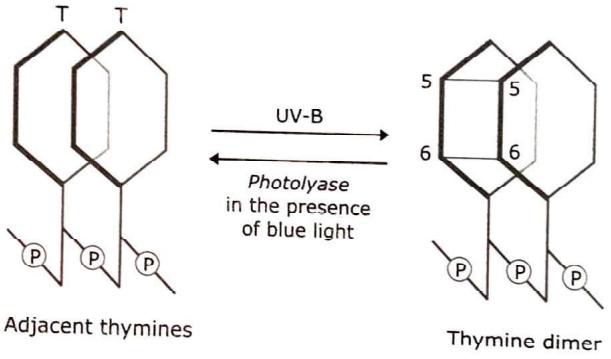
6.14 DNA repair

Although the genetic variation is important for evolution, the survival of the individual demands genetic stability also. Maintaining genetic stability requires not only an extremely accurate mechanism for replicating DNA, but also mechanisms for repairing the many accidental lesions that occur continually in DNA. Most such spontaneous changes in DNA are temporary because they are immediately corrected by a set of processes that are collectively called as *DNA repair*. Without repair systems, a genome would not be able to maintain its essential cellular functions. Most cells possess four different categories of DNA repair system: Direct repair, Excision repair, Mismatch repair and Recombinational repair.

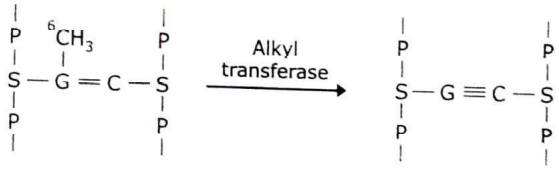
6.14.1 Direct repair

Direct repair systems act directly on damaged nucleotides, converting each one back to its original structure. But only a few types of damaged nucleotide can be repaired directly. One very common type of UV radiation mediated damages, *thymine dimers*, are repaired by a light-dependent direct system called **photoreactivation**. In *E. coli*, the process involves the enzyme called **DNA photolyase**. When stimulated by light with a wavelength between 300 and 500 nm, the enzyme binds to pyrimidine dimers and converts them back to the original monomeric nucleotides. Photoreactivation is a widespread but not universal type of repair.



Adjacent thymines — Thymine dimer

Another example is the repair of O^6-methylguanine, which forms in the presence of alkylating agents and is a common and highly mutagenic lesion. It tends to pair with thymine rather than cytosine during replication. Direct repair of O^6-methylguanine is carried out by O^6-methylguanine DNA methyltransferase (an alkyl transferase), which catalyzes the transfer of the methyl group of O^6-methylguanine to a specific *Cys* residue in the same protein.



6.14.2 Excision repair

Excision repair involves the excision of a nucleotide or segment of the polynucleotide containing a damaged site, followed by the resynthesis of the correct nucleotide sequence by a DNA polymerase. These pathways fall into two categories:

Base excision repair

Base excision repair involves removal of a damaged nucleotide base, excision of a nucleotide and resynthesis with a DNA polymerase. It is used to repair many minor damages like alkylation and deamination resulting from

exposure to mutagenic agents. Enzyme **DNA glycosylase** initiates the repair process. A DNA glycosylase does not cleave phosphodiester bonds, instead cleave the *N*-glycosidic bonds, liberating the altered base and generating an *apurinic* or an *apyrimidinic site*, both called **AP sites**.

The resulting AP site is then repaired by an AP endonuclease repair pathway. All cells have endonucleases that attack the sites left after the spontaneous loss of single purine or pyrimidine residues. The **AP endonucleases** are vital to the cell, because spontaneous depurination is a relatively frequent event. These enzymes introduce chain breaks by cleaving the phosphodiester bonds at AP sites. This bond cleavage initiates an excision-repair process with the help of three enzymes – an exonuclease, DNA polymerase I and DNA ligase.

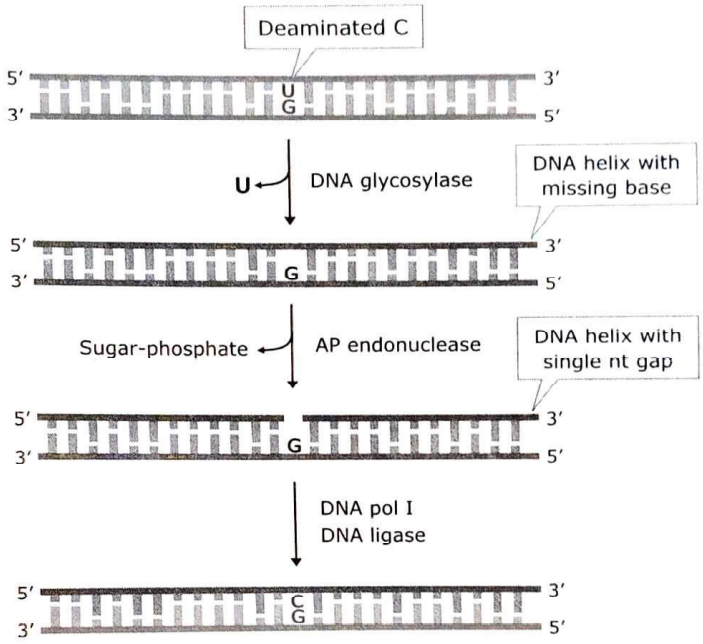


Figure 6.109 Base excision repair.

