases the base from the sugar
    - This is then called an **apurinic site**
  - Fortunately, apurinic sites can be repaired
    - However, if the repair system fails, a mutation may result
  - Refer to Figure 18.7



(a) Depurination



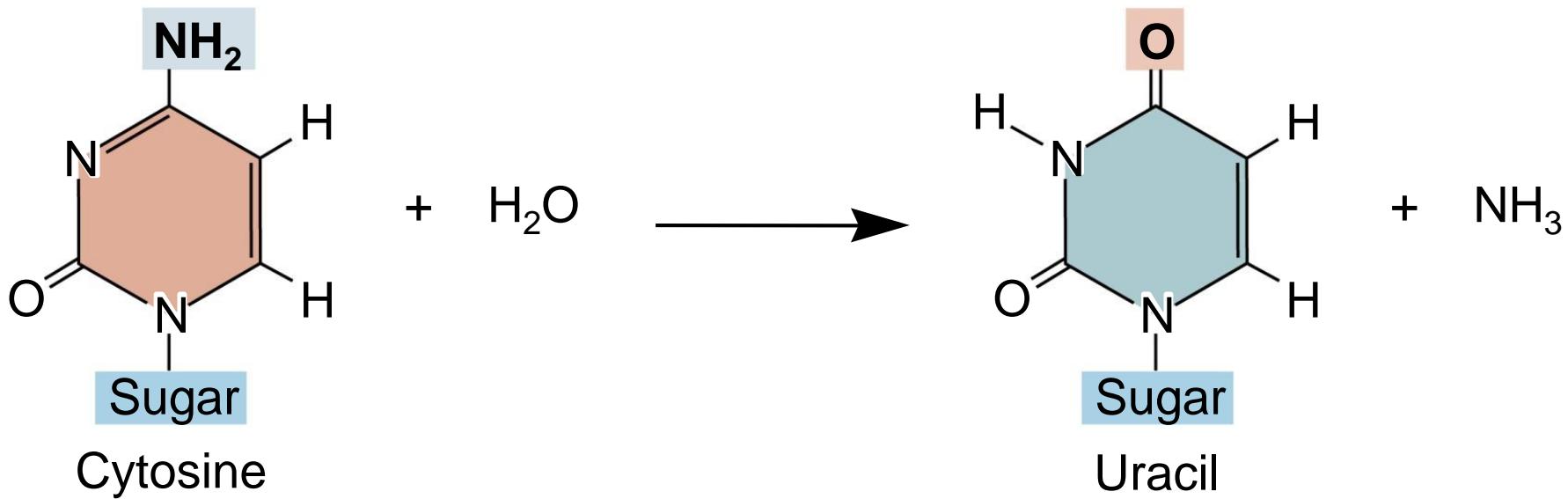
(b) Replication over an apurinic site

A, T and G are incorrect. There's a 75% chance of a mutation

Figure 18.7

- **Deamination**
  - Removal of an amino group from the cytosine base
  - The other bases are not readily deaminated
  - DNA repair enzymes can recognize uracil as an inappropriate base in DNA and remove it
  - If repair system fails to correct the problem, a mutation could result during subsequent rounds of DNA replication
  - Refer to Figure 18.8a

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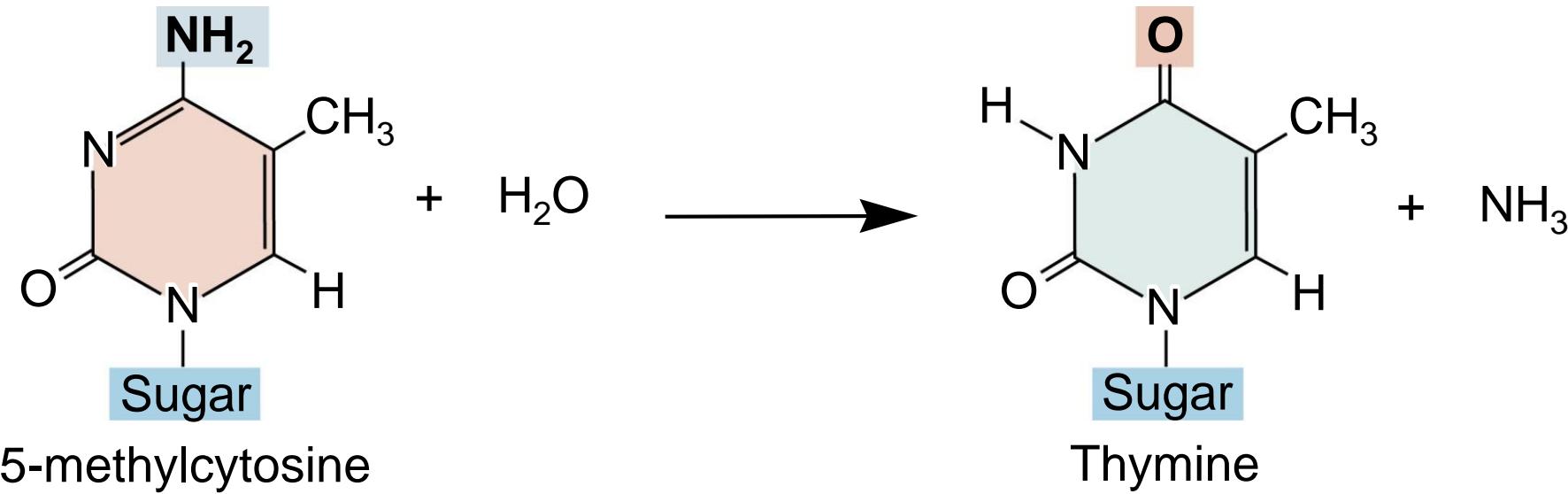


**(a) Deamination of cytosine**

Figure 18.8a

- **Deamination of 5-methyl cytosine** can also occur
- However deamination of 5-methyl cytosine does not result in uracil
  - It results in thymine – a normal constituent of DNA
    - This poses a problem for repair enzymes
    - They cannot determine which of the two bases on the two DNA strands is the incorrect base
    - For this reason, methylated cytosine bases tend to create hot spots for mutation
- Refer to Figure 18.8b

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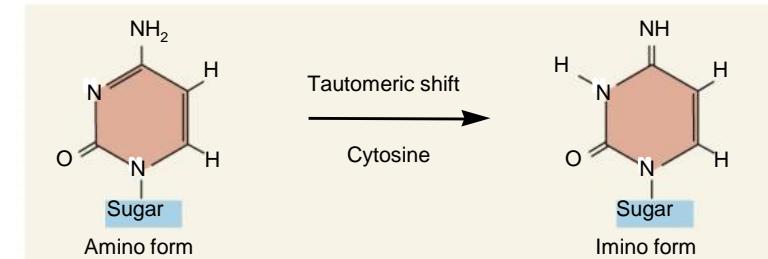
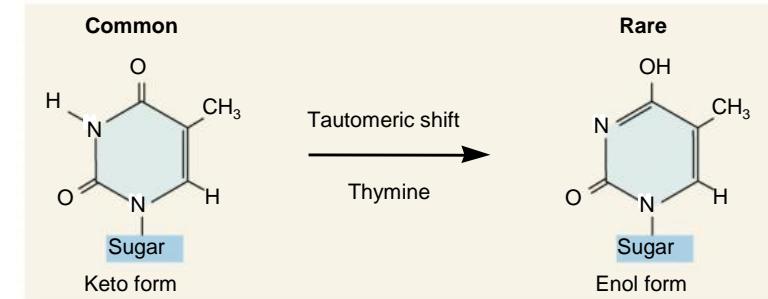
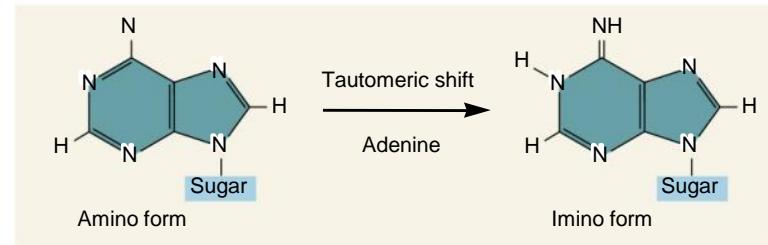
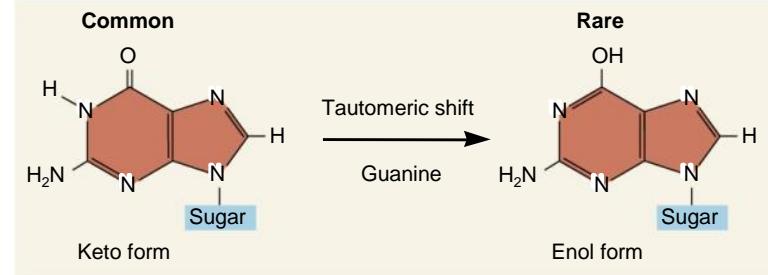


**(b) Deamination of 5-methylcytosine**

**Figure 18.8b**

- **Tautomeric shifts**
  - Involves a temporary change in base structure
  - The common, stable form of thymine and guanine is the **keto form**
    - At a low rate, T and G can interconvert to an enol form
  - The common, stable form of adenine and cytosine is the **amino form**
    - At a low rate, A and C can interconvert to an imino form
  - These rare forms promote AC and GT base pairs
  - To cause a mutation it must occur immediately prior to DNA replication
  - Refer to Figure 18.9a, b and c

