Lecture 28:

Sources of mutations

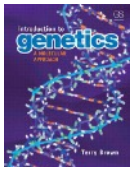
Course 281

AIMS

- Understand

The Causes of Mutations

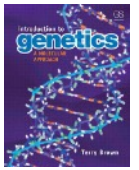
- **Errors in replication** are a source of point mutations
- Replication errors can also lead to insertion and deletion mutations
- **Mutagens** are one type of environmental agent that causes damage to cells
- There are many types of **chemical mutagen**
- There are also several types of **physical mutagen**



Errors in Replication

- The *in vitro* error rate during DNA replication is 5–10% which is unacceptable in a living cell.

- The accuracy of DNA replication in the cell is increased by the 3`→ 5` exonuclease activity of the DNA polymerases (proof reading)



Template-dependent DNA Replication

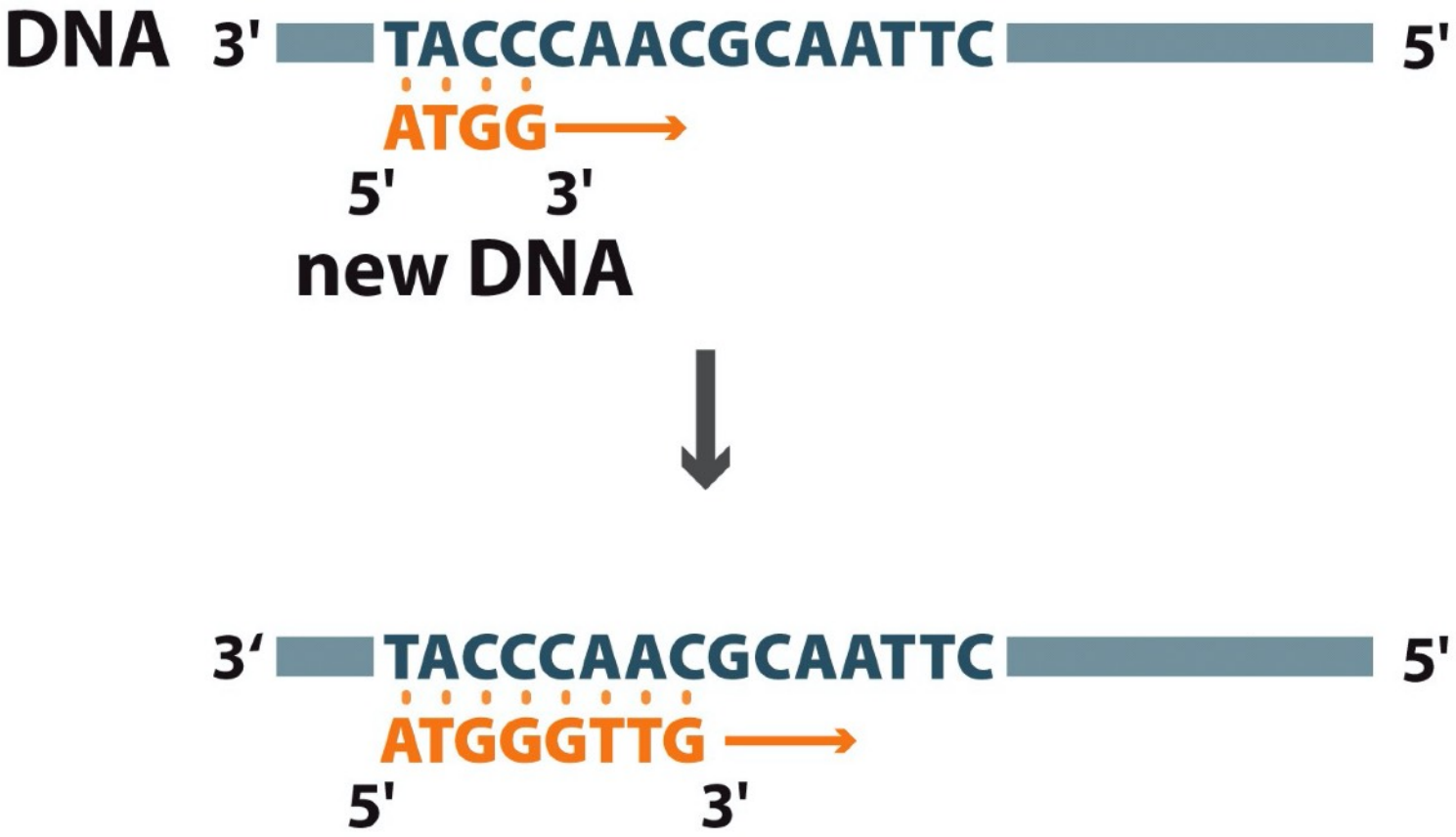


Figure 16.4 Introduction to Genetics (© Garland Science 2012)

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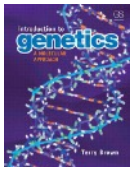

DNA Polymerase Inserts the Correct Nucleotide

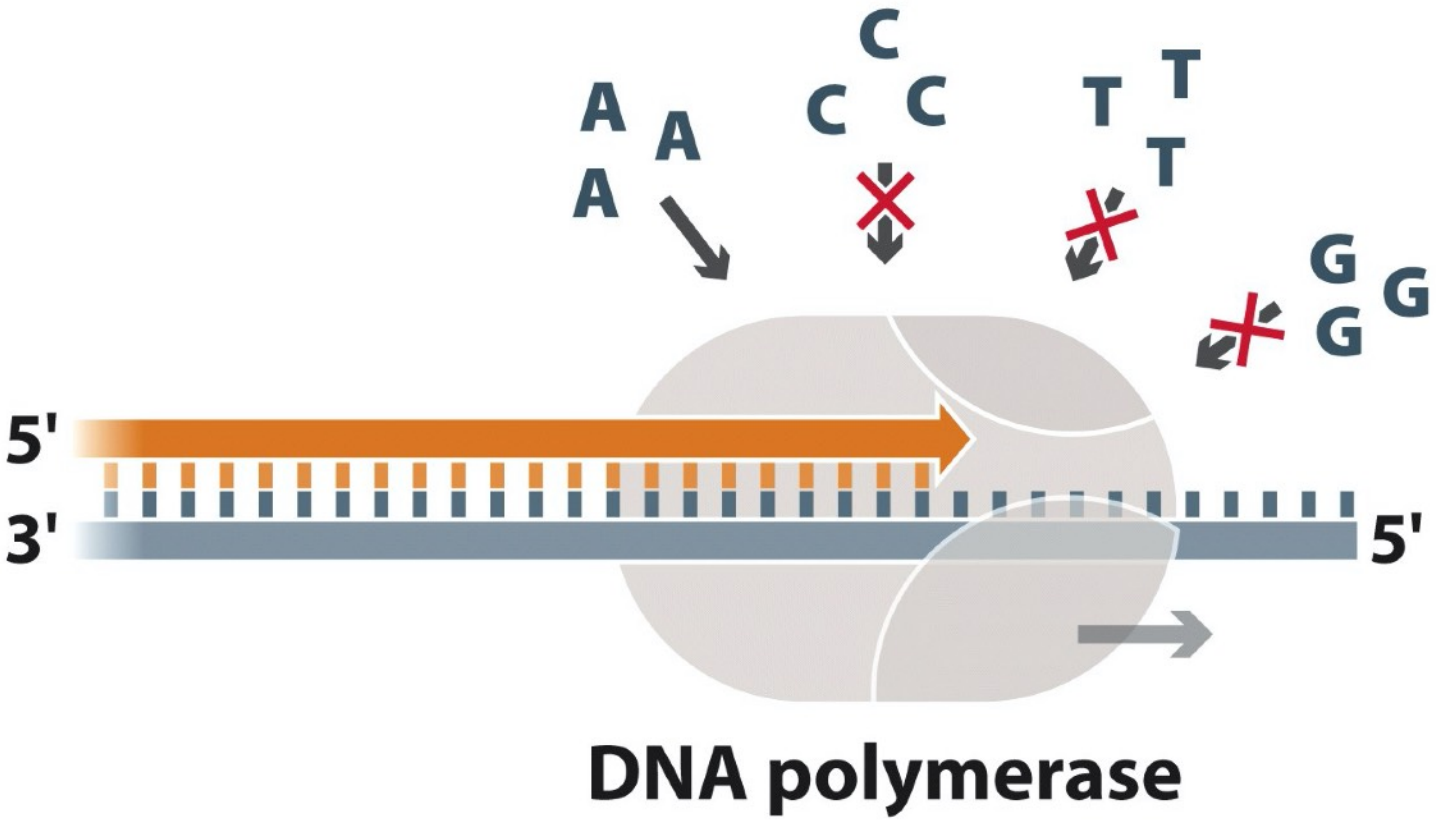

Figure 16.5 Introduction to Genetics (© Garland Science 2012)

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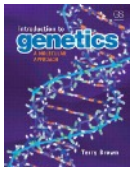


