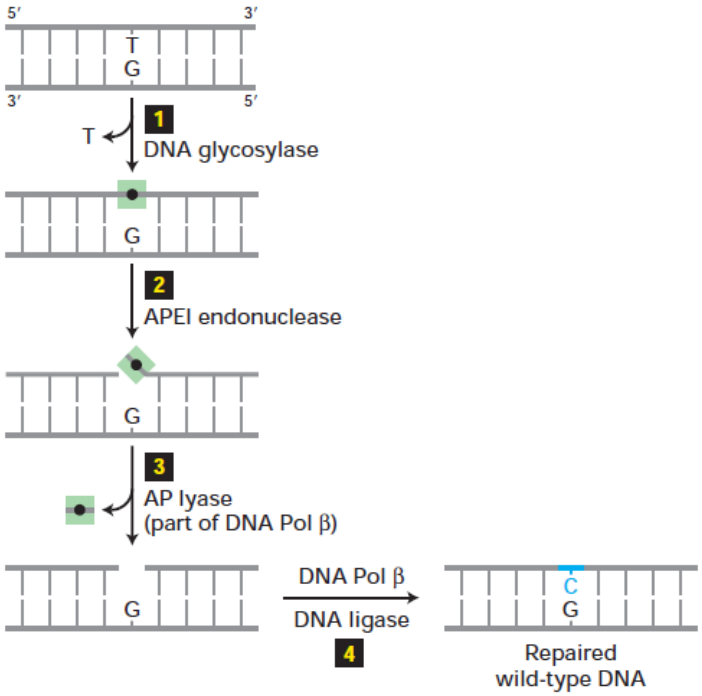
DNA Damage and Repair system

As a cell multiplies and divides, in most cases the genome is accurately copied and information is passed on to the next generation with minimal error. When DNA polymerases do make mistakes, their proofreading activity generally, but not always, corrects the error. However, forms of DNA damage unrelated to the process of replication occur relatively commonly. Such damage is induced by exposure to a number of different types of agents, for example, oxygen free radicals, ultraviolet or ionizing radiation, and various chemicals. DNA damage poses a continuous threat to genomic integrity. To cope with this problem cells have evolved a range of DNA repair enzymes and repair polymerases as complex as the DNA replication apparatus itself, which indicates their importance for the survival of a cell.