Common



Rare

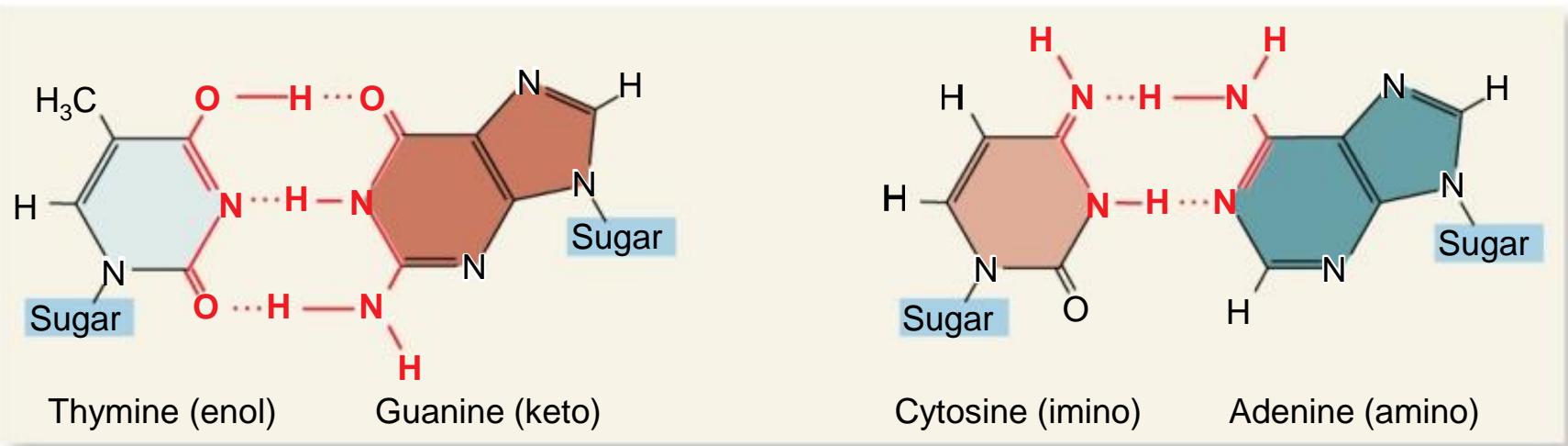


(a) Tautomeric shifts that occur in the 4 bases found in DNA

Figure 18.9a



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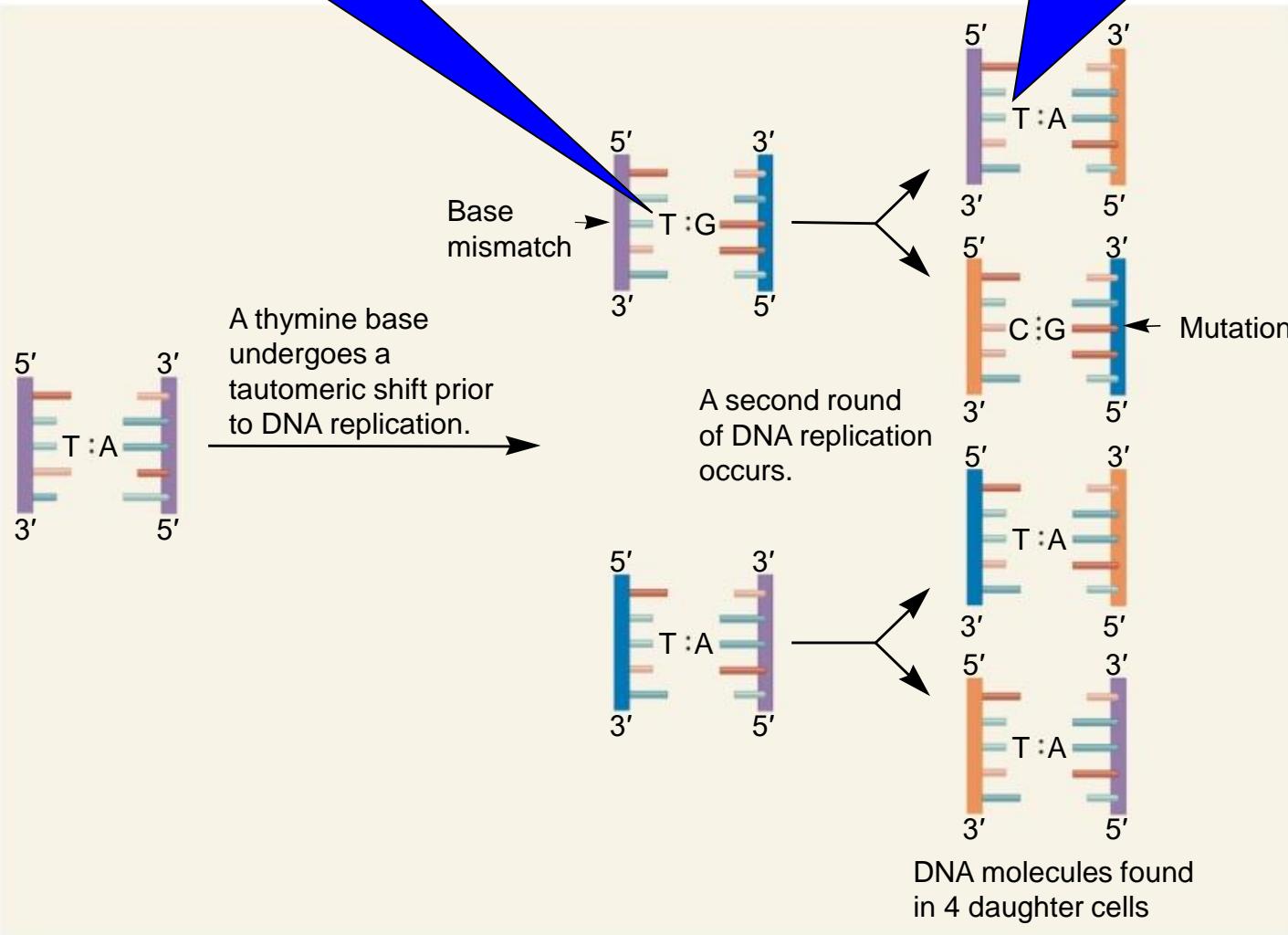
(b) Mis-base pairing due to tautomeric shifts

Figure 18.9b

## Temporary tautomeric shift

## Shifted back to its normal form

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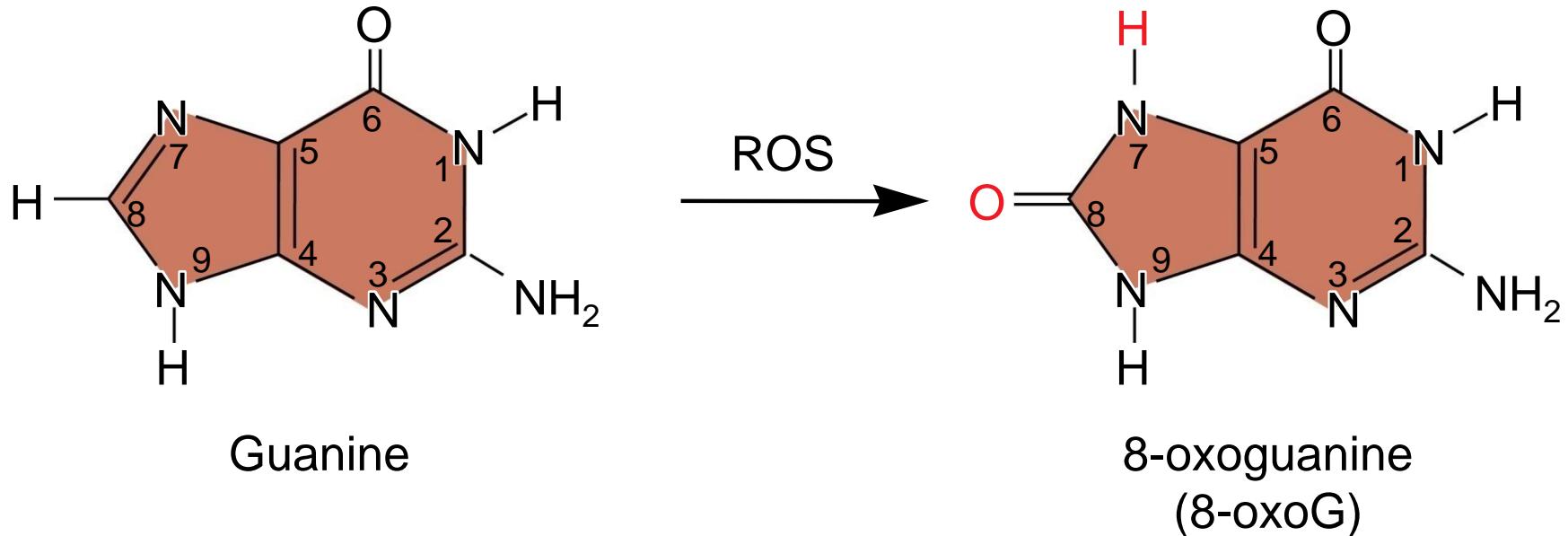


(c) Tautomeric shifts and DNA replication can cause mutation.

Figure 18.9c

# Oxidative stress and DNA damage

- Aerobic organisms use oxygen as terminal electron acceptor
- **Reactive oxygen species** (ROS) generated by normal metabolism
  - ROS used by immune system to kill invading cells
- **Oxidative stress** is an imbalance between synthesis and destruction of ROS
  - ROS can damage DNA and other molecules if not removed – called **oxidative DNA damage**
  - Refer to Figure 18.10



**Figure 18.10**

- 8-oxoG
  - Base pairs with adenine during DNA replication
  - G-C base pair becomes A-T base pair

# Mutations Due to Trinucleotide Repeats

- Certain regions of the chromosome contain trinucleotide sequences repeated in tandem
- Several human genetic diseases are caused by an unusual form of mutation called **trinucleotide repeat expansion (TNRE)**
  - Trinucleotide sequences increase from one generation to the next
  - These diseases include spinal and bulbar muscular atrophy (SBMA), Huntington disease (HD), Fragile X syndrome (FRAXA), spinocerebellar ataxia (SCA1), and myotonic muscular dystrophy

- In normal individuals, trinucleotide sequences are transmitted from parent to offspring without mutation
- But in TNRE disorders, the length of a trinucleotide repeat increases above a certain critical size
  - It becomes prone to frequent expansion
  - Example: The trinucleotide repeat **CAG**

CAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG

*n* = 11

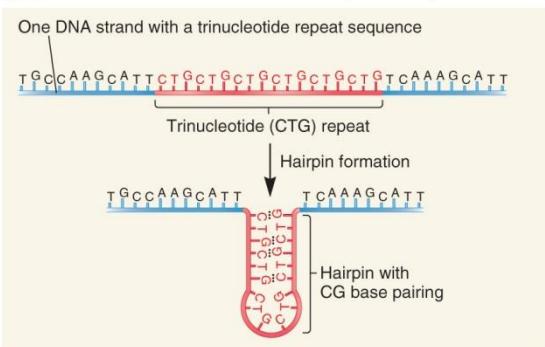
CAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG

*n* = 18



- Expansion may be within the coding sequence of the gene
  - Typically CAG (glutamine)
  - Encoded protein will contain **long tracks of glutamine**
    - Causes the proteins to aggregate with each other
    - Aggregation correlated with disease progression
- Expansions may be located in noncoding regions
  - Hypothesized to cause abnormal changes in RNA structure
  - May produce methylated CpG islands to silence the gene