Nucleotide excision repair

Nucleotide excision repair is similar to base excision repair, but is not preceded by the removal of a damaged base and can act on more substantially damaged areas of DNA. This repair system includes the breaking of a phosphodiester bond on either side of the lesion, on the same strand, resulting in the excision of an oligonucleotide. This excision leaves a gap that is filled by repair synthesis, and a ligase seals the breaks.

The *uvr* system of excision repair in *E. coli* is the best-studied example. It involves the removal of relatively short, usually 12 nucleotides in length. The key enzyme is made up of three subunits, products of the *uvr*A, *uvr*B and *uvr*C genes, and is called the **ABC excinuclease**. ABC excinuclease binds to DNA at the site of a lesion. First **UvrA** and **UvrB** attach to the DNA at the damaged site. UvrA recognizes the damage. Departure of UvrA allows **UvrC** to bind, forming UvrBC dimer which cleaves the damaged strand at the eighth phosphodiester bond on the 5′ side of the lesion and at the fourth or fifth phosphodiester bond on the 3′ side. **UvrD** is a helicase (also called helicase II) that helps to unwind the DNA to allow release of the single strand between the two cuts. The resulting gap is filled in by DNA polymerase I and sealed by ligase.

In humans, Xeroderma pigmentosum and Cockayne syndrome are caused by genetically defective nucleotide excision repair. Xeroderma pigmentosum, a rare inherited disease, is mainly characterized by the inability of skin cells to repair UV-induced DNA lesions (pyrimidine dimer). Individuals suffering from this autosomal recessive condition are extremely sensitive to sunlight.

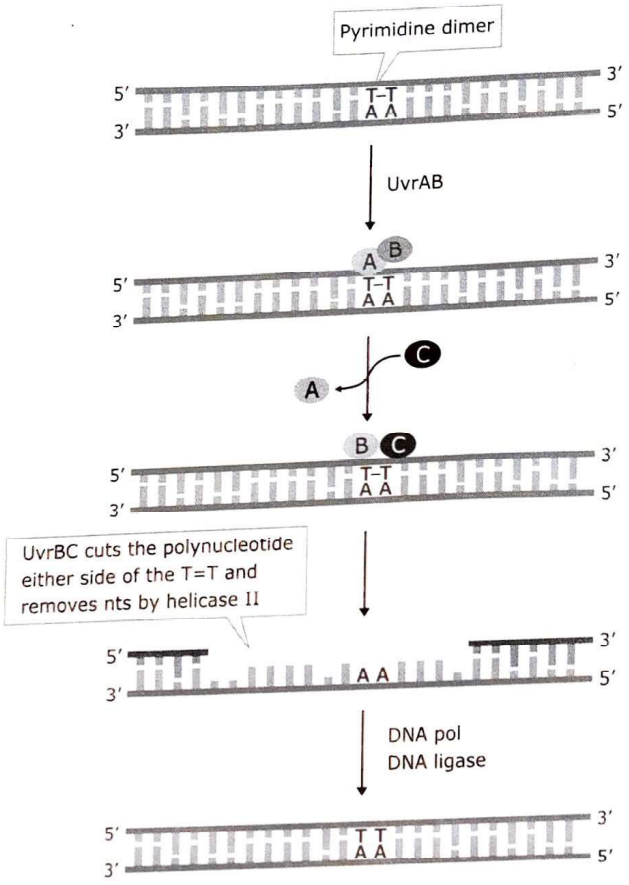


Figure 6.110 Nucleotide excision repair (A-UvrA, B-UvrB, C-UvrC).

6.14.3 Mismatch repair

The mismatch repair system can detect mismatches that occur in DNA replication. Enzyme systems involved in mismatch repair are as follows:

1. Recognize mismatched base pair(s).
2. Determine which base in the mismatch is the incorrect one.
3. Excise the incorrect base and carry out repair synthesis.

The repair must be made in the daughter polynucleotide because it is in this newly synthesized strand that the error has occurred; the parent polynucleotide has the correct sequence. How does the repair process know which strand is which? When mismatch errors occur during replication in *E. coli*, it is possible to distinguish the original strand of DNA. Immediately after replication of methylated DNA, only the original parental strand carries the methyl groups.

During the period while the newly synthesized strand awaits the introduction of methyl groups, the two strands can be distinguished. In *E. coli*, the answer is that the daughter strand is, at this stage, undermethylated and can be distinguished from the parent polynucleotide, which has a full complement of methyl groups. *E. coli* DNA is methylated because of the activities of the *DNA adenine methylase* (**Dam**), which converts adenines to 6-methyladenines in the sequence 5′-GATC-3′, and the *DNA cytosine methylase* (**Dcm**), which converts internal cytosines to 5-methylcytosines in 5′-CCAGG-3′ and 5′-CCTGG-3′. These methylations are not mutagenic, the modified nucleotides having the same base-pairing properties as the unmodified versions. There is a delay between DNA replication and methylation of the daughter strand, and it is during this window of opportunity that the repair system scans the DNA for mismatches and makes the required corrections in the undermethylated, daughter strand.