DNA Polymerase Proofreading Activity

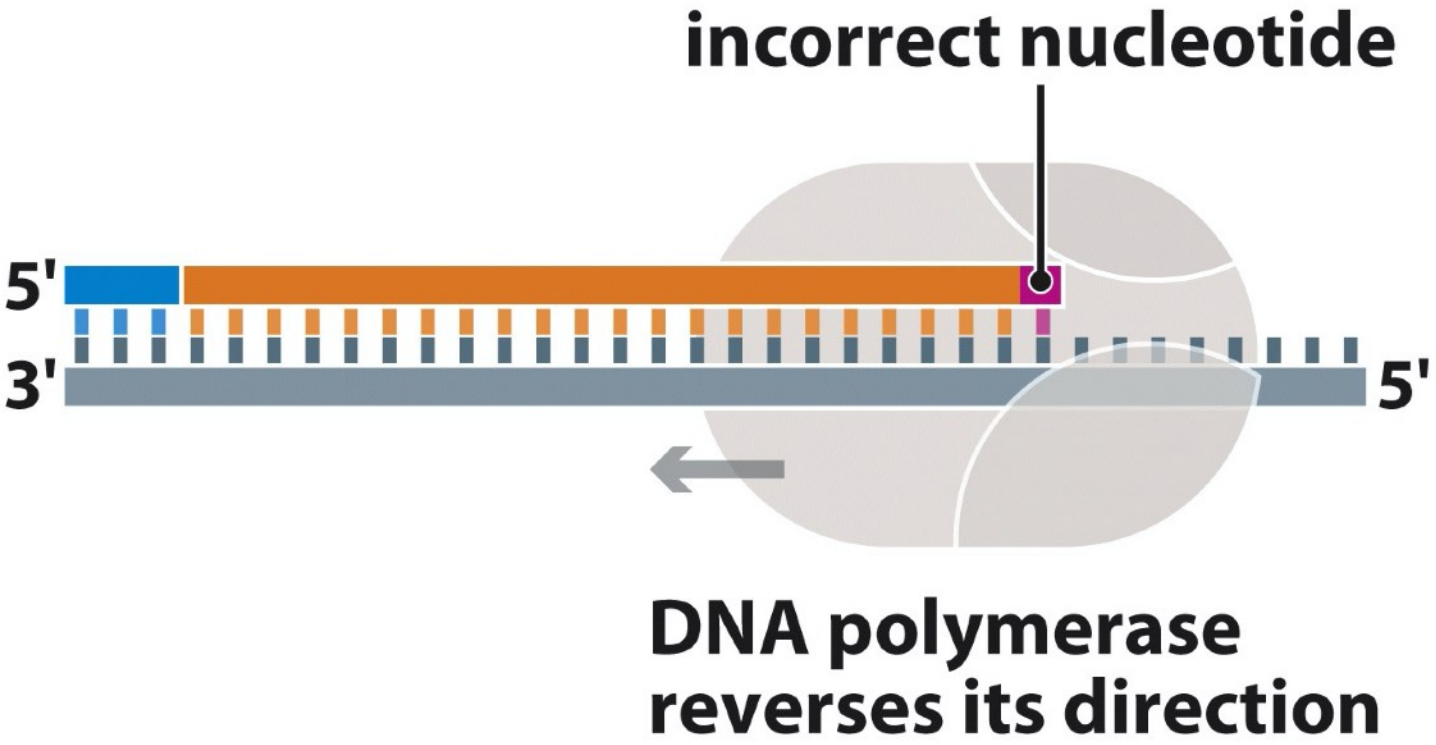
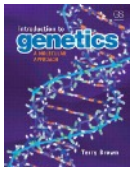


Figure 16.6 Introduction to Genetics (© Garland Science 2012)



Tautomeric Shift

tau·to·mer | ˈtôdəmər |

noun *Chemistry*

each of two or more isomers of a compound which exist together in equilibrium, and are readily interchanged by migration of an atom or group within the molecule.

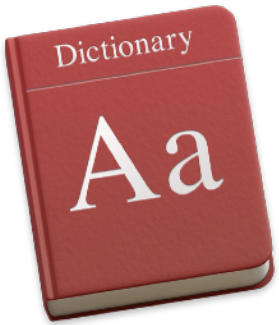
DERIVATIVES

tautomeric | ˌtôtəˈmerik | adjective

tautomerism | tôˈtämәˌrizәm | noun

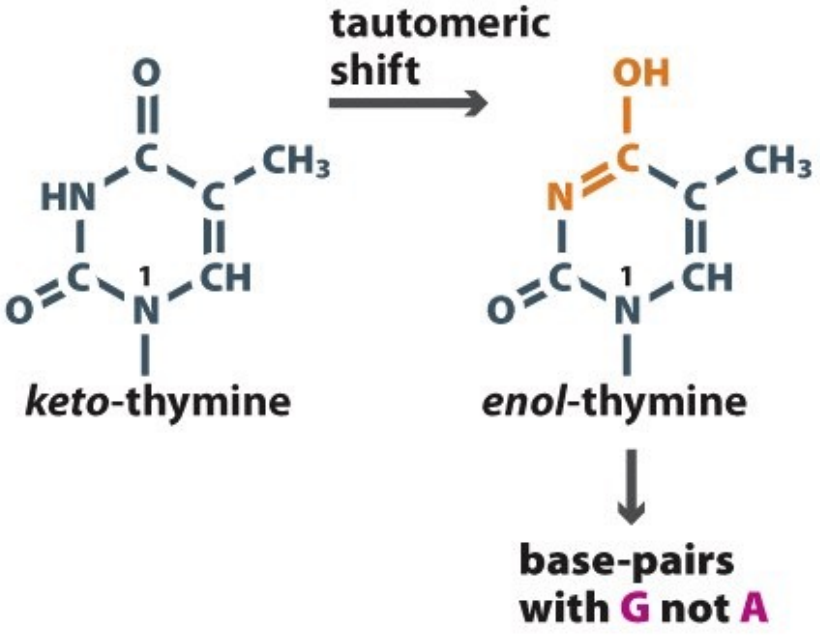
ORIGIN

early 20th century: blend of tauto- ‘same’ and isomer.

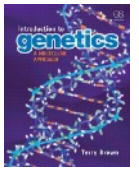


Tautomeric Shift

- Some error occur due to a **tautomeric shift** form the **keto** to the **enol** form; the equilibrium is biased toward the keto form.



- The **enol** form of thymine base pairs with G rather than A



Tautomeric Shift

- The **enol** form of G pairs with T

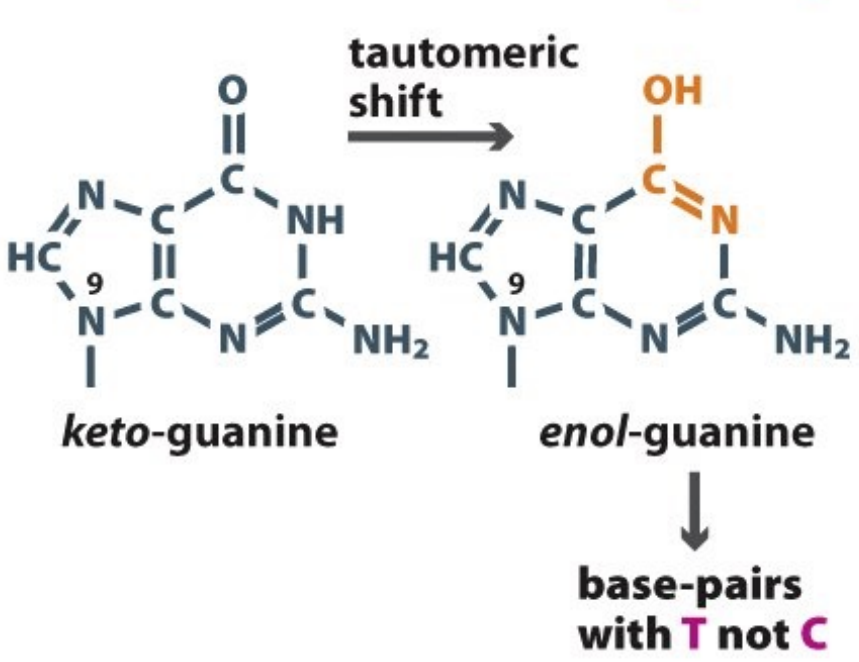
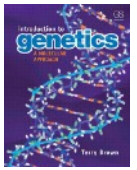
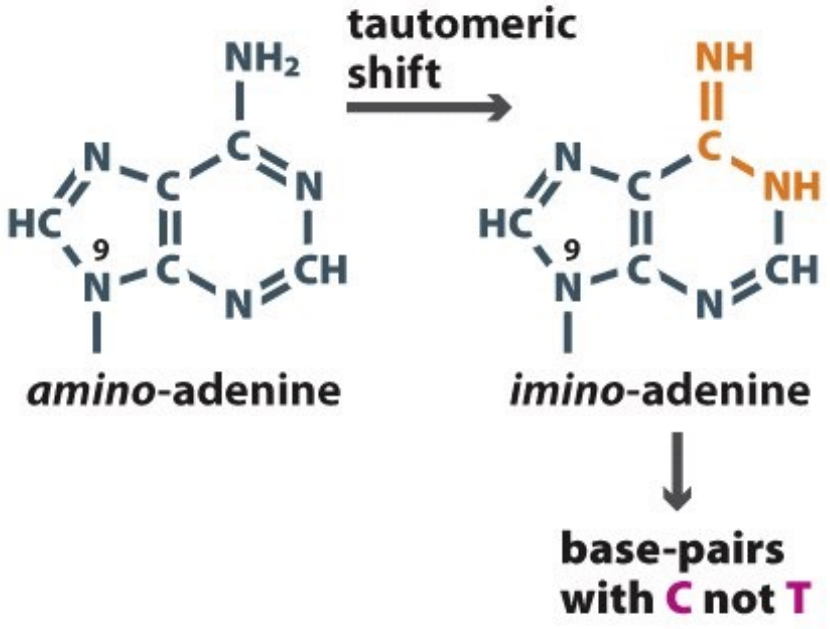


