Lecture 29:

DNA Repair

Course 281

Lessons for life

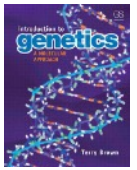
AIMS

- Understand

DNA Repair

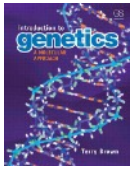
- Without repair systems key genes became inactivated by DNA damage within a few hours.

- Similarly, replication errors can accumulate rendering the genome dysfunctional after a few thousand rounds of cell division.



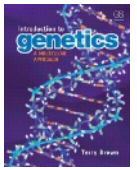
DNA Repair is Very Efficient

- The error rate for DNA synthesis in *E. coli* is 1 mistake for every 10^7 bp that are replicated
- The overall error rate for replication of the *E. coli* genome is only 1 in 10^{11} to 1 in 10^{10}
  - Due entirely to the mismatch repair system
- The repair enzymes scan newly replicated DNA and correct the errors of replication
- Repair is highly efficient; on average only one uncorrected replication error occurs every 1000 times the *E. coli* genome is copied



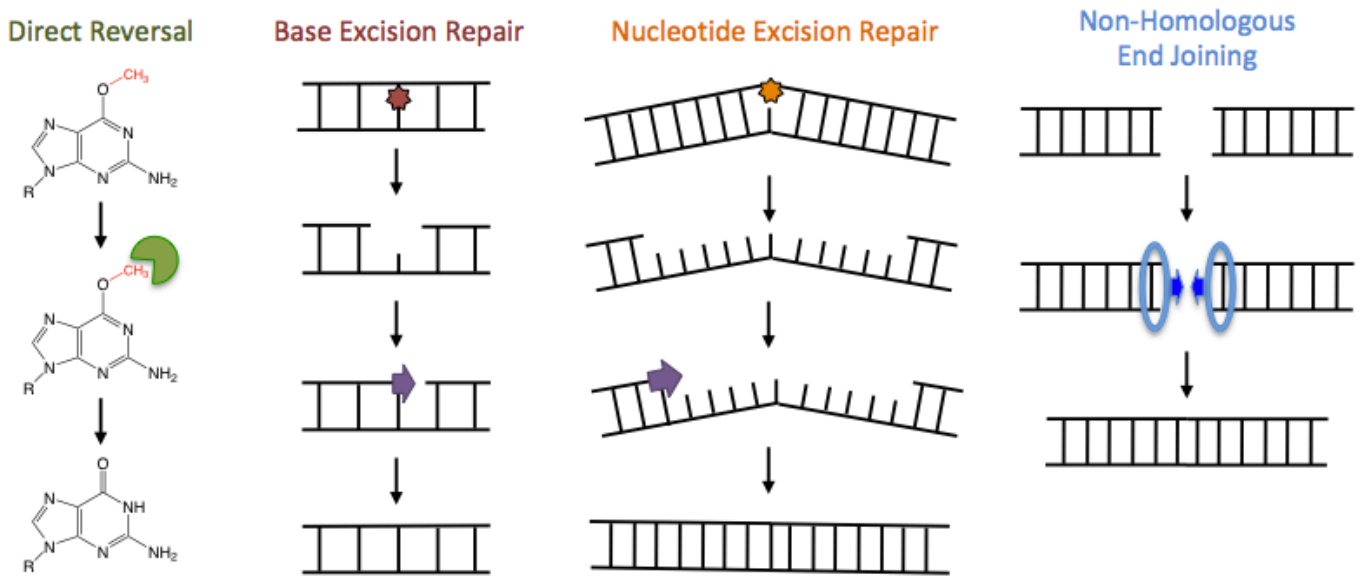
DNA Repair

- **Direct repair systems** fill in nicks and correct some types of nucleotide modification.
- Many types of damaged nucleotide can be repaired by **base excision**.
- **Nucleotide excision repair** is used to correct more extensive types of damage.
- **Mismatch repair** corrects errors of replication
- DNA breaks can also be repaired
- In an emergency, DNA damage can be bypassed during genome replication
- Defects in DNA repair underlie human diseases, including cancers



Types of DNA Repair Systems

- **Direct repair systems**.
- **Excision repair**.
- **Mismatch repair.**
- **Nonhomologous end joining (NHEJ)**.