- Two unusual features of TNRE disorders
  1. The severity of the disease tends to worsen in future generations - called **anticipation** or **dynamic mutation**
  2. Severity depends on whether it is inherited from the father or mother
    - In Huntington disease, the TNRE is more likely to occur if inherited from the father
    - In myotonic muscular dystrophy, the TNRE is more likely to occur if inherited from the mother
- A key aspect of TNRE is that the triplet repeat can form a hairpin (or stem-loop)
  - Leading to errors in DNA replication
  - Refer to Figure 18.11



(a) Formation of a hairpin with a trinucleotide (CTG) repeat sequence

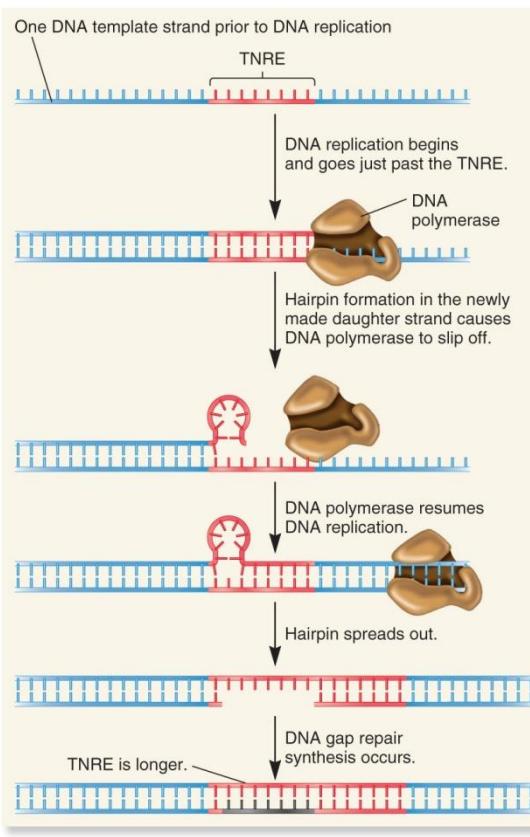


Figure 18.11

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**TABLE 18.5**

**TNRE Disorders**

| Disease                                     | SBMA               | HD                 | SCA1               | FRAXA             | FRAXE             | DM                 |
|---|--------------------|--------------------|--------------------|-------------------|-------------------|--------------------|
| Repeat Sequence                             | CAG                | CAG                | CAG                | CGG               | GCC               | CTG                |
| Location of Repeat                          | Coding sequence    | Coding sequence    | Coding sequence    | 5'-UTR            | 5'-UTR            | 3'-UTR             |
| Number of Repeats in Unaffected Individuals | 11–33              | 6–37               | 6–44               | 6–53              | 6–35              | 5–37               |
| Number of Repeats in Affected Individuals   | 36–62              | 27–121             | 43–81              | >200              | >200              | >200               |
| Pattern of Inheritance                      | X-linked           | Autosomal dominant | Autosomal dominant | X-linked          | X-linked          | Autosomal dominant |
| Disease Symptoms                            | Neuro-degenerative | Neuro-degenerative | Neuro-degenerative | Mental impairment | Mental impairment | Muscle disease     |
| Anticipation*                               | None               | Male               | Male               | Female            | None              | Female             |

\*Indicates the parent in which anticipation occurs most prevalently.

SBMA, spinal and bulbar muscular atrophy; HD, Huntington disease; SCA1, spinocerebellar ataxia; FRAXA and FRAXE, fragile X syndromes; DM, dystrophia myotonica (myotonic muscular dystrophy).

## 18.4 Induced Mutations

- ❑ Definition of mutagen
- ❑ Chemical and physical mutagens
- ❑ Definition of mutation rate
- ❑ Analysis of the results of an Ames test

# Induced Mutations

- An enormous array of agents can act as **mutagens** to permanently alter the structure of DNA
- Mutagens are often involved in the development of human cancers
- Mutagenic agents are usually classified as chemical or physical mutagens

# **Mutagens Alter DNA Structure in Different Ways**

- Chemical mutagens come in three main types

- 1. Base modifiers**

- 2. Intercalating agents**

- 3. Base analogs**

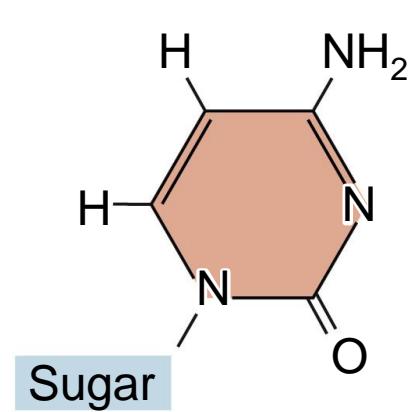
**TABLE 18.6**

**Examples of Mutagens**

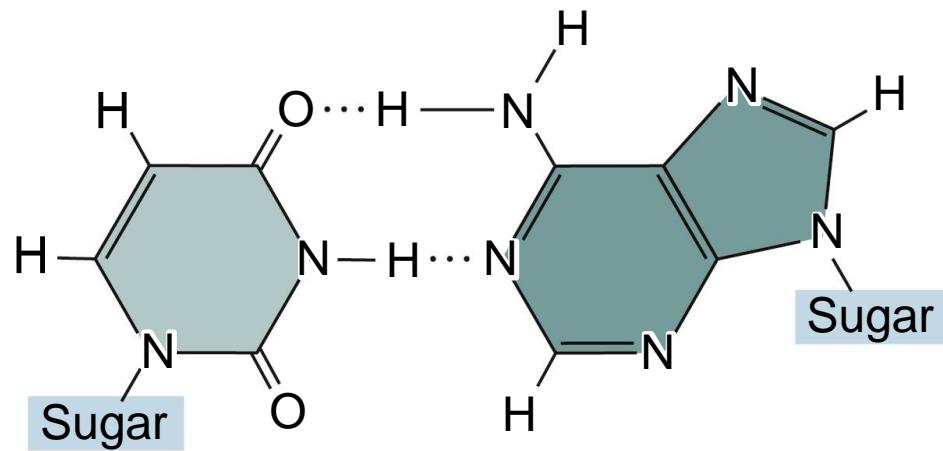
| Mutagen                | Effect(s) on DNA Structure   |
|------------------------|--|
| <b><i>Chemical</i></b> |  |
| Nitrous acid           | Deaminates bases   |
| Nitrogen mustard       | Alkylating agent   |
| Ethyl methanesulfonate | Alkylating agent   |
| Proflavin              | Intercalates within DNA helix  |
| 5-Bromouracil          | Base analog  |
| 2-Aminopurine          | Base analog  |
| <b><i>Physical</i></b> |  |
| X-rays                 | Cause base deletions, single-stranded nicks in DNA, crosslinking, and chromosomal breaks |
| UV light               | Promotes pyrimidine dimer formation, such as thymine dimers                              |

- **Base modifiers** covalently modify the structure of a nucleotide
  - For example, **nitrous acid**, replaces amino groups with keto groups ( $-\text{NH}_2$  to  $=\text{O}$ )
  - This can change cytosine to uracil and adenine to hypoxanthine
    - These modified bases do not pair with the appropriate nucleotides in the daughter strand during DNA replication
    - Refer to Figure 18.12

