MECHANISM OF DNA REPAIR

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An estimate suggests that human cells suffer with more than seventy thousand DNA damages per day. The lesions may either be in the form of single-strand break (SSB) or double-strand breaks (DSB) within the DNA. Some changes occur during duplication of genome due to the errors in DNA replication but most of them are induced later due to occupational hazards or life style. The ultraviolet radiations, environmental chemicals, thermal fluctuations, reactive metabolites or reactive oxygen species cause either deletion or substitution of one or more base pairs. The pyrimidine dimers are formed as a result of exposure to UV light. Ionising radiations from radioactive elements such as uranium, radium, plutonium and X-rays also damage DNA. The superoxide and hydroxyl radicals damage DNA by either oxidation of sugar and base or breaking of the strands.

Most of the changes that occur in DNA are instantly corrected by DNA repair processes. Accuracy in DNA replication along with repairing accidental lesions is essential for maintaining the genetic stability. The Mismatch repair fixes mispaired bases just after DNA replication. The DNA damage repair pathways detect and correct damage throughout the cell cycle. The excision repair mechanism targets the removal of bulky DNA adducts, UV-induced photoproducts, base-pair alterations, purine loss, DNA mismatches, single- and double-strand DNA breaks. Several proteins and enzymes are required during DNA damage response (DDR) and repair signalling pathways. We shall

The damages may be either in the genomic DNA or mitochondrial DNA or in both of them.

12.2.1 Genomic DNA Damage

Genomic DNA that stores the genetic information is highly susceptible to chemicals and physical insults. It gets damaged by environmental and internal hazards, such as irradiation, various types of chemicals, reactive oxygen species and replication errors. These factors and stresses can generate numerous lesions (Fig. 12.1) on base pairs and ribose sugar-phosphate backbone of DNA. Among DNA lesions, DNA double-strand break (DSB) is the most deleterious type of lesion. If it is not repaired, DSB can cause genomic instability and several other genetic diseases. Loss of DNA damage repair leads to the accumulation of DNA lesions and damage response. Several proteins like **cohesin** and **BRCA** participate in DNA damage and repair response. The cohesin, a multi protein complex, serves as critical component during separation of sister chromatids and activation of DNA damage check point. The Breast Cancer type 1 susceptibility protein, BRCA1 plays an important role in maintaining genomic stability. Mutations of BRCA1 impair DNA damage repair and also cause familial breast and ovarian tumors.