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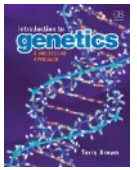
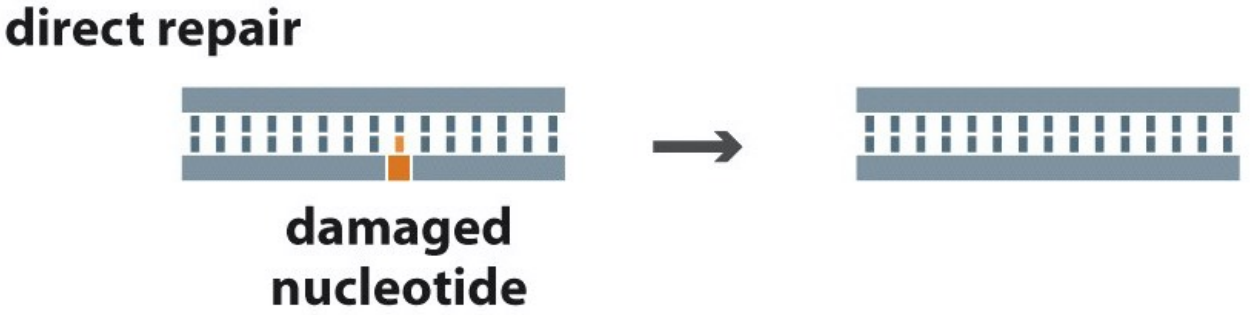
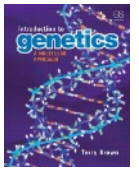


Types of DNA Repair Systems

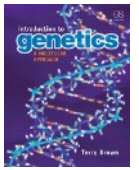
- **Direct repair systems**: act directly on damaged nucleotides converting each one back to its original structure





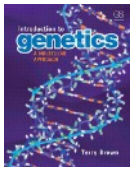
Direct Repair Systems

- Direct repair systems fill in nicks and correct some types of nucleotide modification
- Most of DNA damage, caused by chemical or physical mutagens, can be repaired only by excision of the damaged nucleotide
- A few types of damage can be repaired directly without removal of the nucleotide
- Some of the products of alkylation are enzymatically reversible



Direct Repair Systems

- The **Ada enzyme** of *E. coli* is activated in response to DNA damage
- Ada removes alkyl groups attached to the oxygen atoms at positions 4 of T and 6 of G
- It can also repair methylated phosphodiester bonds
- The human *O*6-methylguanine-DNA methyltransferase **(MGMT)**, has a more restricted specificity
- MGMT removes alkyl groups only from position 6 of G

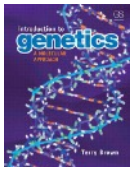


Direct Repair Systems

- Cyclobutyl dimers, resulting from UV damage, can be repaired directly by a light-dependent system called **photoreactivation**

- The *E. coli* enzyme **DNA photolyase** is stimulated by light with a wavelength between 300 - 500 nm

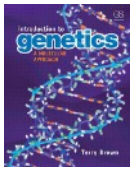
- The enzyme binds to cyclobutyl dimers and converts them back to the original nucleotides



Direct Repair Systems

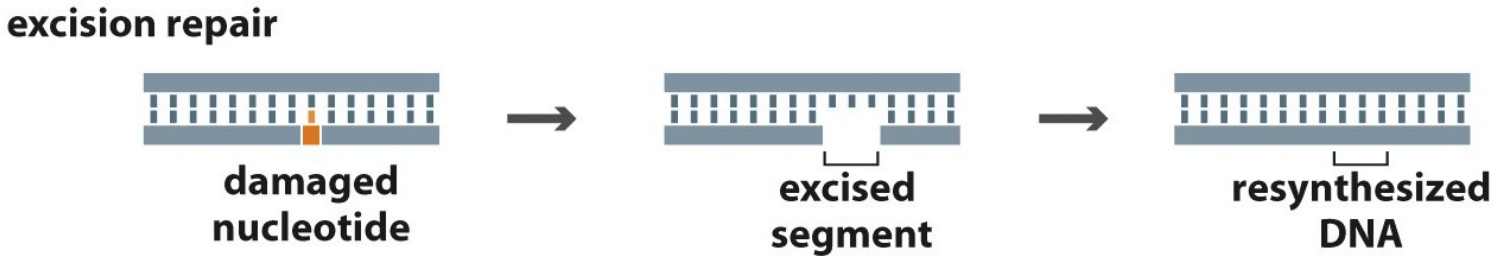
- Most bacteria and a few eukaryotes including some vertebrates are capable of photoreactivation; it is absent in humans and other placental mammals

