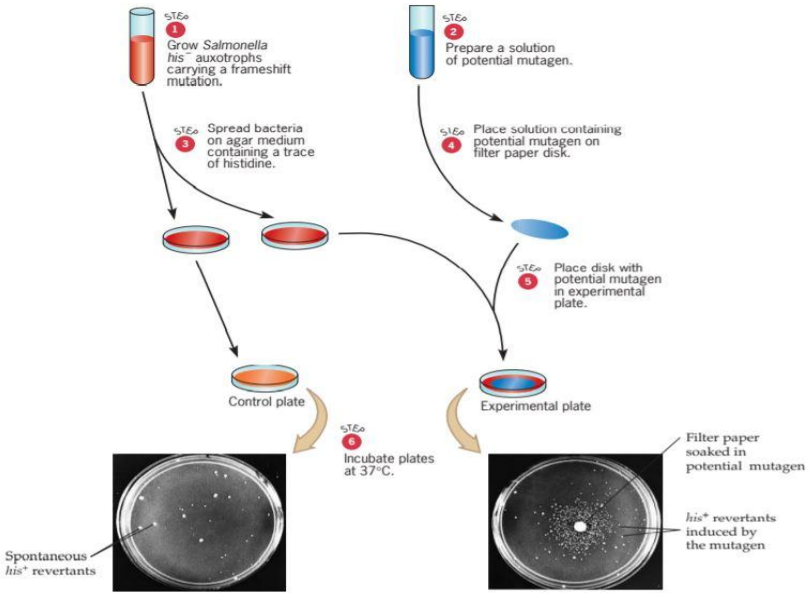
Ames test

A method for detecting the mutagenicity of chemicals.Mutagenic agents are also carcinogens; that is, theyinduce cancers.

Bruce Ames and his associates developed sensitive techniques that allow the mutagenicity of large numbers of chemicals to be tested quickly at relatively low cost.

Technique:

- They constructedauxotrophic strains of the bacterium *Salmonella typhimurium* carrying various types of mutations—transitions, transversions, and frameshifts—in genes required for the biosynthesis of the amino acid histidine.
- They monitored the reversion of these auxotrophic mutants to prototrophy by placing a known number of mutant bacteria on medium lacking histidine and scoring the number of colonies produced by prototrophic revertants. Because some chemicals are mutagenic only to replicating DNA, they added asmall amount of histidine—enough to allow a few cell divisions but not the formation of visible colonies—to the medium.
- They measured the mutagenicity of a chemical by comparing the frequency of reversion in its presence with the spontaneous reversion frequency.
- They assessed its ability to induce different types of mutations by using a set of tester strains that carry different types of mutations—one strain with a transition, one with a frameshift mutation, and so forth.



■ FIGURE 13.24 The Ames test for mutagenicity. The medium in each petri dish contains a trace of histidine and a known number of *his*⁻ cells of a specific *Salmonella typhimurium* "tester strain" harboring a frameshift mutation. The control plate shown on the left provides an estimate of the frequency of spontaneous reversion of this particular tester strain. The experimental plate on the right shows the frequency of reversion induced by the potential mutagen, in this case, the carcinogen 2-aminofluorene.

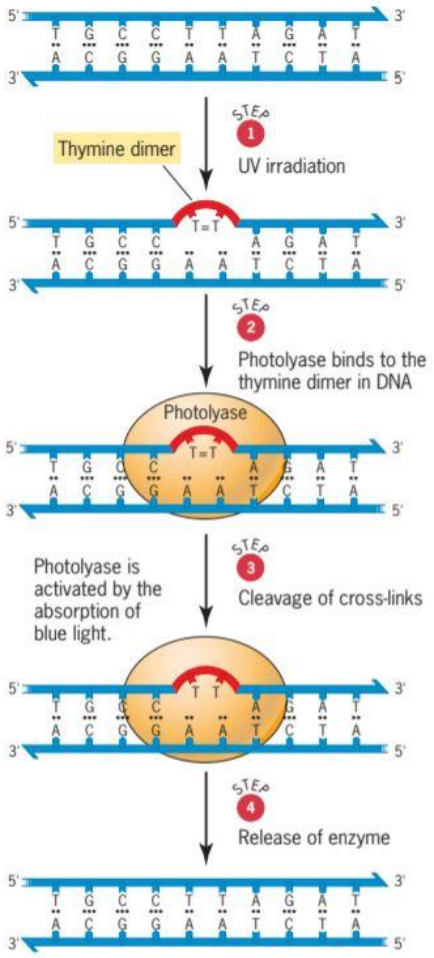
DNA Repair Mechanisms

Living organisms contain many enzymes that scan their DNA for damage and initiate repair processes when damage is detected.