## Template strand



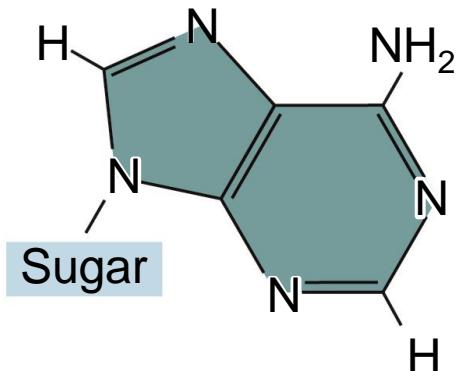
## After replication



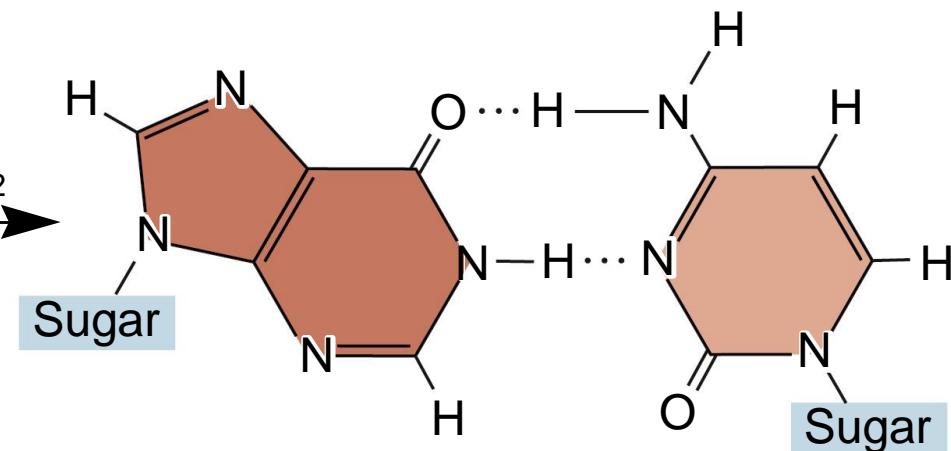
Cytosine

Uracil

Adenine



Adenine



Hypoxanthine

Cytosine

**Figure 18.12**

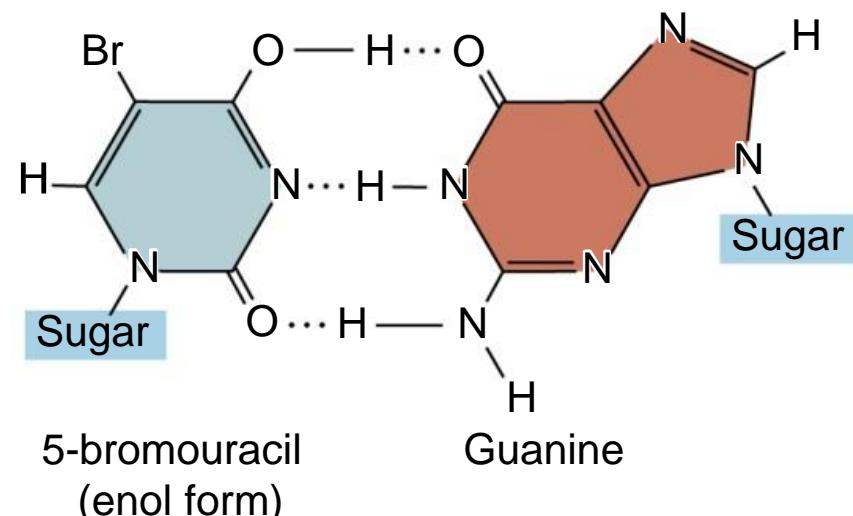
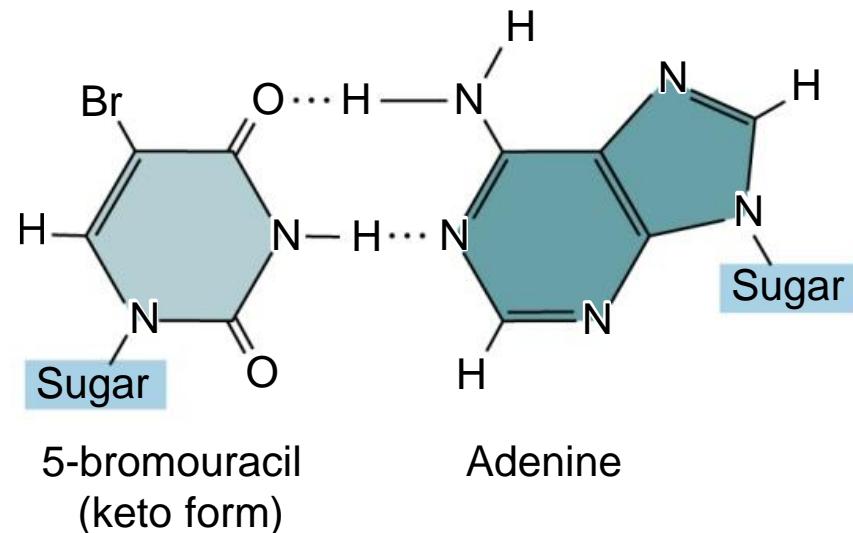


- Some chemical mutagens disrupt the appropriate pairing between nucleotides by alkylating bases within the DNA
  - Examples: **Nitrogen mustard** and **ethyl methanesulfonate (EMS)**

- **Intercalating agents** contain flat planar structures that intercalate themselves into the double helix
  - This distorts the helical structure
  - When DNA containing these mutagens is replicated, the daughter strands may contain single-nucleotide additions and/or deletions resulting in frameshifts
  - Examples:
    - **Acridine dyes**
    - **Proflavin**

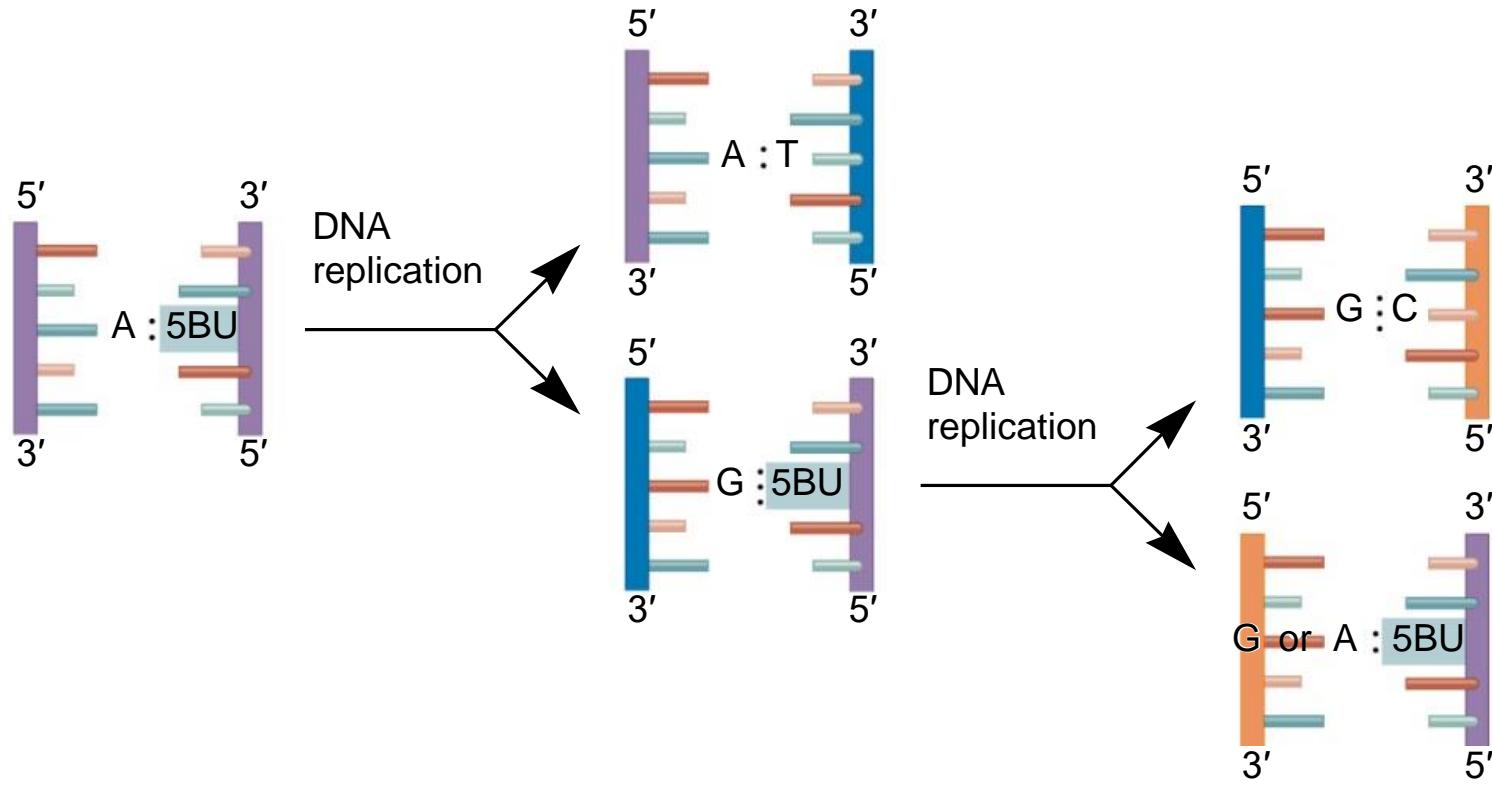
- **Base analogs** become incorporated into daughter strands during DNA replication
  - For example, 5-bromouracil is a thymine analogue
    - It can be incorporated into DNA instead of thymine

Refer to Figure 18.13a and b



**Figure 18.13a**

**(a) Base pairing of 5BU (a thymine analog) with adenine or guanine**



**(b) How 5BU causes a mutation in a base pair during DNA replication**

## Figure 18.13b

- Physical mutagens come into two main types
  1. Ionizing radiation
  2. Nonionizing radiation

- **Ionizing radiation**
  - Includes X-rays and gamma rays
  - Has short wavelength and high energy
  - Can penetrate deeply into biological materials
  - Creates chemically reactive molecules termed free radicals
  - Can cause
    - Base deletions
    - Single nicks in DNA strands
    - Cross-linking
    - Oxidized bases
    - Chromosomal breaks

- **Nonionizing radiation**
  - Includes UV light
  - Has less energy
  - Cannot penetrate deeply into biological molecules material
  - Causes the formation of cross-linked **thymine dimers**
    - Thymine dimers may cause mutations when that DNA strand is replicated
  - Refer to Figure 18.14

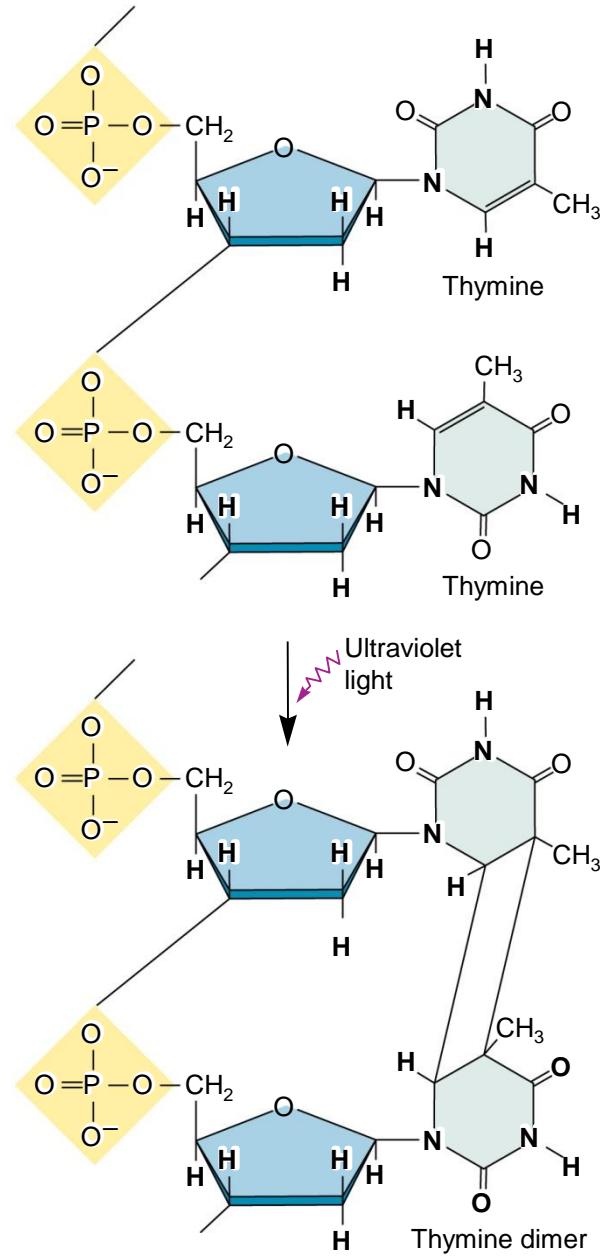


Figure 18.14

# Mutation Rate

- The term **mutation rate** is the likelihood that a gene will be altered by a new mutation
  - It is commonly expressed as the number of new mutations in a given gene per cell generation
  - It is in the range of  $10^{-5}$  to  $10^{-9}$  per cell generation
- The mutation rate for a given gene is not constant
  - It can be increased by the presence of mutagens
- Mutation rates vary substantially between species and even within different strains of the same species

# Testing Methods for Mutagens

- Many different kinds of tests have been used to evaluate mutagenicity
  - One commonly used test is the **Ames test**
    - The test uses a strain of *Salmonella typhimurium* that cannot synthesize the amino acid histidine
      - It has a point mutation in a gene involved in histidine biosynthesis
    - Test is to see if an agent increases the reversion rate
    - Refer to Figure 18.15

Mix together the suspected mutagen, a rat liver extract, and a *Salmonella* strain that cannot synthesize histidine. The suspected mutagen is omitted from the control sample.