Figure 16.7 Introduction to Genetics (© Garland Science 2012)



Tautomeric Shift

- The rare **imino** tautomer of A preferentially pairs with C
- After replication, the rare tautomer reverts to its more common form; leads to a **mismatch mutation**



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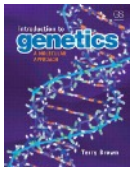
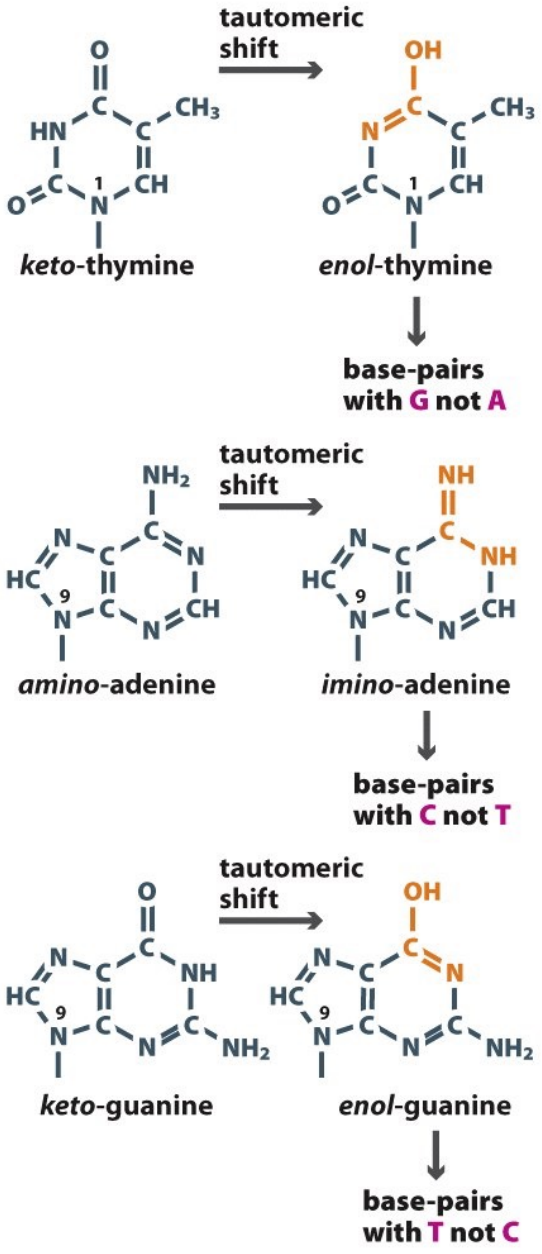


Tautomeric Shifts

- The two tautomeric forms of the nitrogen bases have different hydrogen bonding properties
- The amino and **imino** tautomers of C both pair with G



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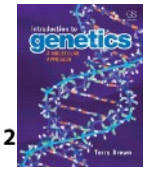
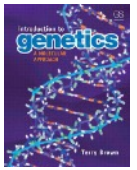


Replication Slippage

- Short DNA repeated sequences induce **replication slippage**
- The template strand and its copy shift their relative positions so that part of the template is either copied twice or missed
- The new polynucleotide has a larger or smaller number of the repeat units



Replication Slippage

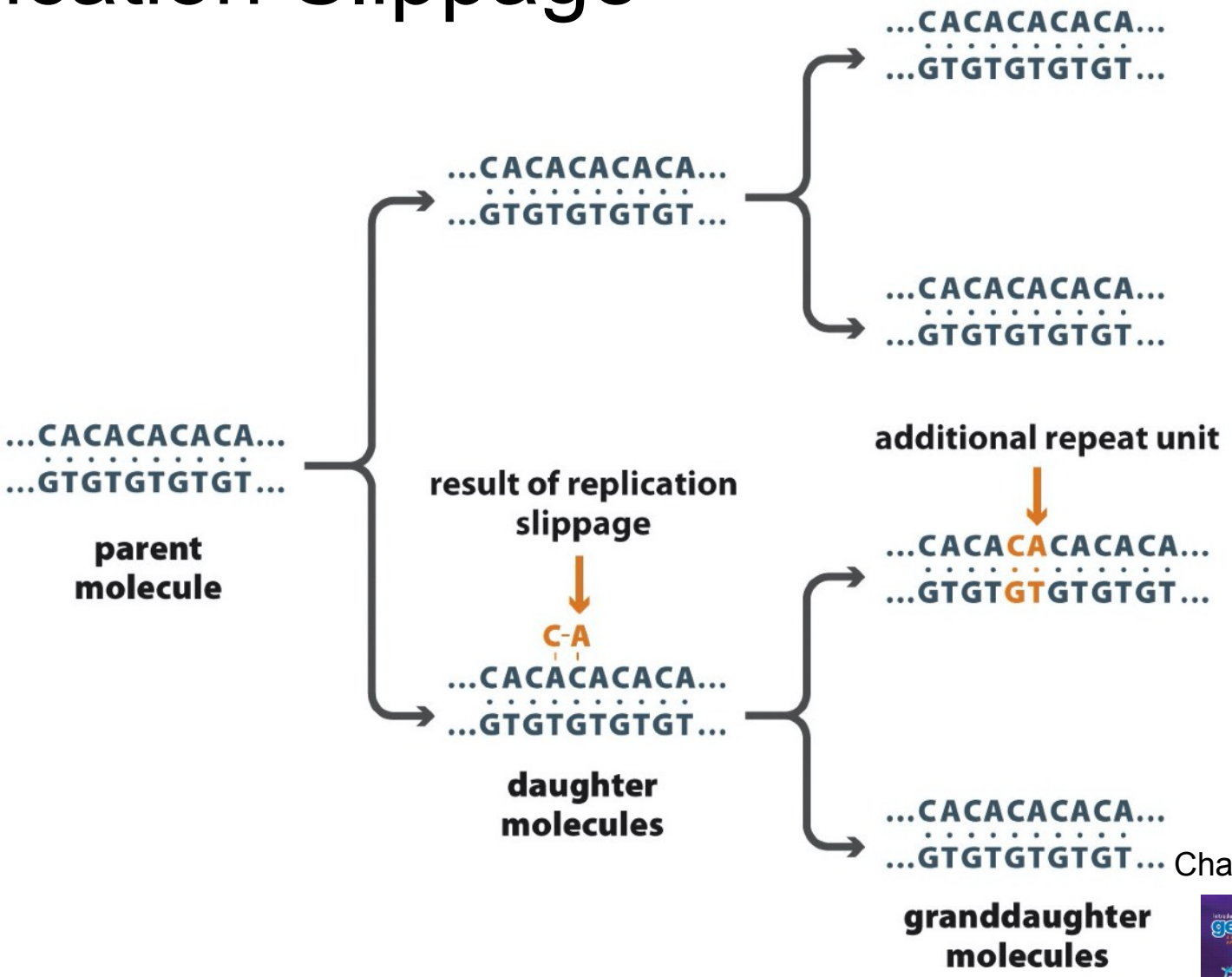
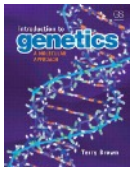


Figure 16.9 Introduction to Genetics (© Garland Science 2012)



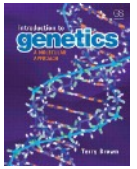
The Trinucleotide Repeat Expansion Diseases

- Replication slippage responsible for the **trinucleotide repeat expansion diseases**

- Human neurodegenerative diseases caused by expanding trinucleotide repeat copy numbers to two or more times its normal length

- The human *HD* gene contains the sequence 5'-CAG-3' (glutamine) repeated 6 - 35 times in tandem

- In **Huntington's disease,** this repeat expands 36 – 121 copies; the longer polyglutamine tract results in protein dysfunctional