E. coli cells possess five well-characterized mechanisms for the repair of defects in DNA: (1) light-dependent repair or photoreactivation, (2) excision repair, (3) mismatch repair, (4) post-replication repair, and (5) the error-prone repair system (SOS response). Moreover, there are at least two different types of excision repair, and the excision repair pathways can be initiated by several different enzymes, each acting on a specific kind of damage in DNA. Mammals seem to possess all of the repair mechanisms found in *E. coli* except photoreactivation. Because most mammalian cells do not have access to light,photoreactivation would be of relatively little value to them.

Light-dependent repair

- Light-dependent repair or photoreactivation of DNA in bacteria is carried out by a lightactivated enzyme called DNA photolyase.
- When DNA is exposed to ultraviolet light, thymine dimers are produced by covalent cross-linkages between adjacent thymine residues.
- DNA photolyase recognizes and binds to thymine dimers in DNA, and uses light energy to cleave the covalent cross-links.
- Photolyase will bind to thymine dimers in DNA in the dark, but it cannot catalyze cleavage of the bonds joining the thymine moieties without energy derived from visible light, specifically light within the blue region of the spectrum.
- Photolyase also splits cytosine dimers and cytosine-thymine dimers.
- Thus, when ultraviolet light is used to induce mutations in bacteria, the irradiated cells are grown in the dark for a few generations to maximize the mutation frequency.



Excision repair

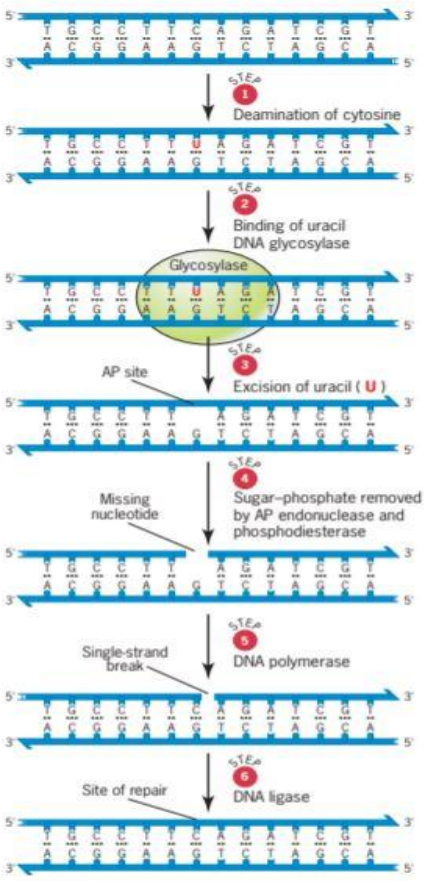
Excision repair of damaged DNA involves at least three steps.

1. DNA repair endonuclease or endonuclease-containing enzyme complex recognizes, binds to, and excises the damaged base or bases in DNA.
2. DNA polymerase fills in the gap by using the undamaged complementary strand of DNA as template.
3. The enzyme DNA ligase seals the break left by DNA polymerase to complete the repair process.

There are two major types of excision repair: **base excision repair** systems remove abnormal or chemically modified bases from DNA, whereas **nucleotide excision repair** pathways remove larger defects like thymine dimers. Both excision pathways are operative in the dark, and both occur by very similar mechanisms in *E. coli* and humans.

Base excision repair can be initiated by any of a group of enzymes called DNA glycosylases that recognize abnormal bases in DNA.