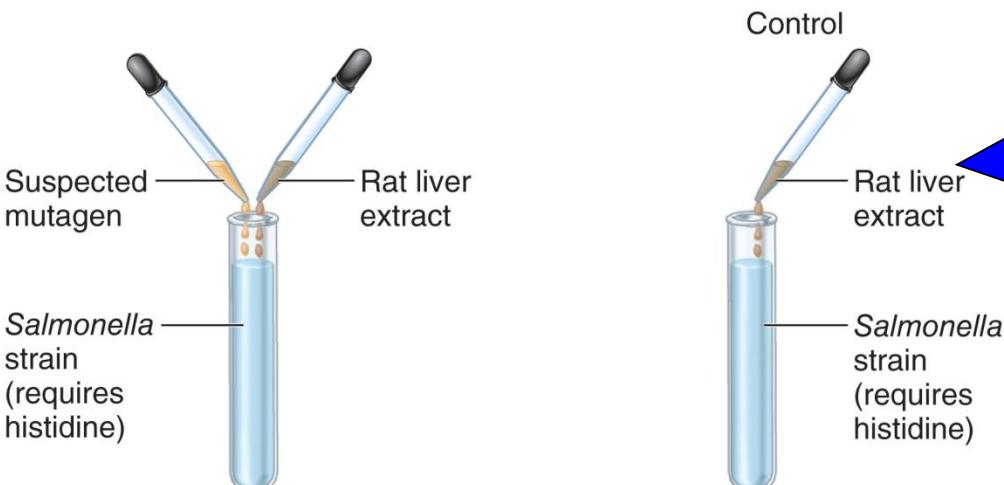
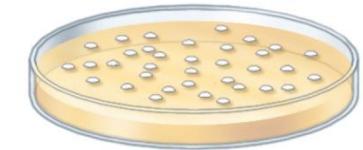


Plate the mixtures onto petri plates that lack histidine.

Incubate overnight to allow bacterial growth.



A large number of colonies suggests that the suspected mutagen causes mutation.

Provides a mixture of enzymes that may activate a mutagen

The control plate indicates that there is a low level of spontaneous mutation

Figure 18.15

# 18.5 DNA Repair

- ❑ Different types of DNA repair mechanisms
- ❑ How specialized DNA polymerases are able to synthesize DNA over a damaged region

# DNA Repair

- Since most mutations are deleterious, DNA repair systems are vital to the survival of all organisms
  - Living cells contain several DNA repair systems that can fix different types of DNA alterations
- In most cases, DNA repair is a multi-step process
  1. An irregularity in DNA structure is detected
  2. The abnormal DNA is removed
  3. Normal DNA is synthesized

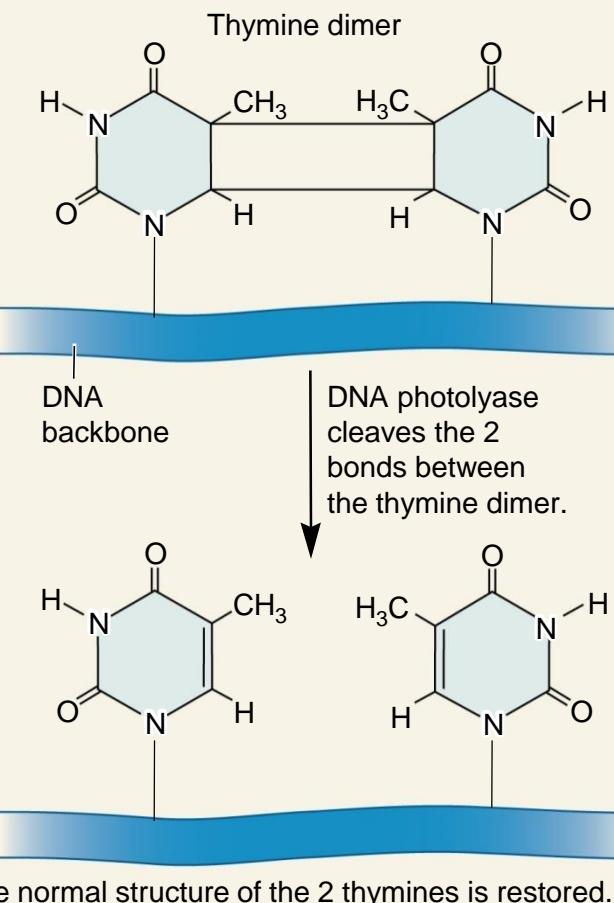
- Common types of DNA repair
  - Direct repair
  - Base excision and nucleotide excision repair
  - Mismatch repair
  - Homologous recombination repair
  - Non-homologous end joining

**TABLE 18.7**  
**Common Types of DNA Repair Systems**

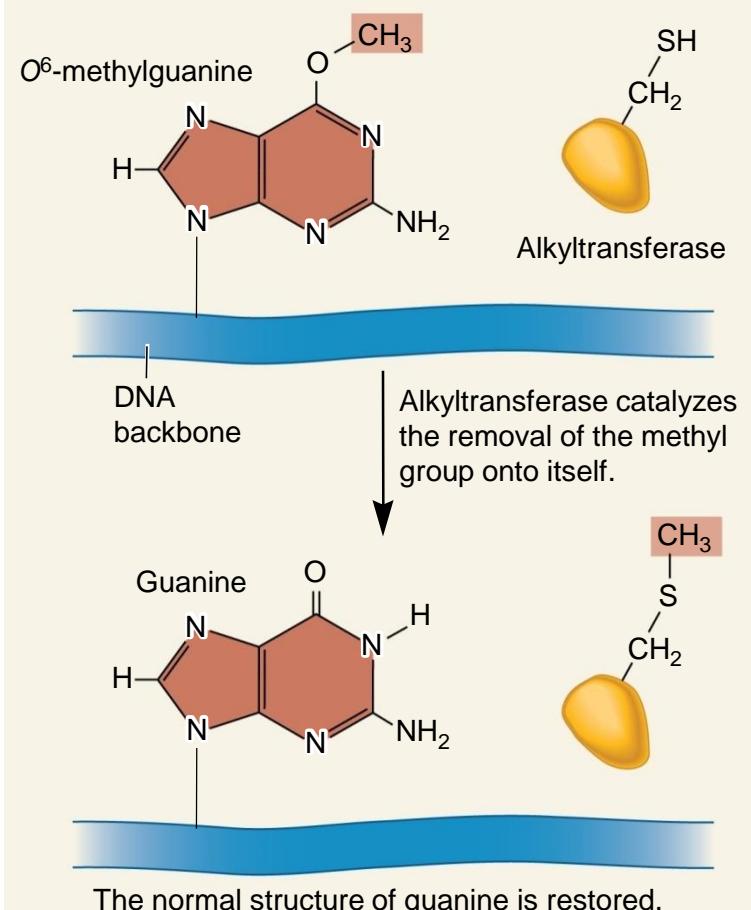
| System  | Description  |
|---|--|
| Direct repair                                       | An enzyme recognizes an incorrect alteration in DNA structure and directly converts it back to a correct structure.  |
| Base excision repair and nucleotide excision repair | An abnormal base or nucleotide is first recognized and removed from the DNA, and a segment of DNA in this region is excised, and then the complementary DNA strand is used as a template to synthesize a normal DNA strand.  |
| Mismatch repair                                     | Similar to excision repair except that the DNA defect is a base pair mismatch in the DNA, not an abnormal nucleotide. The mismatch is recognized, and a segment of DNA in this region is removed. The parental strand is used as a template to synthesize a normal daughter strand of DNA. |
| Homologous recombination repair                     | Occurs at double-strand breaks or when DNA damage causes a gap in synthesis during DNA replication. The strands of a normal sister chromatid are used to repair a damaged sister chromatid.  |
| Nonhomologous end joining                           | Occurs at double-strand breaks. The broken ends are recognized by proteins that keep the ends together; the broken ends are eventually rejoined.   |

# Direct Repair

- In a few cases, the covalent modifications of nucleotides can be reversed by specific enzymes
  - **Photolyase** can repair thymine dimers
    - Splits the dimers restoring the DNA to original condition
    - Uses light so called **photoreactivation**
  - **Alkyltransferase** repairs alkylated bases
    - Transfers methyl or ethyl group from base to a cysteine side chain within the alkyltransferase protein
      - This permanently inactivates alkyltransferase
    - Refer to Figure 18.16



(a) Direct repair of a thymine dimer



(b) Direct repair of a methylated base

Figure 18.16

# Base Excision Repair

- **Base excision repair (BER)** involves a category of enzymes known as **DNA N-glycosylases**
  - These enzymes can recognize an abnormal base and cleave the bond between it and the sugar in the DNA
  - **AP endonuclease** then makes a cut on the 5' side
- Depending on the species, this repair system can eliminate abnormal bases such as
  - Uracil; Thymine dimers
  - 3-methyladenine; 7-methylguanine
- Refer to Figure 18.17

Depending on whether a purine or pyrimidine is removed, this creates an apurinic or an apyrimidinic site, respectively