The Genetic Basis of Huntington's Disease

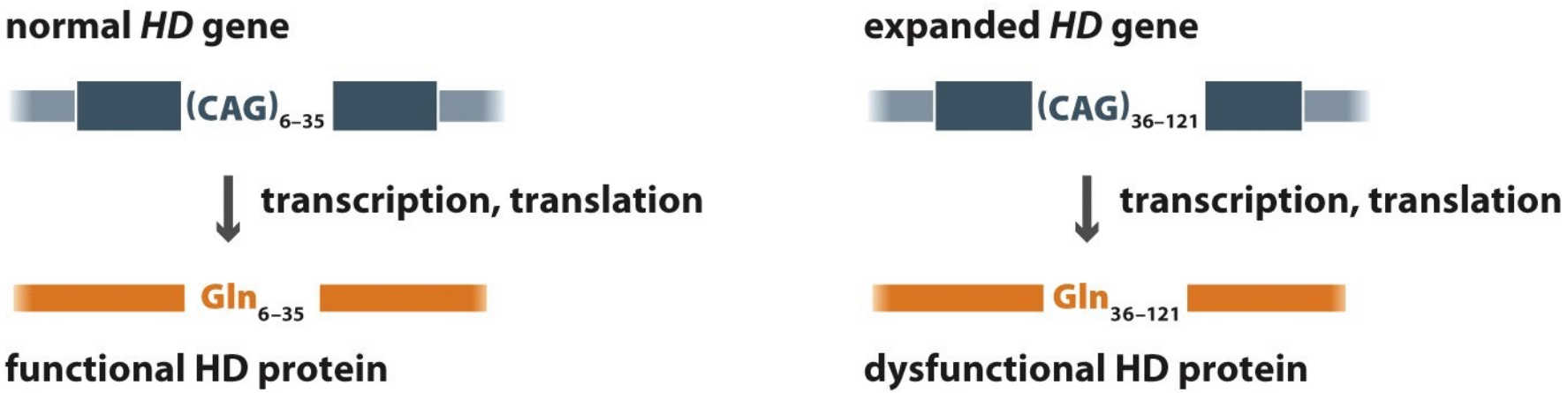
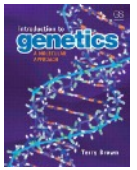
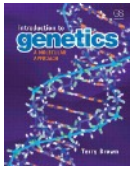


Figure 16.10 Introduction to Genetics (© Garland Science 2012)



Environmental Agents That Cause Damage to Cells

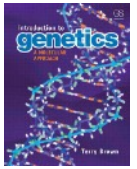
- Naturally occurring chemicals
- Industrial wastes
- Physical agents such as radiation



Mutagens Cause Mutations

- A **mutagen** is a chemical or physical agent that causes mutations

- Mutagens cause mutations in three different ways;
    1. **Base analogs** are mistakenly used as substrates in DNA replication
    2. Other mutagens react **directly** with DNA, causing diverse **structural changes** that lead to errors in DNA replication
    3. Some mutagens act **indirectly** on DNA. They do not affect DNA structure but cause the cell to synthesize chemicals **(e.g.; peroxides)** that have a direct mutagenic effect



Mutagens Cause Mutations in Three Different Ways

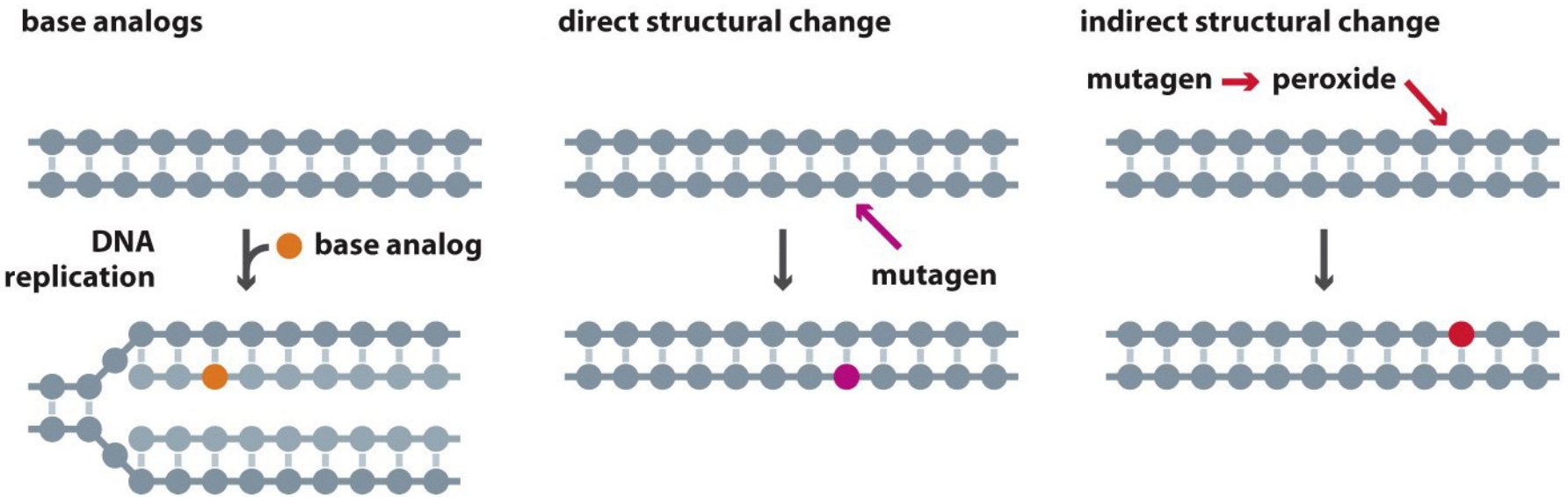
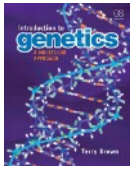
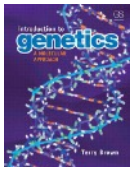


Figure 16.11 Introduction to Genetics (© Garland Science 2012)



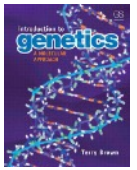
Some Environmental Agent Damage Cells Without Causing Mutations

- It is distinct from environmental agent that cause damage to cells in ways other than mutations;
  - **Carcinogens**: cause cancer
  - **Oncogens**: cause tumor formation
  - **Teratogens**: cause developmental abnormalities



Some Environmental Agent Damage Cells Without Causing Mutations

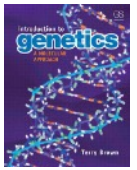
- Some mutagens are also carcinogens, but each agent has a distinct biological effect
- **Clastogens**: agents that damage DNA without causing mutations
- Cause DNA breaks and subsequent chromosome fragmentation; this blocks replication and cell death



Important Types of Chemical Mutagen

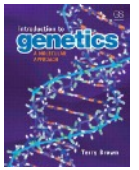
Type	Examples
Base analogs	5-Bromouracil, 2-aminopurine
Deaminating agents	Nitrous acid, sodium bisulfite
Alkylating agents	Ethylmethane sulfonate, dimethylnitrosamine, methyl halides
Intercalating agents	Ethidium bromide

Table 16.1 Introduction to Genetics (© Garland Science 2012)



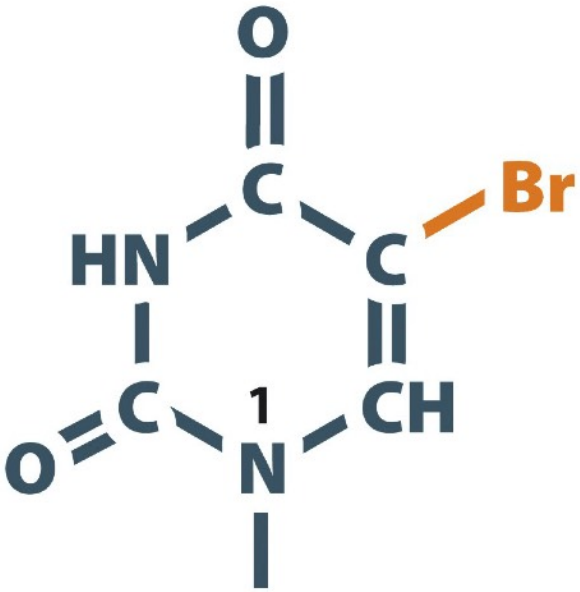
Base Analogs

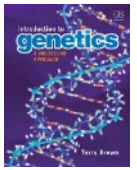
- Purine and pyrimidine bases that are similar to the standard bases
- Incorporated into nucleotides and can be used as substrates for DNA synthesis during replication



Base Analogs

- **5-bromouracil** (**5-bu**) has the same base-pairing properties as thymine
  - The equilibrium is biased toward the rarer enol form which pairs with G rather than A **(point mutation)**