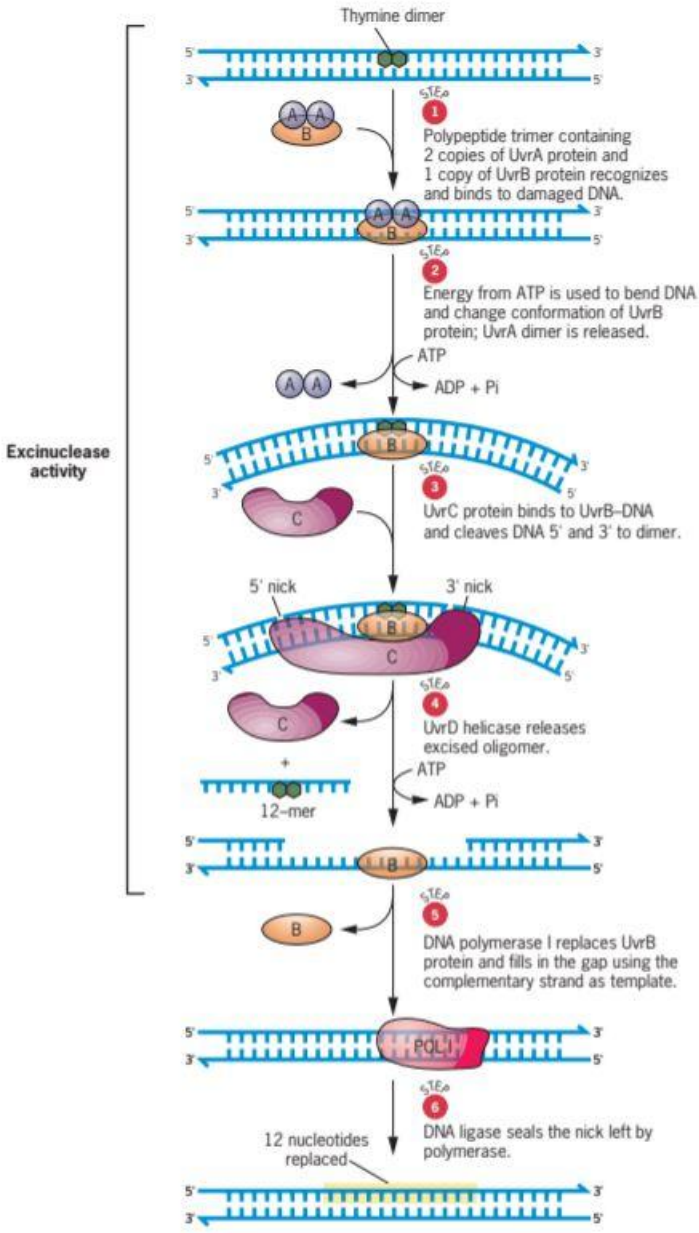
- Each glycosylase recognizes a specific type of altered base, such as deaminated bases, oxidized bases, and so on.
- The glycosylases cleave the glycosidic bond between the abnormal base and 2-deoxyribose, creating apurinic or apyrimidinic sites (AP sites) with missing bases. AP sites are recognized by enzymes called AP endonucleases, which act together with phosphodiesterases to excise the sugar-phosphate groupsat these sites.
- DNA polymerase then replaces the missing nucleotide according to the specifications of the complementary strand, and DNA ligase seals the nick.



Nucleotide excision repair removes larger lesions like thymine dimers and bases with bulky side-groups from DNA.

- In nucleotide excision repair, a unique excision nuclease activity produces cuts on either side of the damaged nucleotide(s) and excises an oligonucleotide containing the damaged base(s). This nuclease is called an excinuclease to distinguish it from the endonucleases and exonucleases that play other roles in DNA metabolism.
- In *E. coli*, excinuclease activity requires the products of three genes, uvrA, uvrB, and uvrC (designated uvr for UV repair).
- A trimeric protein containing two UvrA polypeptides and one UvrB polypeptide recognizes the defect in DNA, binds to it, and uses energy from ATP to bend the DNA at the damaged site.
- The UvrA dimer is then released, and the UvrC protein binds to the UvrB/DNA complex.
- The UvrC protein cleaves the fourth or fifth phosphodiester bond from the damaged nucleotide(s) on the 3’ side and the eighth phosphodiester linkage from the damage on the 5’ side.
- The uvrD gene product, DNA helicase II, releases the excised dodecamer.
- In the last two steps of the pathway, DNA polymerase I fills in the gap, and DNA

ligase seals the remaining nick in the DNA molecule.



Nucleotide excision repair in humans occurs through a pathway similar to the one in *E. coli*, but it involves about four times as many proteins. In humans, the excinuclease activity contains 15 polypeptides. Protein XPA (for xeroderma pigmentosum protein A) recognizes and binds to the damaged nucleotide(s) in DNA. It then recruits the other proteins required for excinuclease activity. In humans, the excised oligomer is 24 to 32 nucleotides long rather than the 12-mer removed in E. coli. The gap is filled in by either DNA polymerase or ε in humans, and DNA ligase completes the job.