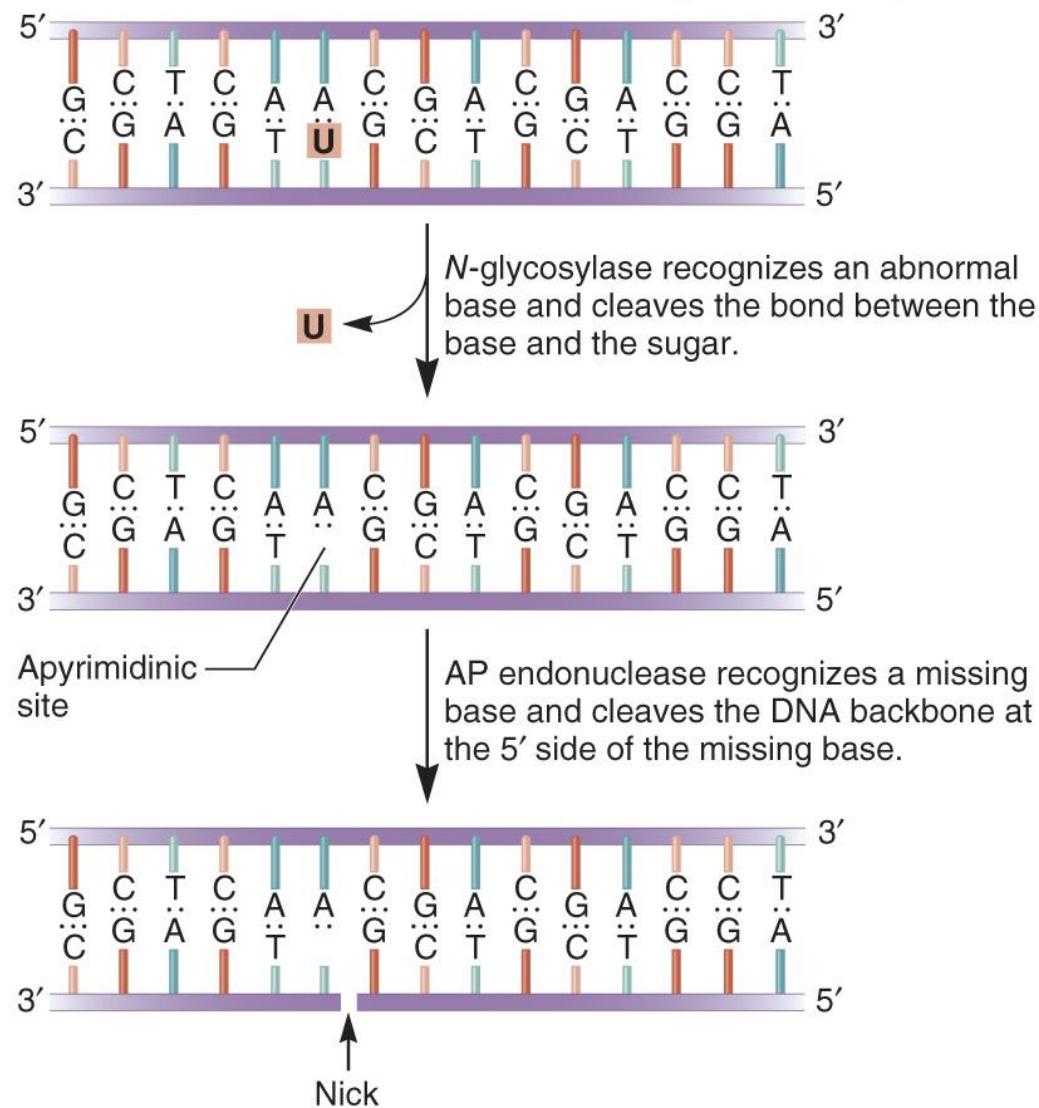


Figure 18.17



Depending on whether a purine or pyrimidine is removed, this creates an apurinic or an apyrimidinic site, respectively

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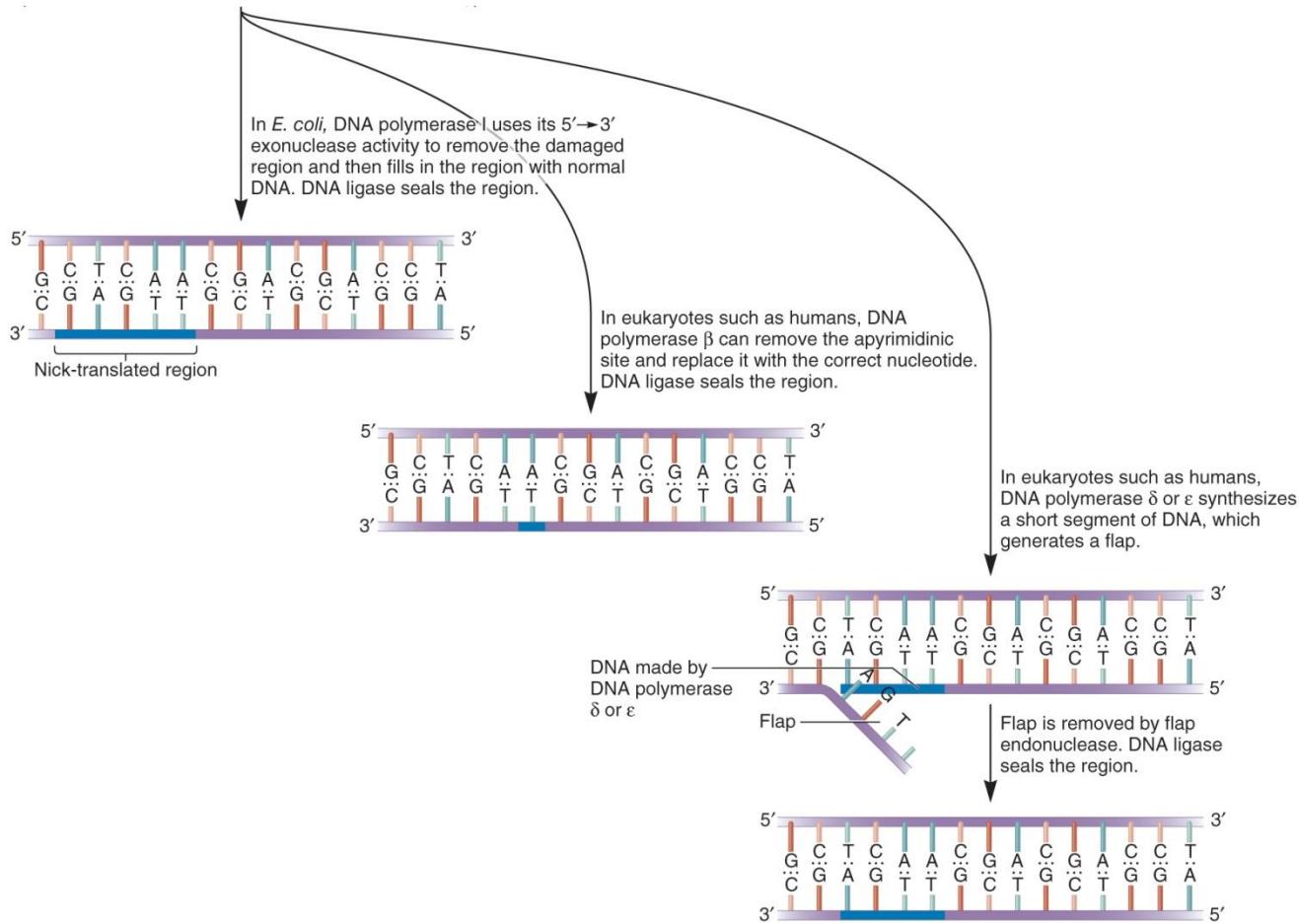


Figure 18.17

# Nucleotide Excision Repair

- Can repair many types of DNA damage, including
  - Thymine dimers and chemically modified bases
  - Missing bases, some types of cross-link
- NER is found in all eukaryotes and prokaryotes
  - Molecular mechanism best understood in prokaryotes

- In *E. coli*, the NER system requires four key proteins
  - **UvrA, UvrB, UvrC** and **UvrD**
    - Named as such because they are involved in Ultraviolet light repair of pyrimidine dimers
      - They are also important in repairing chemically damaged DNA
  - UvrA, B, C, and D recognize and remove a short segment of damaged DNA
  - DNA polymerase and ligase finish the repair job
  - Refer to Figure 18.18

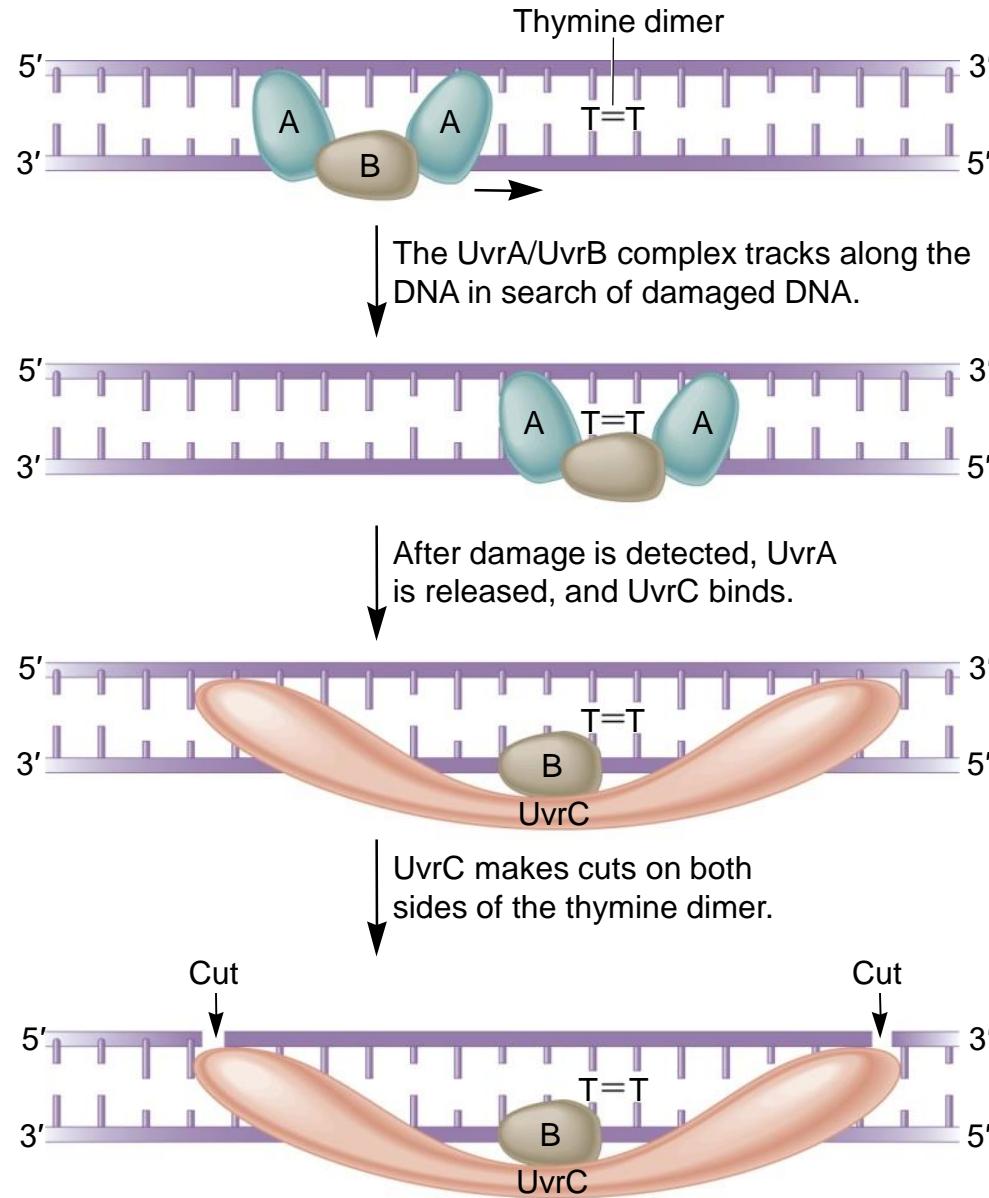


Figure 18.18 (top)