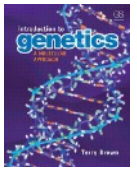
- A variety of organisms have a photoreactivation system which involves the **(6–4) photoproduct photolyase** that repairs (6–4) lesions



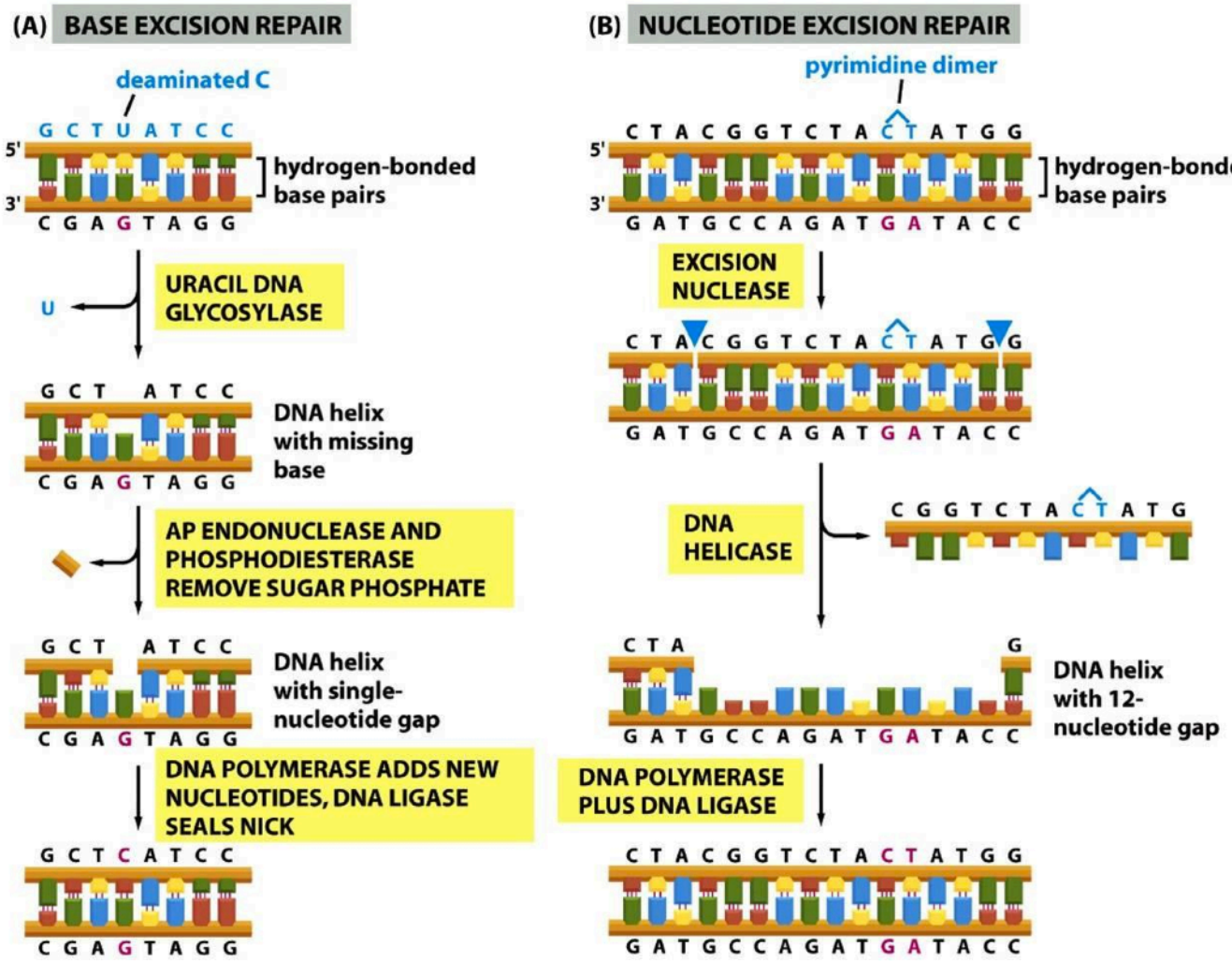
Types of DNA Repair Systems

- **Excision repair**: involves excision of the damaged site (one nucleotide much longer) followed by resynthesis of the correct sequence by a DNA polymerase.





Types of DNA Repair Systems



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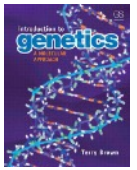
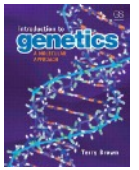


Base Excision Repair

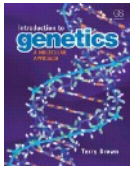
- It involves removal of one or more damaged nucleotides followed by resynthesis of DNA to seal the resulting gap
- It is used to repair bases damage by exposure to alkylating agents or ionizing radiation



Base Excision Repair

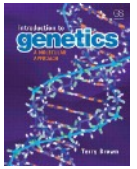
- The process is initiated by a **DNA glycosylase**, which cleaves the β-*N*-glycosidic bond between a damaged base and the sugar component of the nucleotide

- Each DNA glycosylase has a limited specificity, the specificities of the glycosylases possessed by a cell determining the range of damaged nucleotides that can be repaired in this way



Other Mutations Repaired by Base Excision Repair

- Deaminated bases such as uracil (deaminated C) and hypoxanthine (deaminated A)
- Oxidation products such as 5-hydroxycytosine and thymine glycol
- Methylated bases such as;
  - 3-methyladenine
  - 7-methylguanine
  - 2-methylcytosine