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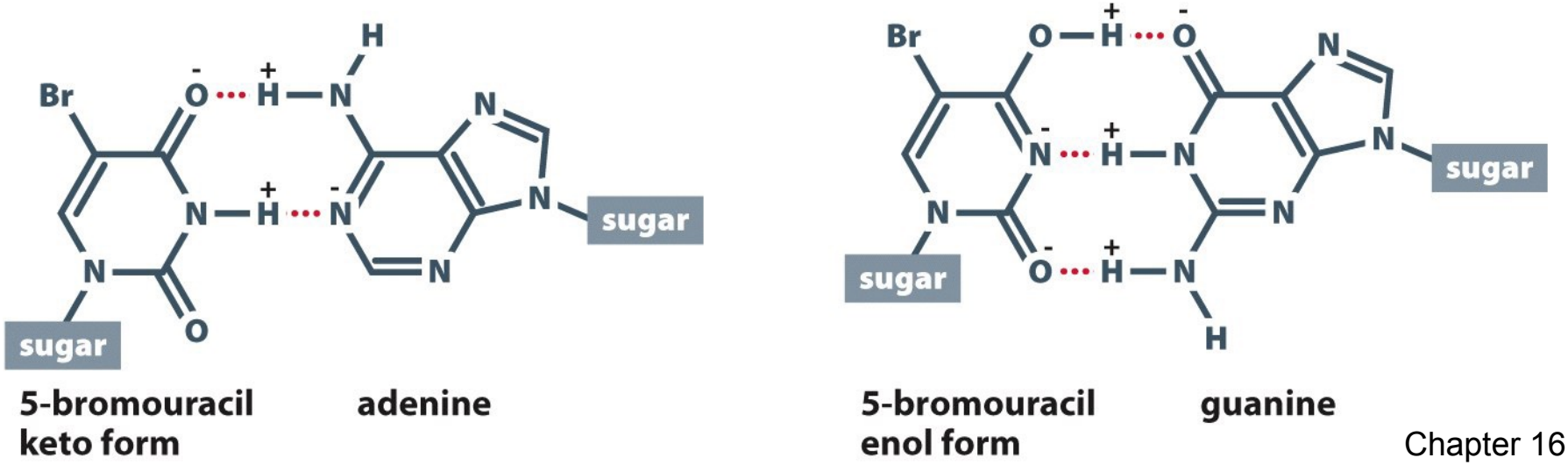
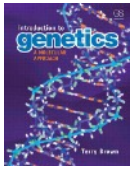


Figure 16.12b Introduction to Genetics (© Garland Science 2012)



The Mutagenic Effect of 5-Bromouracil

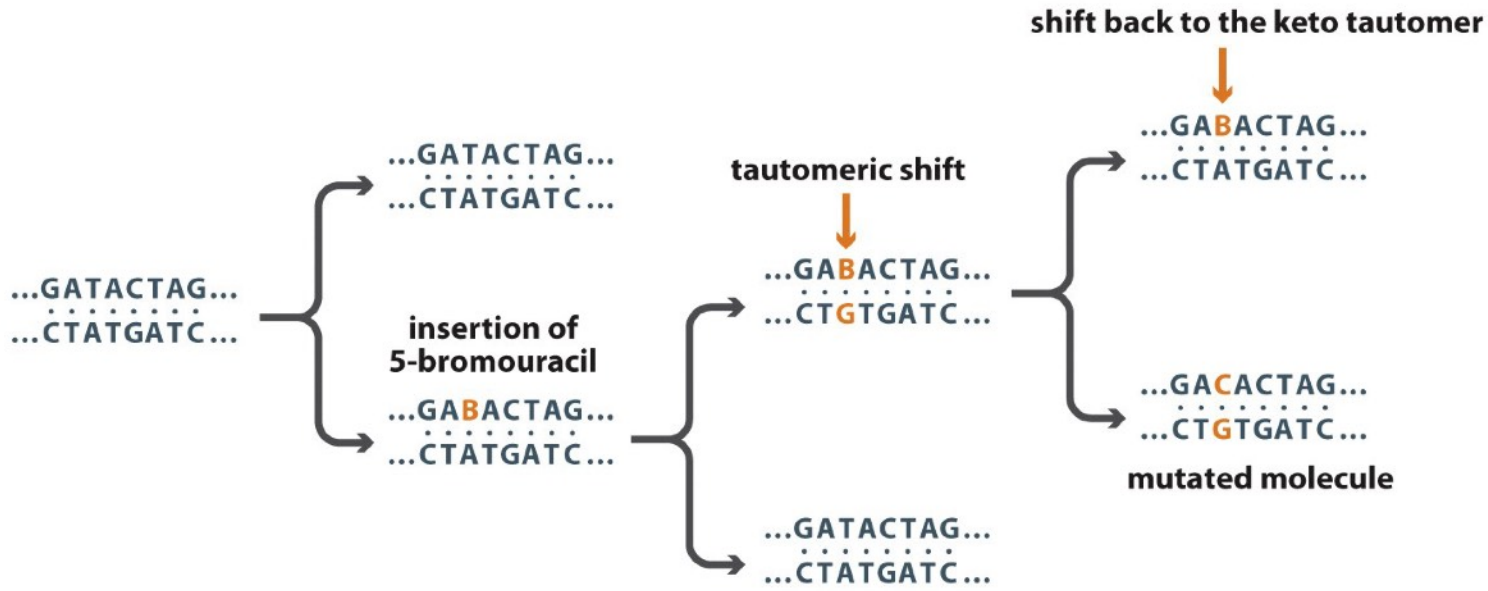
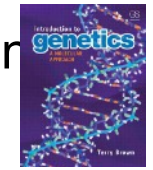


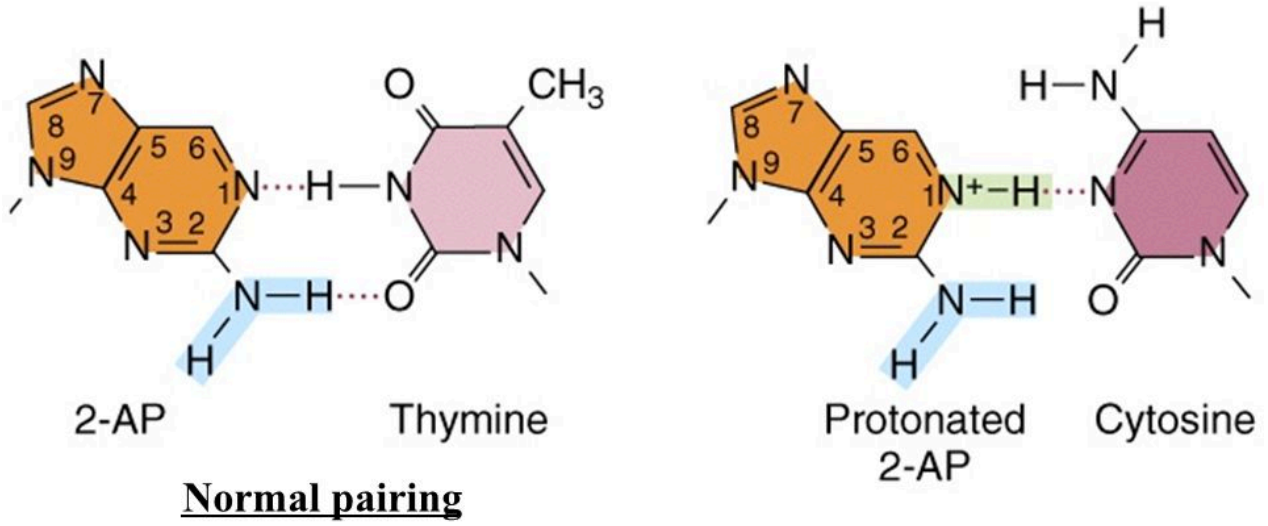
Figure 16.12c Introduction to Genetics (© Garland Science 2012)

- During the first cycle of replication, 5-bromouracil acts as a base analog and replaces a thymine nucleotide in one of the daughter molecules
- During the second cycle, the *enol* tautomer of 5-bromouracil leads to a base-pairing change.
- The third round of replication converts this error into a point mutation

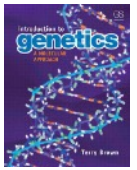


Base Analogs

- **2-aminopurine** is an analog of A
  - The *imino* form is more common and induces T to C transitions during DNA replication

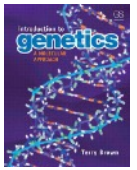


Normal pairing

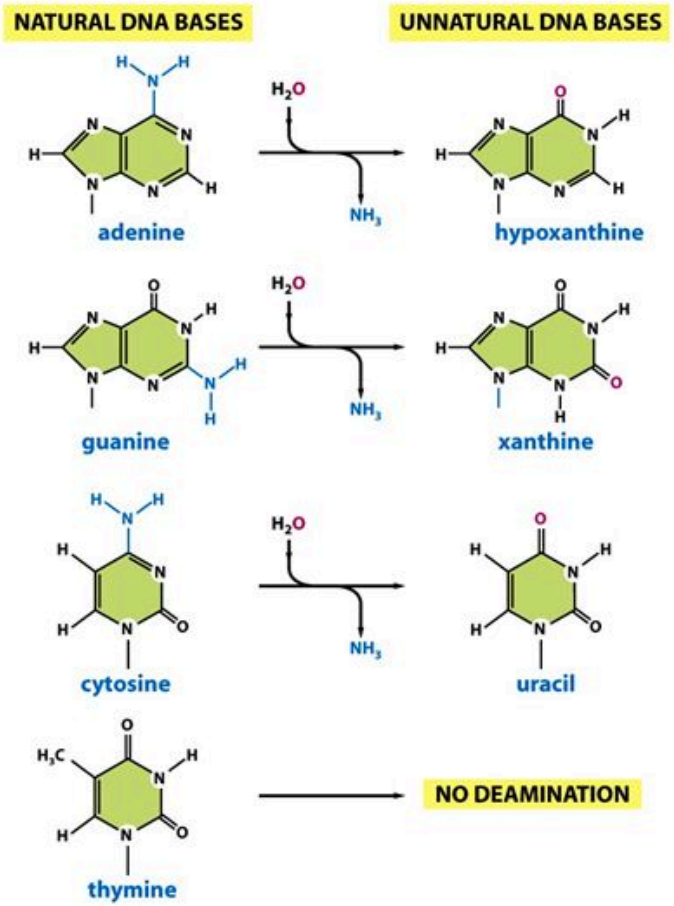


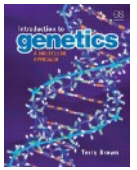
Deaminating Agents

- Base deamination (removal of an amino group) is a spontaneous process
- Chemicals such as **nitrous acid** increase the rate of deamination of A, C, and G
- **Sodium bisulfite** acts only on C.
- Thymine has no amino group and cannot be deaminated