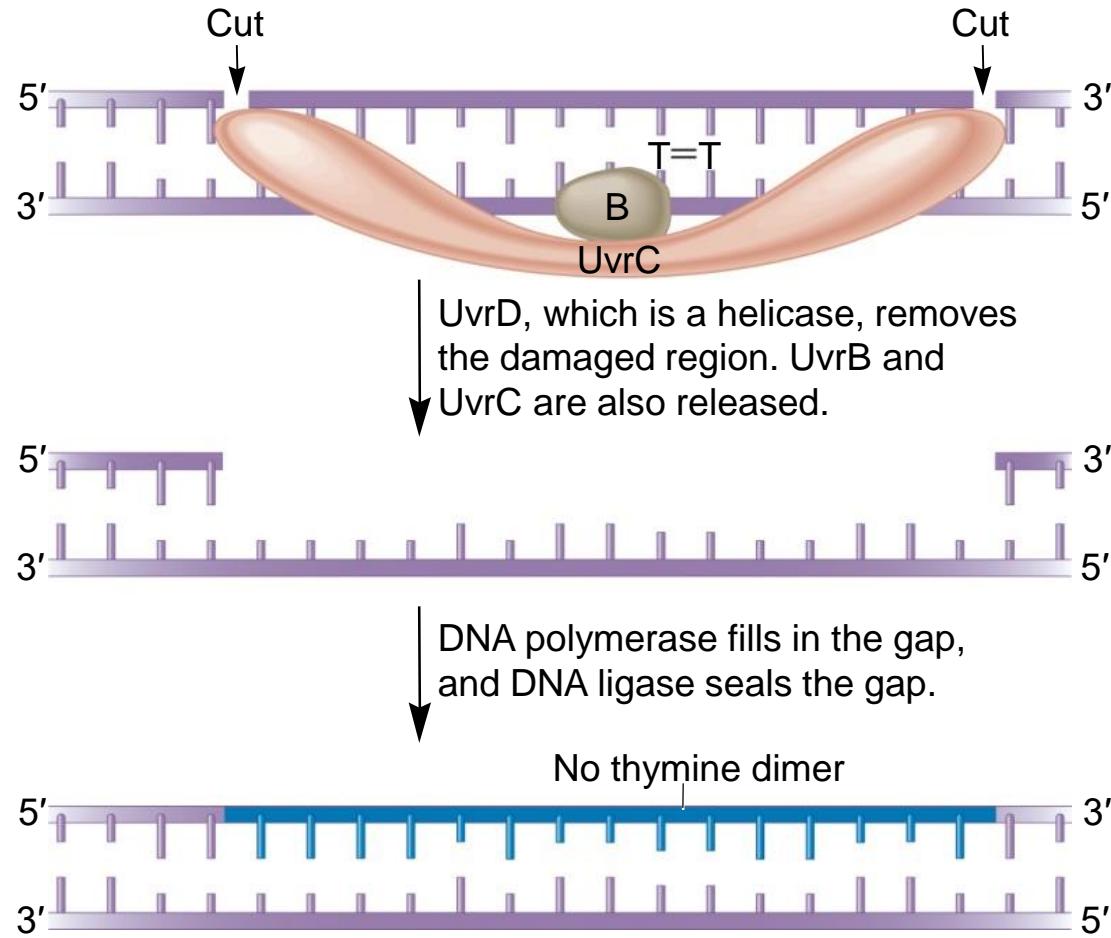


Figure 18.18 (bottom)

- Several **human diseases** have been shown to involve inherited defects in genes involved in NER
  - These include xeroderma pigmentosum (XP) and Cockayne syndrome (CS)
    - A common characteristic in both syndromes is an increased sensitivity to sunlight
    - **Figure 18.19** shows an individual affected with XP
  - Xeroderma pigmentosum can be caused by defects in seven different NER genes

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## Table 18.19

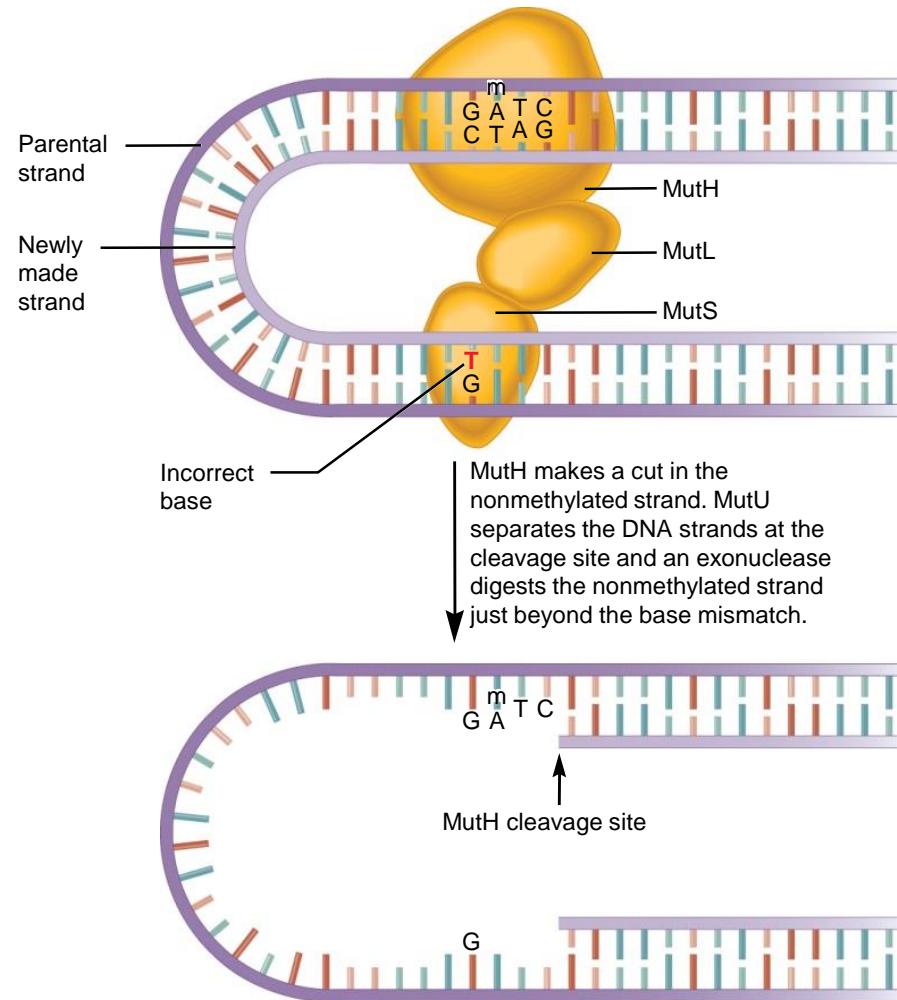
# Mismatch Repair

- The structure of the DNA double helix obeys the AT/GC rule of base pairing
  - However, during DNA replication an incorrect base may be added to the growing strand by mistake
  - Creating a **base pair mismatch**
- DNA polymerases have a 3' to 5' proofreading ability that can detect base mismatches and fix them

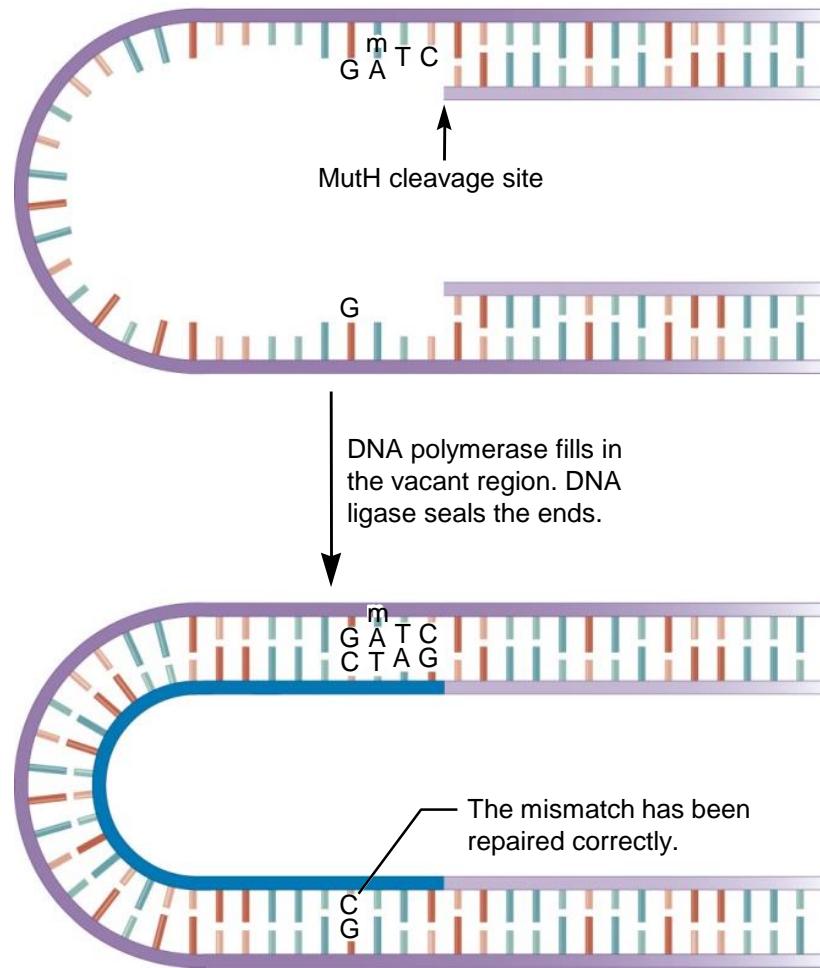
- If proofreading fails, the **mismatch repair system** comes to the rescue
- Mismatch repair systems are found in all species
- In humans, mutations in the system are associated with particular types of cancer

- Mismatch repair has been studied extensively in *E. coli*
  - **MutL, MutH** and **MutS** detect the mismatch and direct its removal from the newly made strand
  - MutH can distinguish between the parental strand and the daughter strand
    - Prior to replication, both strands are methylated
    - Immediately after replication, the parental strand is methylated but the daughter strand is not
    - Refer to Figure 18.20

The MutS protein finds a mismatch. The MutS/MutL complex binds to MutH, which is already bound to a hemimethylated sequence.