Removal of a Damaged Base by DNA Glycolysis

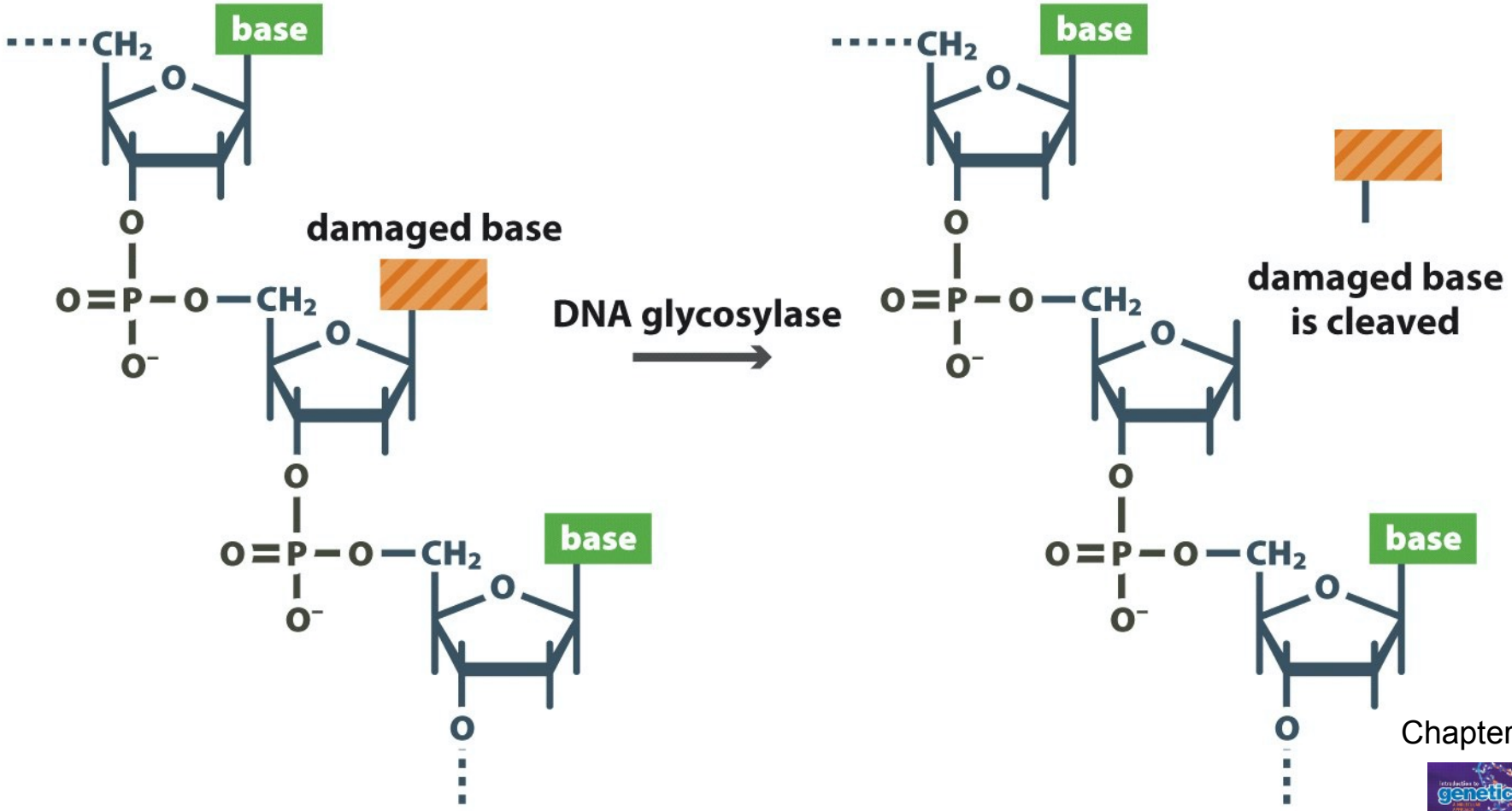


Figure 16.18a Introduction to Genetics (© Garland Science 2012)

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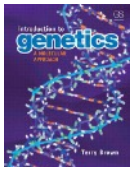
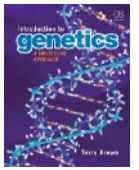


Excision Repair by DNA Glycosylase

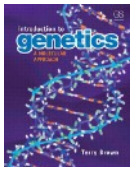
- A DNA glycosylase “flips” the damaged base to a position outside of the helix and then detaches it from the polynucleotide

- This creates an AP, or baseless, site that is converted into a single-nucleotide gap in the second step of the repair pathway



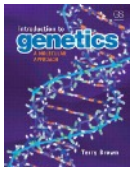
Excision Repair by DNA Glycosylase

- An **AP endonuclease** cuts the phosphodiester bond on the 5’ side of the AP site
- Some AP endonucleases can also remove the sugar from the AP site,
- Others lack this ability; a separate **phosphodiesterase** remove the sugar



Excision Repair by DNA Glycosylase

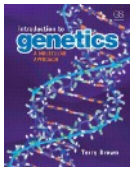
- An alternative pathway for converting the AP site into a gap utilizes the endonuclease activity of some **DNA glycosylases**
- DNA is cut at the 3’ side of the AP site, the damaged base is removed, and the sugar removed by a **phosphodiesterase**



Excision Repair by DNA Glycosylase

- The single-nucleotide gap is filled by **DNA polymerase**, using the undamaged base in the other DNA strand
  - **DNA polymerase I** in *E. coli*
  - **DNA polymerase β** in mammals