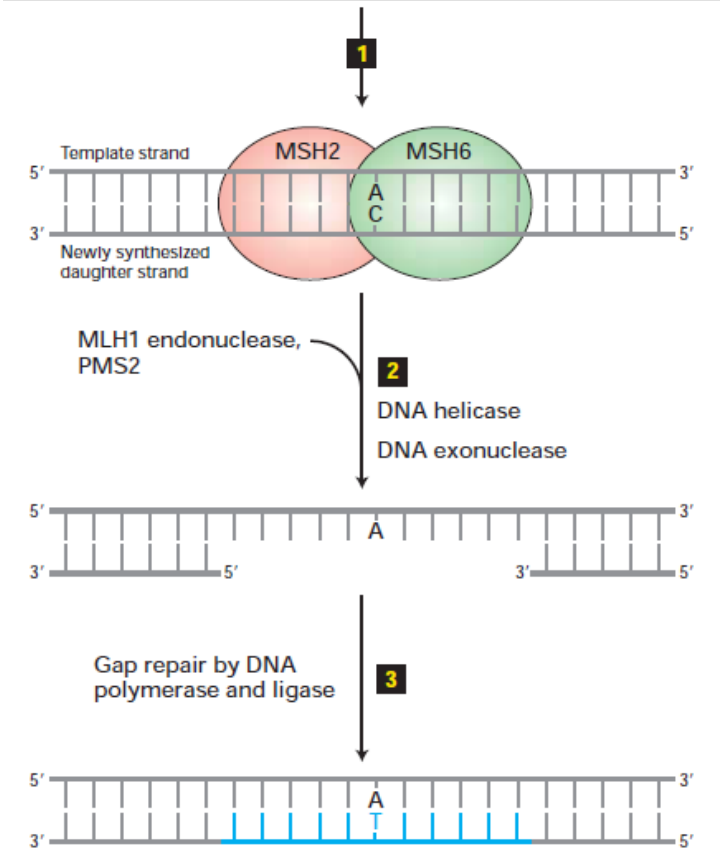
Base excision repair of a G-T mismatch

In humans, the most common type of point mutation is a C to T, which is caused by deamination of 5-methyl C to T (see Figure). The conceptual problem with *base excision repair* is determining which is the normal and which is the mutant DNA strand, and repairing the latter so that it is properly base-paired with the normal strand. But since a G · T mismatch is almost invariably caused by chemical conversion of C to U or 5-methyl C to T, the repair system "knows" to remove the T and replace it with a C. A DNA glycosylase specific for G-T mismatches, usually formed by deamination of 5-methyl C residues (see Figure), flips the thymine base out of the helix and then cuts it away from the sugar-phosphate DNA backbone (step1), leaving just the deoxyribose (black dot). An endonuclease specific for the resultant baseless site then cuts the DNA backbone (step2), and the deoxyribose phosphate is removed by an endonuclease associated with DNA polymerase β (step3). The gap is then filled in by DNA Pol β and sealed by DNA ligase (step4), restoring the original G-C base pair. [After O. Schärer, 2003, *Angewandte Chemie*]



Mismatch excision repair of newly replicated DNA in human cells

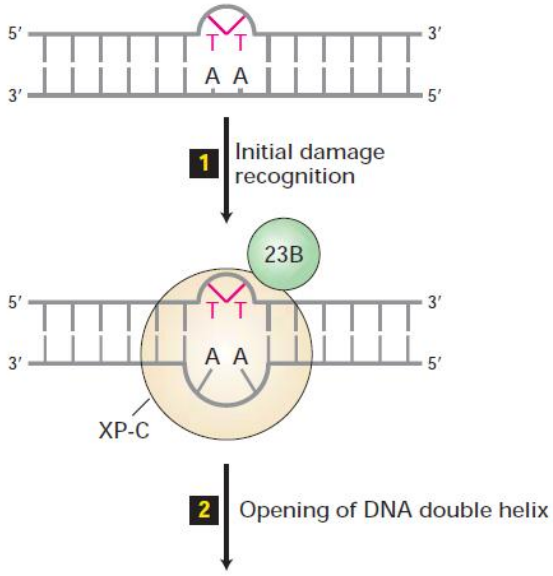
Another process, also conserved from bacteria to man, principally eliminate base-pair mismatches, deletions, and insertions that are accidentally introduced by polymerases during replication. As with base excision repair of a T in a T · G mismatch, the conceptual problem with *mismatch excision repair* is determining which is the normal and which is the mutant DNA strand, and repairing the latter. How this happens in human cells is not known with certainty. It is thought that the proteins that bind to the mismatched segment of DNA distinguish the template and daughter strands; then the mispaired segment of the daughter strand—the one with the replication error—is excised and repaired to become an exact complement of the template strand (Figure 23-28). Hereditary nonpolyposis colorectal cancer, arising from a common inherited predisposition to cancer, results from an inherited loss-of-function mutation in one allele of either the *MLH1* or the *MSH2* gene; the MSH2 and MLH1 proteins are essential for DNA mismatch repair (see Figure 23-28). A complex of the MSH2 and MSH6 proteins binds to a mispaired segment of DNA in such a way as to distinguish between the template and newly synthesized daughter strands (step1). This triggers binding of the MLH1 endonuclease, as well as other proteins such as PMS2, which has been implicated in oncogenesis through mismatch-repair mutations, although its specific function is unclear. A DNA helicase unwinds the helix and the daughter strand is cut; an exonuclease then removes several nucleotides, including the mismatched base (step2). Finally, as with base excision repair, the gap is then filled in by a DNA polymerase (Pol δ, in this case) and sealed by DNA ligase (step3).