Deaminating Agents

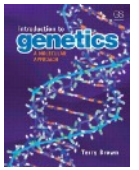




Deaminating Agents

- Deamination of A and C result in **point mutations**;
  - Deamination of A gives **hypoxanthine** which pairs with C rather than T
  - Deamination of C gives U, which pairs with A rather than G

- Deamination of G gives **xanthine** which blocks replication;
  - This is not a mutagenic effect according to our definition of mutation

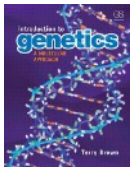


Alkylating Agents

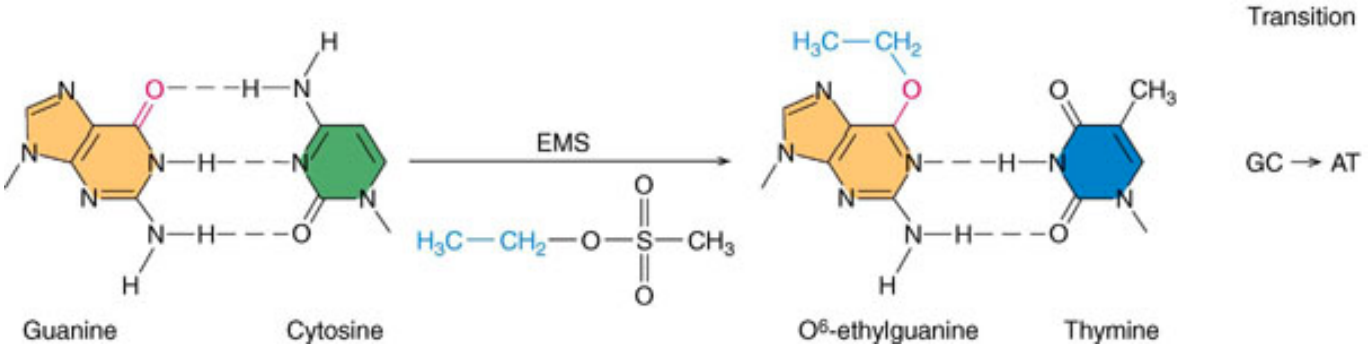
- Some chemicals add alkyl groups to nucleotides in DNA
  - Ethylmethane sulfonate (EMS)
  - Dimethylnitrosamine

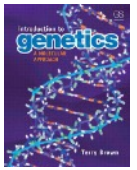
- Alkylation may result in crosslinking the DNA strands, or adding large alkyl groups, that block replication

- Methylating agents such as methyl halides, the products of nitrite metabolism, also cause point mutations



Alkylating Agents

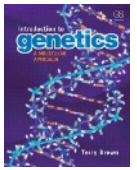




Alkylating Agents

- The effect of alkylation depends on;
  - The position at which the nucleotide is modified
  - The type of alkyl group that is added

- Methylations often result in modified nucleotides and lead to point mutations

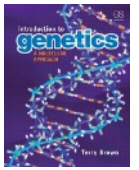


Intercalating Agents

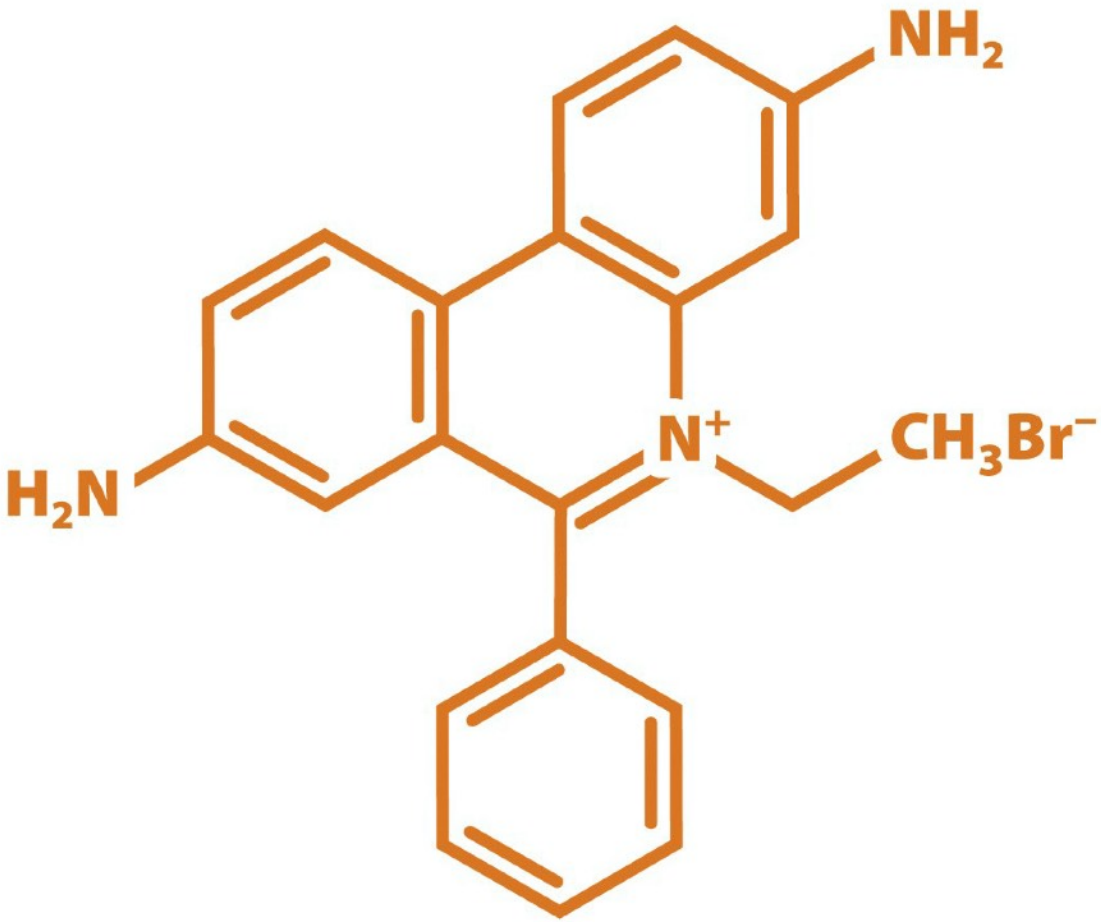
- **Ethidium bromide** and other intercalating agents are flat molecules that can slip between base pairs in the double helix

- Slightly unwinds the DNA helix which increases the distance between adjacent base pairs
- This is leads to insertion, deletion, and other types of mutation.

- Ethidium bromide is sometimes used as a stain for DNA because it fluoresces when exposed to UV radiation



Ethedium Bromide



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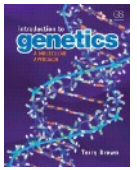
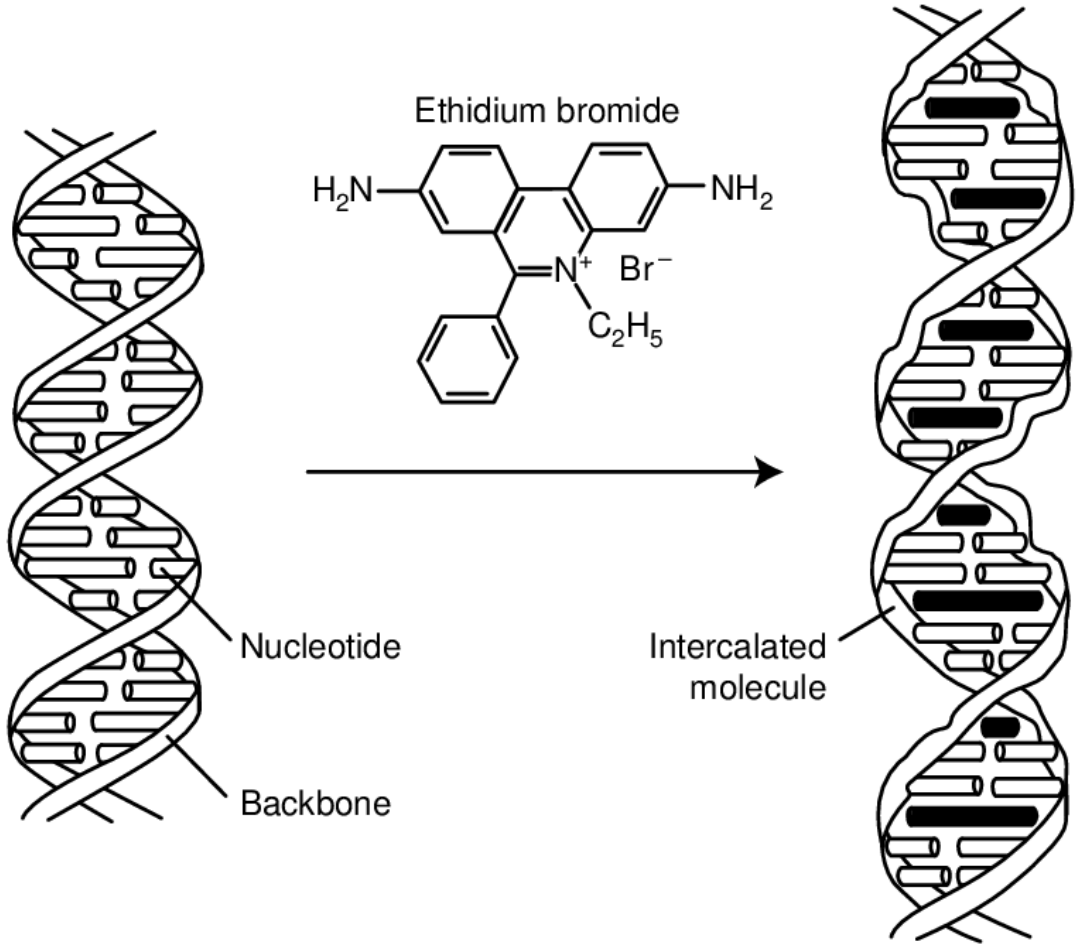