- The final phosphodiester bond is made by **DNA ligase**



Excision Repair Pathway

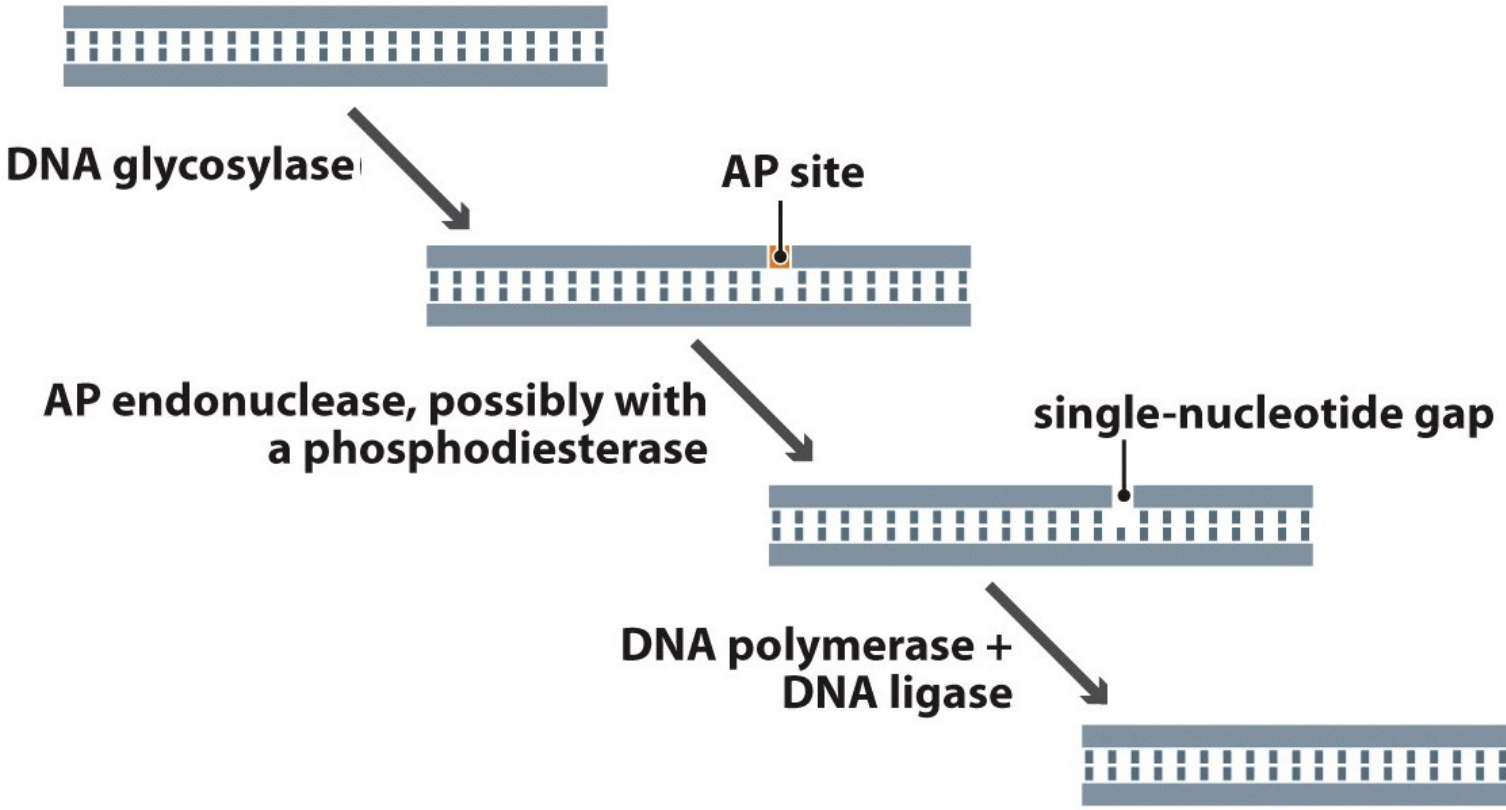
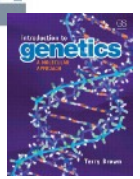


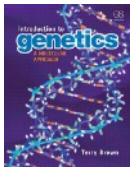
Figure 16.18b Introduction to Genetics (© Garland Science 2012)



Nucleotide Excision Repair Corrects Extensive Damage

- Nucleotide excision repair deals with extreme DNA damage;
  - Intrastrand crosslinks
  - Attachment of large chemical groups to bases

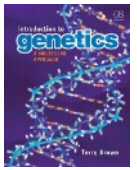
- Can correct cyclobutyl dimers by a **dark repair** process;
  - Important in humans; they lack the photoreactivation system