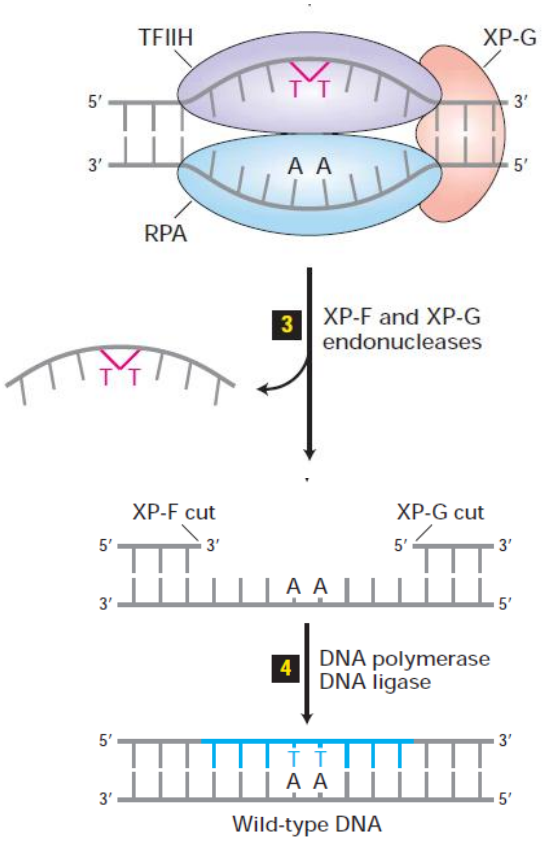


Nucleotide excision repair in human cells.

Cells use *nucleotide excision repair* to fix DNA regions containing chemically modified bases, often called chemical adducts, that distort the normal shape of DNA locally. A key to this type of repair is the ability of certain proteins to slide along the surface of a double-stranded DNA molecule look looking for bulges or other irregularities in the shape of the double helix. For example, this mechanism repairs *thyminethymine dimers*, a common type of damage caused by UV light (Figure); these dimers interfere with both replication and transcription of DNA. Nucleotide excision repair also can correct DNA regions containing bases altered by covalent attachment of carcinogens such as benzo(*a*)pyrene and aflatoxin (see Figure), both of which cause G-to-T transversions. Figure-1 illustrates how the nucleotide excisionrepair system repairs damaged DNA. Some 30 proteins are involved in this repair process, the first of which were identified through a study of the defects in DNA repair in cultured cells from individuals with xeroderma pigmentosum, a hereditary disease associated with a predisposition to cancer. Individuals with this disease frequently develop the skin cancers called melanomas and squamous cell carcinomas if their skin is exposed to the UV rays in sunlight. Cells of affected patients lack a functional nucleotide excision-repair system. Mutations in any of at least seven different genes, called *XP-A* through *XP-G*, lead to inactivation of this repair system and cause xeroderma pigmentosum; all produce the same phenotype and have the same consequences.

A DNA lesion that causes distortion of the double helix, such as a thymine dimer, is initially recognized by a complex of the XP-C (*x*eroderma *p*igmentosum *C* protein) and 23B proteins (step1). This complex then recruits transcription factor TFIIH, whose helicase subunits, powered by ATP hydrolysis, partially unwind the double helix. XP-G and RPA proteins then bind to the complex and further unwind and stabilize the helix until a bubble of ≈25 bases is formed (step2). Then XP-G (now acting as an endonuclease) and XP-F, a second endonuclease, cut the damaged strand at points 24–32 bases apart on each side of the lesion (step3). This releases the DNA fragment with the damaged bases, which is degraded to mononucleotides. Finally the gap is filled by DNA polymerase exactly as in DNA replication, and the remaining nick is sealed by DNA ligase (step4). [Adapted from J. Hoeijmakers, 2001, *Nature* **411**:366, and O. Schärer, 2003, *Angewandte Chemie*]