The Mutagenic Effect of Ethedium Bromide



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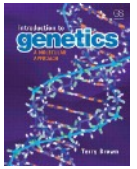
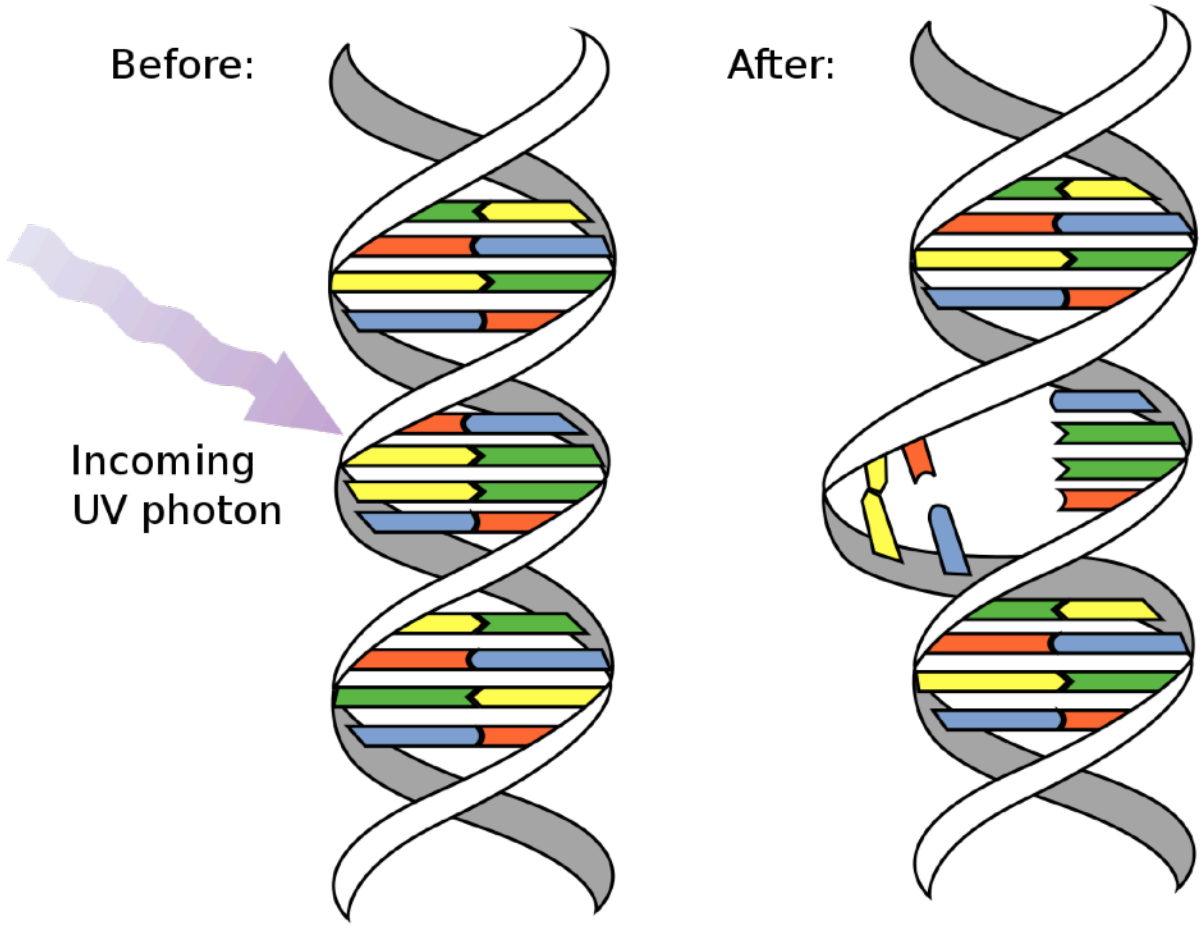
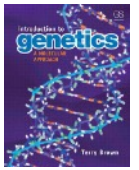


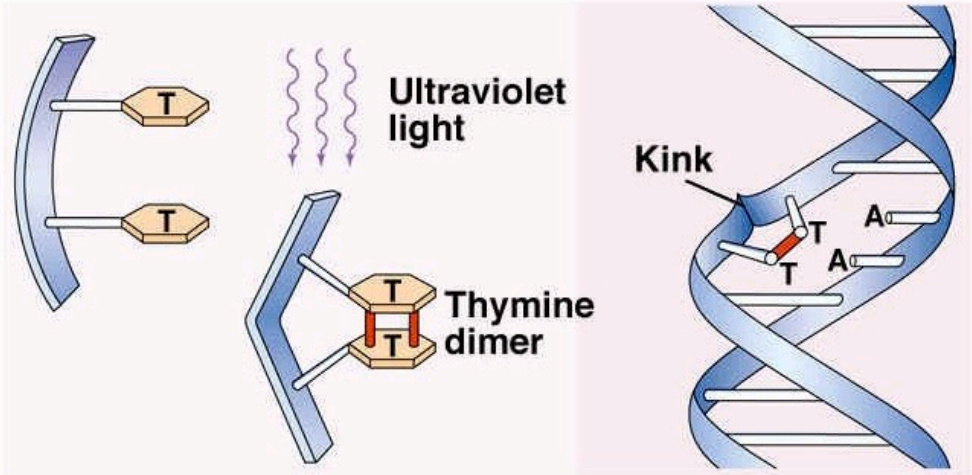
Physical Mutagens
UV Radiation

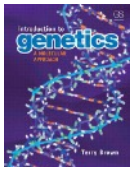




Physical Mutagens
UV Radiation

- Ultraviolet (UV) radiation induces dimerization of adjacent pyrimidine bases, especially thymines, resulting in a **cyclobutyl dimer**



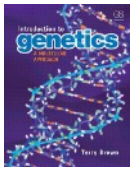


Physical Mutagens
UV Radiation

- Other pyrimidine combinations also form dimers;
  - 5’-CT-3’ > 5’-TC-3’ > 5’-CC-3’

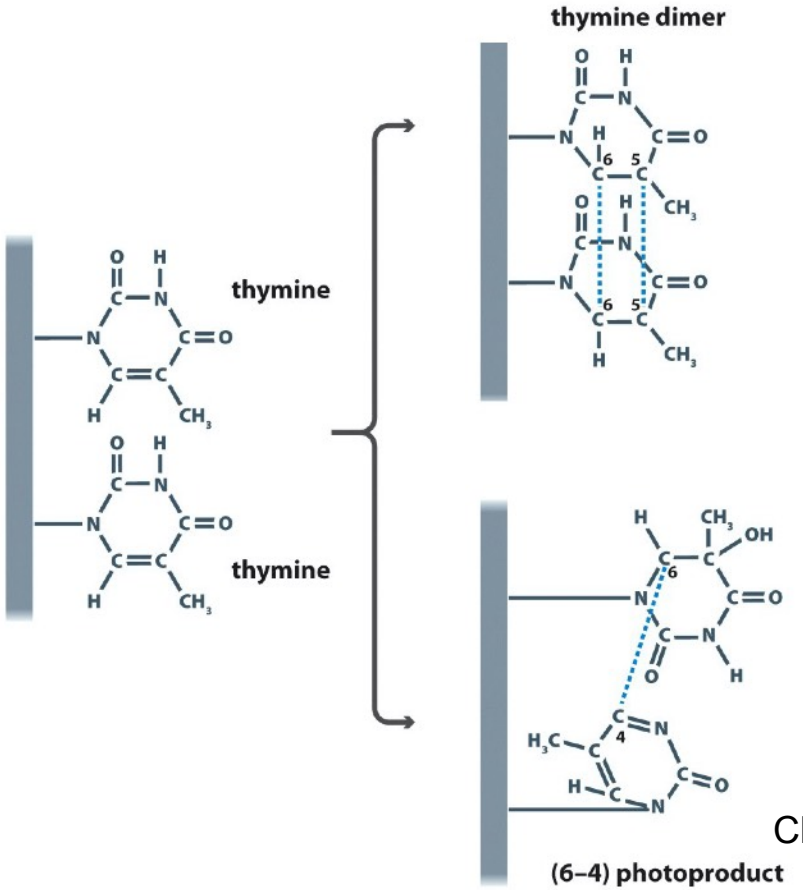
- Purine dimers are less common and UV-induced dimerization results in a deletion mutation