Nucleotide Excision Repair Corrects Extensive Damage

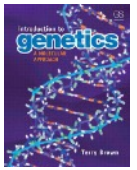
- A segment of single-stranded DNA containing damaged nucleotide(s) is excised and replaced with new DNA

- Similar to base excision repair but;
  - Is not preceded by selective base removal
  - A longer stretch of polynucleotide is cut out



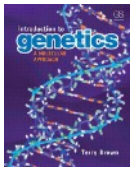
Short-patch Nucleotide Excision Repair in *E. coli*

- A 12 nucleotide segment is excised and “patched” by a multienzyme complex called the **UvrABC endonuclease**
- A trimer comprising two UvrA and one copy of UvrB attaches to the damaged site and searches for distortion of the DNA double helix;
  - UvrA dissociates once the site has been found
  - Must be involved in damage location



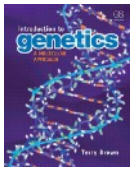
Short-patch Nucleotide Excision Repair in *E. coli*

- Binding of UvrC forms a UvrBC dimer that cuts on either side of the damaged polynucleotidesite; a12-nucleotide segment is excised
- The fifth phosphodiester bond downstream the damaged nucleotide is cut by UvrB
- The eighth phosphodiester bond upstream the damaged nucleotide is cut by UvrC



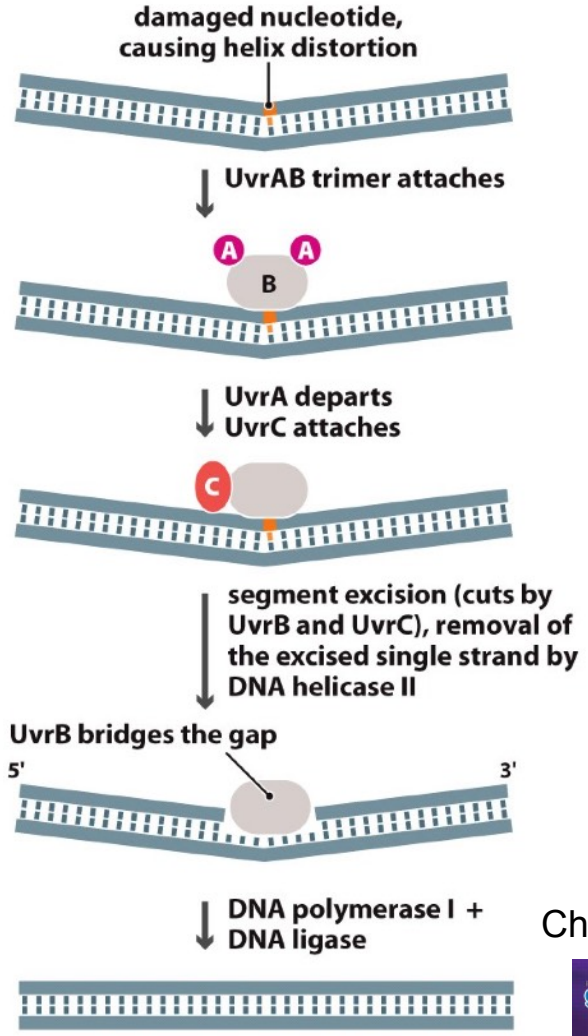
Short-patch Nucleotide Excision Repair in *E. coli*

- The excised segment is removed as an intact piece of DNA by DNA helicase II
- UvrC detaches and UvrB bridges the gap produced by the excision and prevents the exposed single-stranded region from base-pairing with itself
- UvrB may also prevent damage to this strand, and possibly direct DNA polymerase to the site of damage
- The gap is filled by DNA polymerase I and the last phosphodiester bond is synthesized by DNA ligase



Short-patch Nucleotide Excision Repair in *E. coli*

- The damaged nucleotide distorts the helix
- This acts as a recognition signals for the binding of the UvrAB trimer
- UvrA leaves the complex and UvrC joins,
- The UvrBC dimer cuts on either side of the damaged site
- UvrB bridges the gap while it is repaired by DNA polymerase I and DNA ligase



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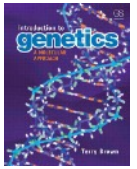
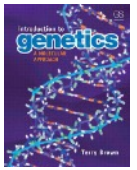