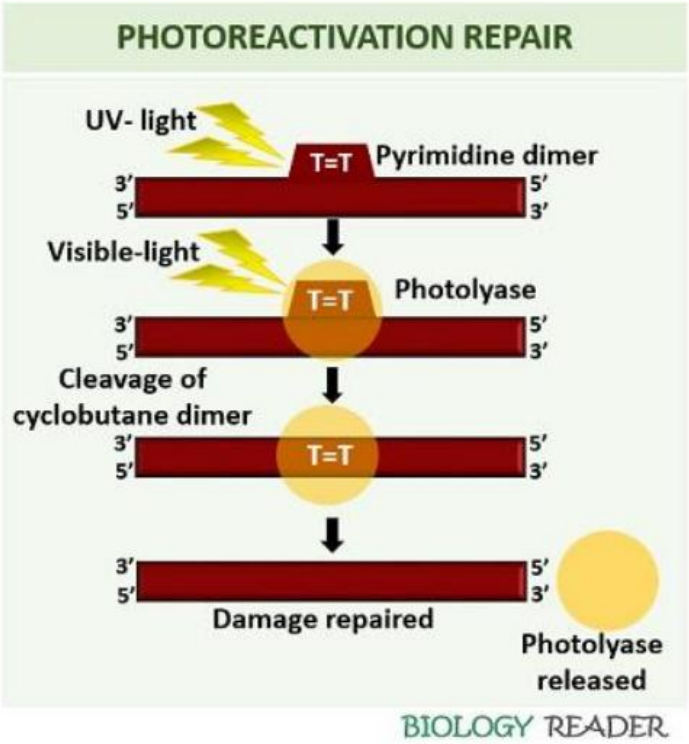


Photoreactivation

Photoreactivation is possibly the simplest DNA repair mechanism currently known. In the photoreactivation mechanism, the cyclobutane pyrimidine dimers (Figure) formed by UV light are reversed by light of a longer wavelength. Since naturally occurring UV damage requires the exposure to sunlight, cells that are so exposed can make use of longer wavelength light as a source of energy for directly restoring dimers to monomeric form. The precise wavelength needed depends on the chromophore or light-absorbing component of the photoreactivating enzyme, the DNA photolyase. In *Escherichia coli*, the enzyme contains two chromophores, a deazaflavin derivative and a folate derivative, but only the latter is used as a light antenna, and photoreactivation peaks at a wavelength of 384 nm.

Formation of Pyrimidine-dimer: The UV-light induces the formation of a covalent bond between the pyrimidine bases either thymine or cytosine. The two adjacent pyrimidine bases bind by the means of **carbon-carbon double bond**. A formation of carbon-carbon double bond between the pyrimidine bases forms a 'Dimeric structure' commonly refers to as '**Cyclobutane pyrimidine dimers**'. Cyclobutane pyrimidine dimers (CBPBs) is a very common product forms after the UV-exposure. In addition to this, **6-4 photoproducts** also produce as a result of UV-damage, where the two bases attach with a single carbon bond to the C-6 of one ring and C-4 of

another ring. Thymine=Thymine dimer usually forms after the UV-exposure, and it appears like a **bubble** in the DNA strand.



Treatment with Photolyase under visible light: Under the visible light of longer wavelength, a photolyase enzyme becomes activated. A photolyase enzyme **scans** the DNA strand and **recognizes** the pyrimidine dimers. After recognition of dimeric forms in the DNA, it directly binds to it.

Breaking of T=T dimer: When a photolyase enzyme binds to the T=T dimer, it starts splitting the carbon-carbon double covalent bond between the cyclobutane rings. Thus, a photolyase enzyme converts the **dimeric** form of the pyrimidines bases into the **monomeric** forms.

Release of Photolyase: After breaking the double covalent bond between the consecutive thymine bases, a photolyase enzyme releases out from the DNA strand, and the DNA fixes.

SOS DNA repair system

The **SOS response** is a global response to DNA damage in which the cell cycle is arrested and DNA repair and mutagenesis is induced. The system involves the RecA protein (Rad51 in eukaryotes). The RecA protein, stimulated by single-stranded DNA, is involved in the inactivation of the repressor (LexA) of SOS response genes thereby inducing the response. It is an error-prone repair system that contributes significantly to DNA changes observed in a wide range of species.During normal growth, the SOS genes are negatively regulated by LexA repressor protein dimers. Under normal conditions, LexA binds to a 20-bp consensus sequence (the SOS box) in the operator region for those genes. Some of these SOS genes are expressed at certain levels even in the repressed state, according to the affinity of LexA for their SOS box. Activation of the SOS genes occurs after DNA damage by the accumulation of single stranded (ssDNA) regions generated at replication forks, where DNA polymerase is blocked. RecA forms a filament around these ssDNA regions in an ATP-dependent fashion, and becomes activated. The activated form of RecA interacts with the LexA repressor to facilitate the LexA repressor's self-cleavage from the operator. Once the pool of LexA decreases, repression of the SOS genes goes down according to the level of LexA affinity for the SOS boxes. Operators that bind LexA weakly are the first to be fully expressed. In this way LexA can sequentially activate different mechanisms of repair.