- Another type of UV-induced **photoproduct** is the **(6–4) lesion**, in which carbons number 4 and 6 of adjacent pyrimidines become covalently linked



Photoproducts Induced by UV Irradiation

- A thymine dimer contains two UV-induced covalent bonds, one linking the carbons at position 6 and the other linking the carbons at position 5
- The (6–4) lesion involves formation of a UV-induced covalent bond between carbons 4 and 6 of the adjacent nucleotides



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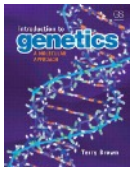
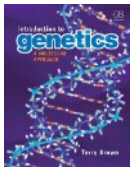


Physical Mutagens
Ionizing Radiation

- The effects of ionizing radiation on DNA depend on the;
  - Type of radiation
  - Intensity of radiation

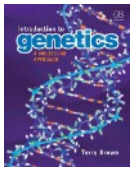
- It leads to point, insertion, and/ or deletion mutations, as well as more severe forms of DNA damage that prevent replication

- Some types of ionizing radiation act directly on DNA, and others act indirectly by stimulating the formation of reactive molecules such as **peroxides** in the cell



Physical Mutagens
Heat

- Heat stimulates the water-induced cleavage of the β-*N*-glycosidic bond
- This occurs more frequently with purines than with pyrimidines
- Results in an **AP** (apurinic/apyrimidinic) or **baseless site**



Heat-induced Hydrolysis of β-N-Glycosidic Bond

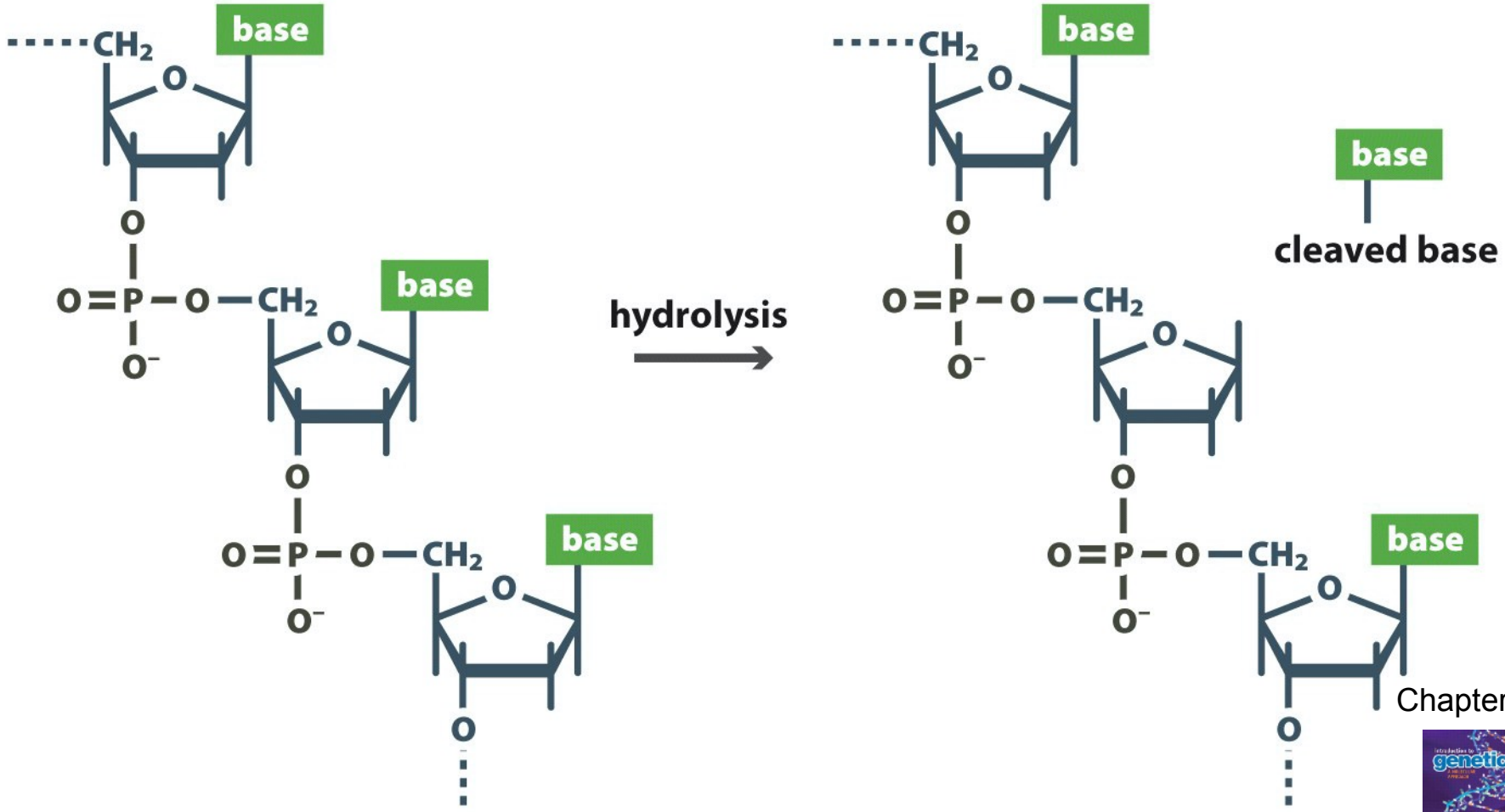


Figure 16.16a Introduction to Genetics (© Garland Science 2012)

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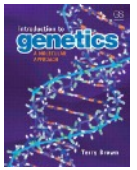


The Effect of Hydrolysis on Double-stranded DNA

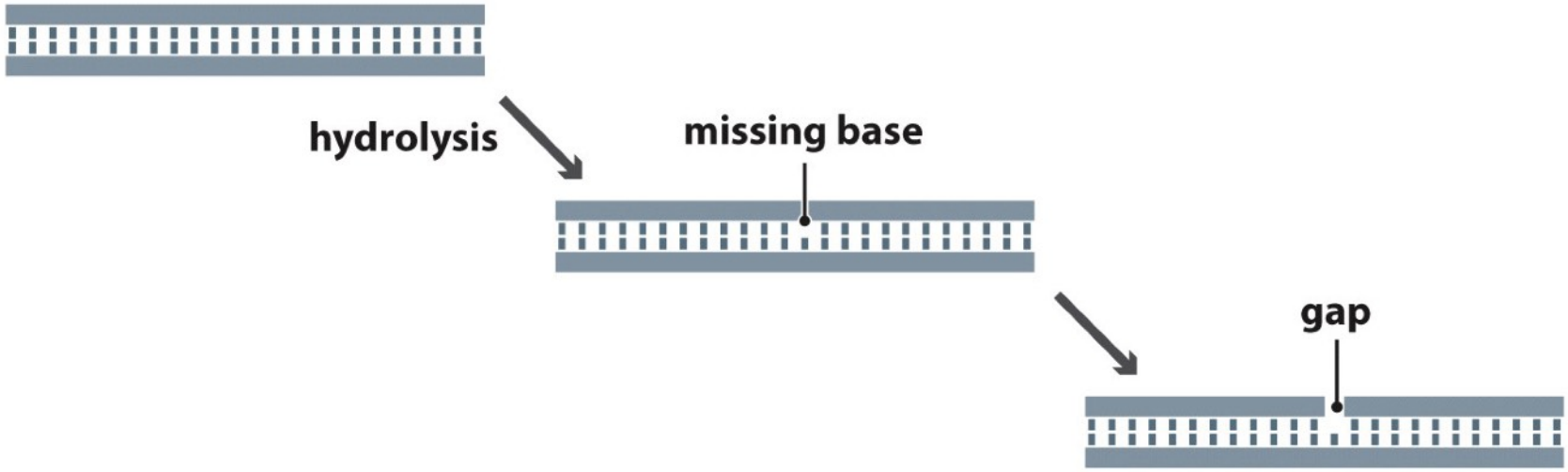
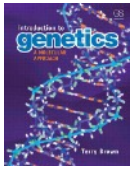
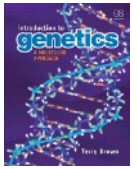


Figure 16.16b Introduction to Genetics (© Garland Science 2012)



Physical Mutagens
Heat

- The unstable sugar–phosphate is rapidly degrades, leaving a gap in the double-stranded DNA molecule
- Approximately 10,000 AP sites are generated in each human cell/day
- However, living cells have effective systems for repairing gaps



To know

keto

enol

Expectations

- You