Long-patch Nucleotide Excision Repair in *E. coli*

- This repair system also involves Uvr proteins but the excised DNA can be anywhere up to 2 kb in length

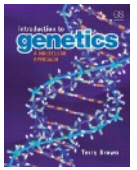
- It is thought to work on more extensive forms of damage, possibly regions where groups of nucleotides are modified



Long-patch Nucleotide Excision Repair in *E. coli*

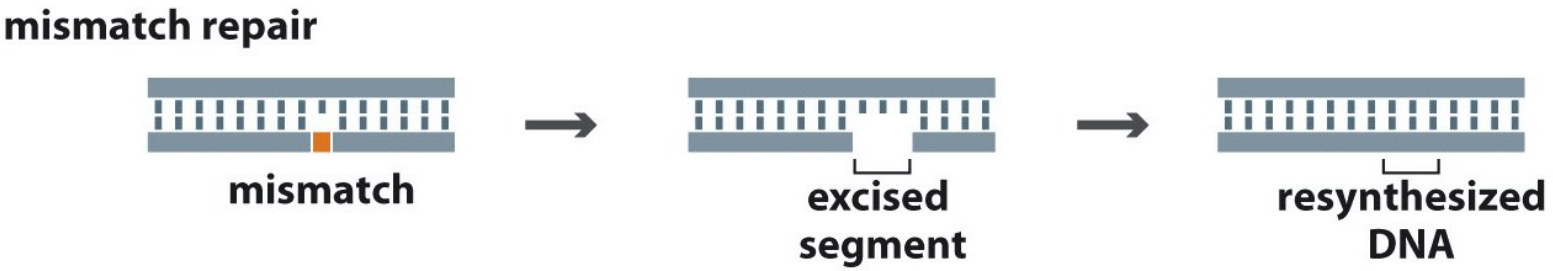
- Long-patch repair has been less well studied and the process is not understood in detail

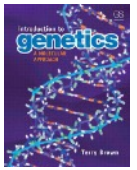
- Eukaryotes have just one type of nucleotide excision repair pathway
  - Replaces 24 - 29 nucleotides of DNA
  - It is unrelated to excision pathways in bacteria



Types of DNA Repair Systems

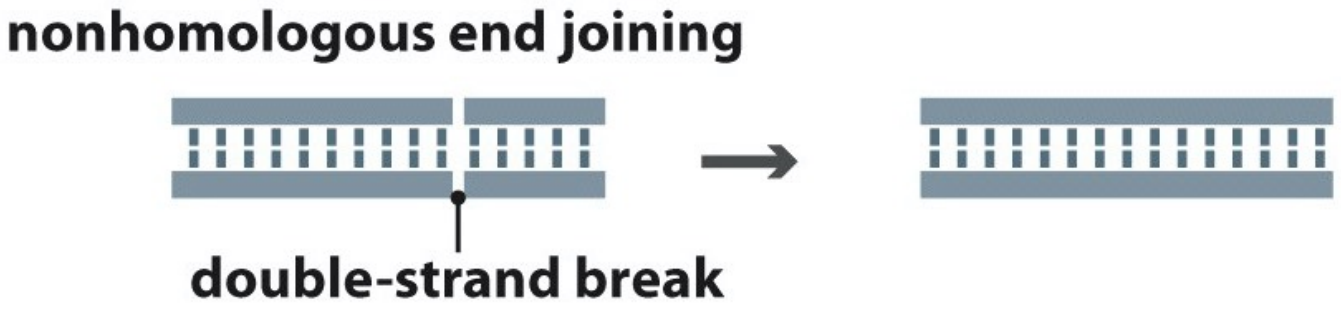
- **Mismatch repair:** is a special type of excision repair which corrects errors of replication by excising a stretch of single-stranded damaged DNA and then repairing the resulting gap





Types of DNA Repair Systems

- **Nonhomologous end joining** (**NHEJ**): is used to repair double-strand breaks



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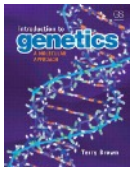

To know

Expectations

- You