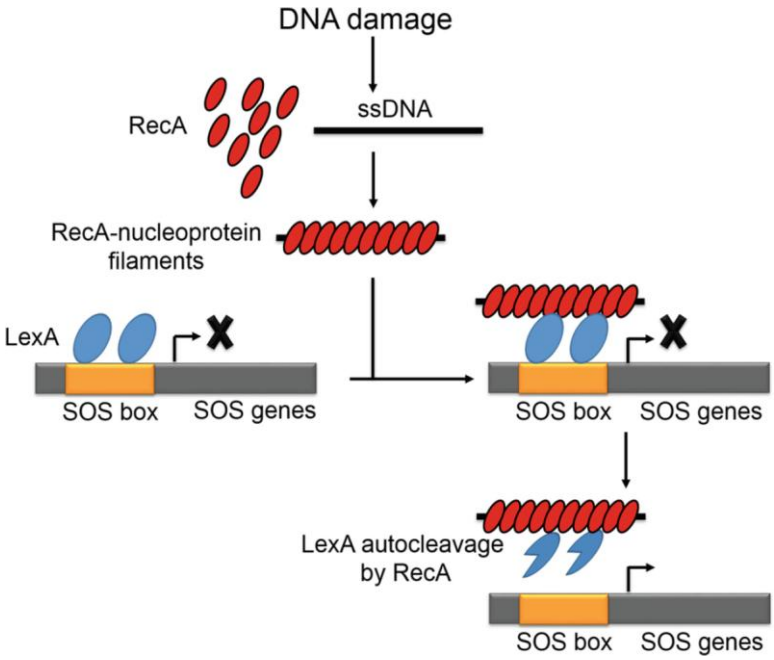


TABLE 23-1 Some Human Hereditary Diseases and Cancers Associated with DNA-Repair Defects

Disease	DNA-Repair System Affected	Sensitivity	Cancer Susceptibility	Symptoms
PREVENTION OF POINT MUTATIONS, INSERTIONS, AND DELETIONS				
Hereditary nonpolyposis colorectal cancer	DNA mismatch repair	UV irradiation, chemical mutagens	Colon, ovary	Early development of tumors
Xeroderma pigmentosum	Nucleotide excision repair	UV irradiation, point mutations	Skin carcinomas, melanomas	Skin and eye photosensitivity, keratoses
REPAIR OF DOUBLE-STRAND BREAKS				
Bloom's syndrome	Repair of double-strand breaks by homologous recombination	Mild alkylating agents	Carcinomas, leukemias, lymphomas	Photosensitivity, facial telangiectases, chromosome alterations
Fanconi anemia	Repair of double-strand breaks by homologous recombination	DNA cross-linking agents, reactive oxidant chemicals	Acute myeloid leukemia, squamous-cell carcinomas	Developmental abnormalities including infertility and deformities of the skeleton; anemia
Hereditary breast cancer, BRCA-1 and BRCA-2 deficiency	Repair of double-strand breaks by homologous recombination		Breast and ovarian cancer	Breast and ovarian cancer

SOURCES: Modified from A. Kornberg and T. Baker, 1992, *DNA Replication*, 2d ed., W. H. Freeman and Company, p. 788; J. Hoeijmakers, 2001, *Nature* **411**:366; and L. Thompson and D. Schild, 2002, *Mutation Res.* **509**:49.

Reference:

1. https://biologyreader.com/photoreactivation-repair.html
2. Lodish H, Berk A, Kaiser CA, Krieger M, Scott MP, Bretscher A, Ploegh H, Matsudaira P, Molecular Cell Biology, W. H. Freeman,