**Figure 18.20 (top)**



**Figure 18.20 (bottom)**

# Recombination Repair

- DNA double-strand breaks are very dangerous
  - Breakage of chromosomes into pieces
  - Caused by ionizing radiation, chemical mutagens and free radicals
  - 10-100 breaks occur each day in a typical human cell
  - Breaks can cause chromosomal rearrangements and deficiencies
- They may be repaired by two systems:
  - **Homologous recombination repair (HRR)**
  - **Nonhomologous end joining (NHEJ)**
  - Refer to Figures 18.21 and 18.22

# Homologous recombination repair

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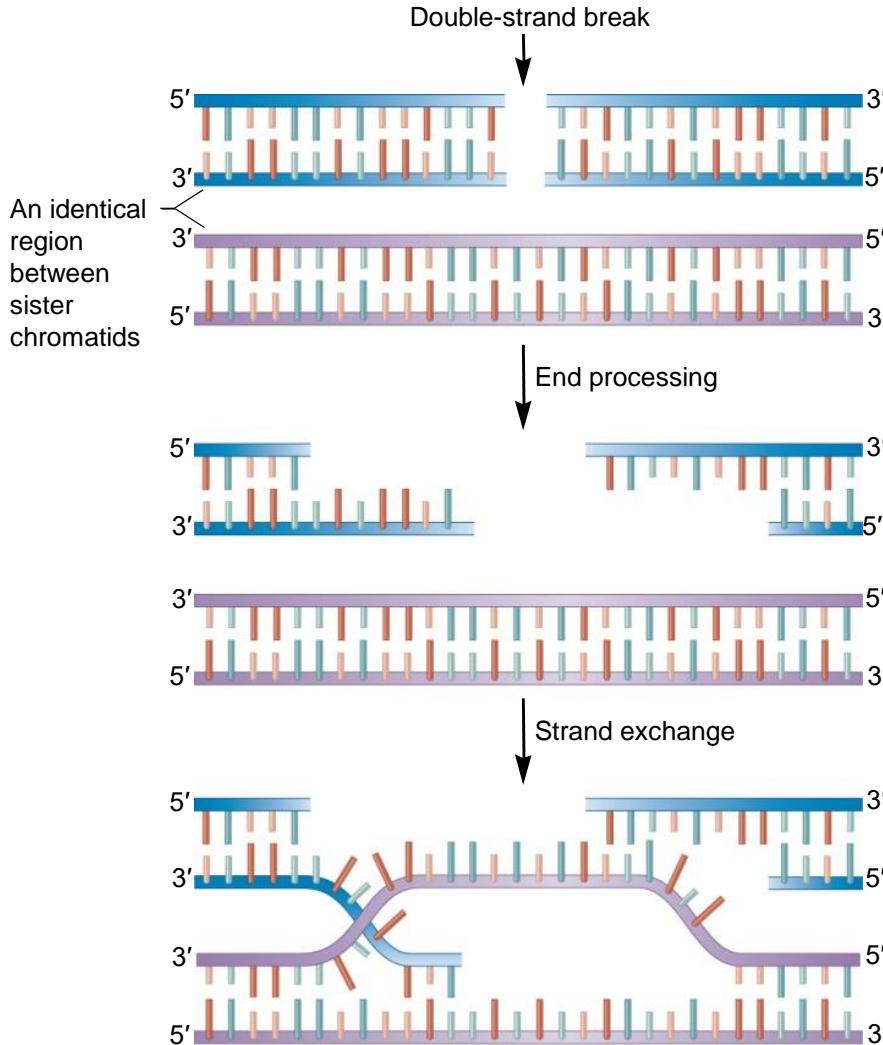


Figure 18.21 (top)

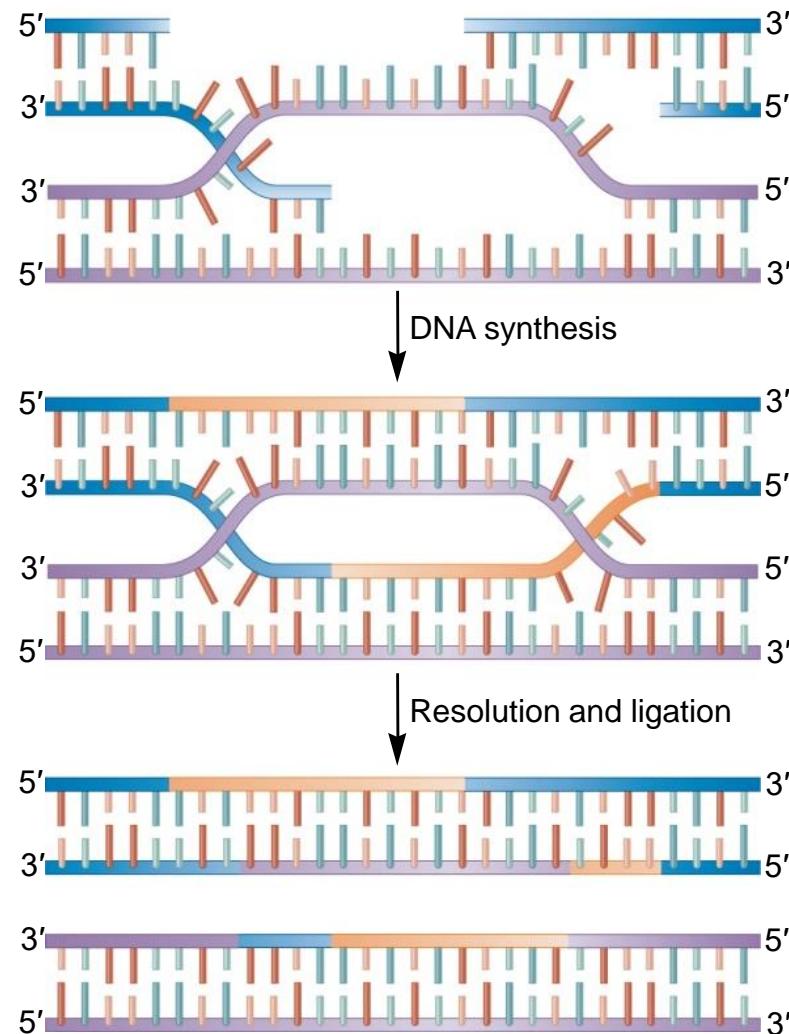


Figure 18.21 (bottom)

# Nonhomologous end joining

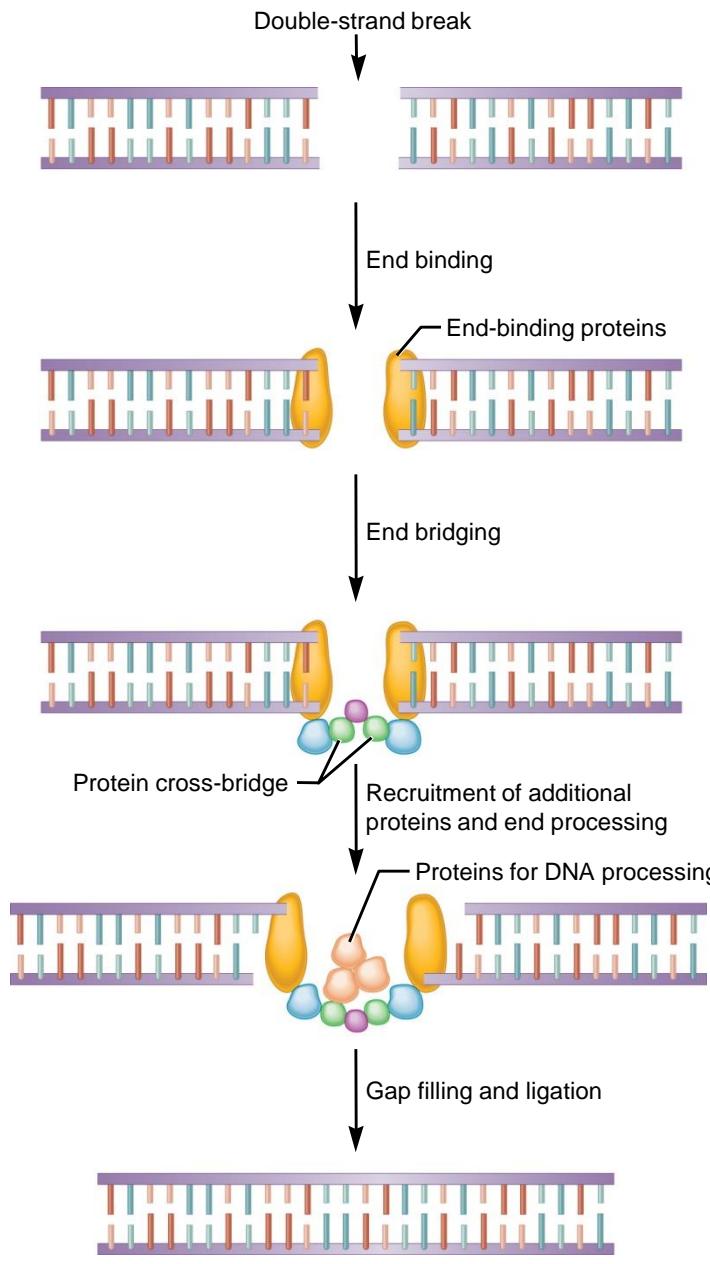


Figure 18.22

# Translesion DNA Polymerases

- Despite all the DNA repair systems, some lesions escape
- Typical DNA polymerases cannot replicate through a region of DNA damage
- Special polymerases help with **translesion synthesis (TLS)**
  - **Translesion polymerases** contain an active site with a loose flexible pocket to accomodate the abnormal structure
    - However, they have low fidelity
    - Their **error-prone replication** has a mutation rate of  $10^{-2}$  to  $10^{-3}$

Replicative DNA polymerase



DNA damage



Pol  $\zeta$   
Pol  $\eta$   
(Pol  $\tau$ )

Pol  $\kappa$   
Rev1\*

TLS DNA polymerase



“Bypass” DNA synthesis