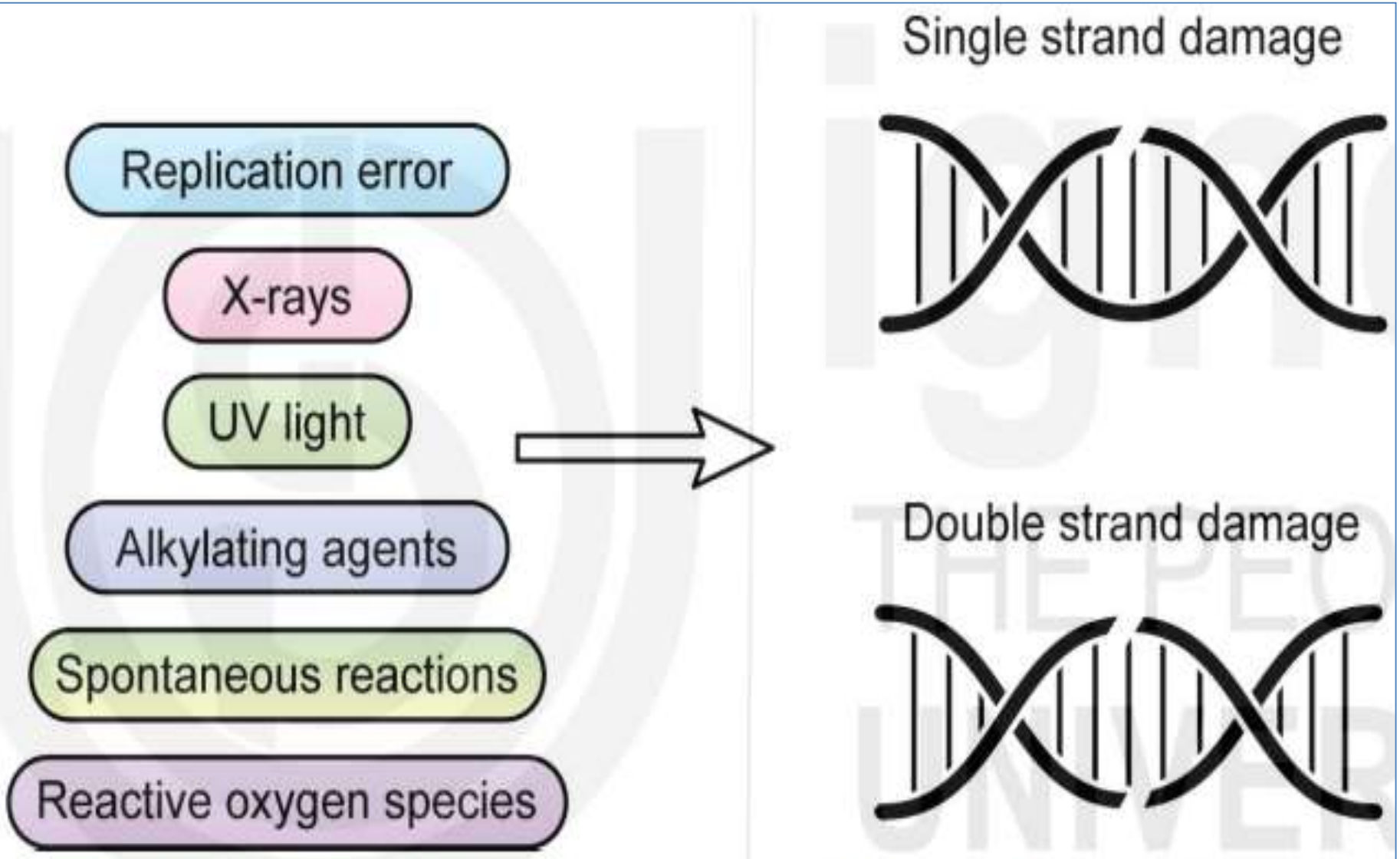

Fig. 12.1: Factors cause either single or double-strand break in Genomic DNA. Replication error, X-ray, UV light, alkylating agents, spontaneous reactions and reactive oxygen species cause DNA break.

12.2.2 Mitochondrial DNA Damage

While studying extranuclear inheritance you learnt about the mitochondrial DNA, Just to recall, mitochondria also contain their own DNA called mitochondrial DNA (mtDNA) which is about one percent of cellular DNA. The mtDNA is circular and double stranded. Here what is important to note is, it is five to ten times more prone to mutations than genomic DNA. The damages in mtDNA have been found responsible for several diseases like stroke, cancer, diabetes and neuro degeneration. The mtDNA also gets damaged by radiation, genotoxic chemicals and reactive oxygen species (ROS). The impact of damage on mtDNA is comparatively more because of errors during DNA replication and lack of conventional histone proteins in mitochondria. The ROS can lead to various types of oxidative damage including base modification or removal, DNA strand breaks and cross linking (Fig. 12.2). Also, the mtDNA DNA polymerase gamma (pol γ) has low frame shift fidelity.

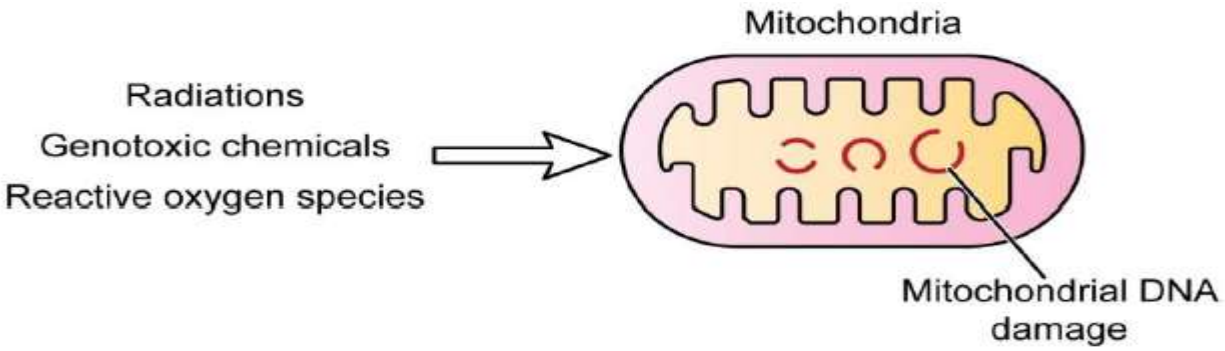

Fig. 12.2: Representation of mitochondrial DNA damage due to toxicants.

Repair mechanisms can be broadly grouped into three categories: direct reversal of the damage, excision repair and post-replication repair mechanism. However we shall consider each repair mechanism separately.

It could be either **direct repair** with the help of methyl transferases or by photolyases using Ultraviolet rays. The DNA polymerases synthesize new DNA strand. Both prokaryotic and eukaryotic cells contain multiple DNA polymerases. The **exonuclease activity** of DNA polymerases excise bases that have been added to DNA incorrectly. In prokaryotes, out of five DNA polymerases DNA polymerase III serves as major replicase. However, DNA polymerase-I, DNA polymerase-II, DNA polymerase-IV and DNA polymerase-V participate in repair of DNA damage (Table 12.1).

Table 12.1: DNA polymeraseswith their associated functions

Enzymes	Functions
DNA polymerase I	Major repair enzyme
DNA polymerase II	Minor repair enzyme
DNA polymerase III	DNA replication
DNA polymerase IV	SOS repair
DNA polymerase V	SOS repair

Table 12.2: EukaryoticDNA polymerases and their associated functions

Enzymes	Functions
DNA polymerase α	Nuclear replication
DNA polymerase δ	Nuclear replication
DNA polymerase ε	Nuclear replication
DNA polymerase γ	Mitochondrial replication
DNA polymerase β	Base excision repair
DNA polymerase ζ	Thymine dimer bypass
DNA polymerase η	Base damage repair
DNA polymerase ι	Required in meiosis
DNA polymerase κ	Deletion and base substitution

12.3.1 Proofreading

The processivity of DNA polymerase slows and exonuclease property gets activated if any wrong or incorrectly paired nucleotide gets added. During DNA replication, the DNA polymerases remove incorrect nucleotide added during replication. The process is described as **proofreading activity of DNA polymerase**. The incorrect nucleotide is removed and replaced with the correct nucleotide before continuing with DNA synthesis.

12.3.2 Direct Repair

Direct repair is rare and involves the reversal or simple removal of the damage. There are some lesions in DNA that can be repaired by direct reversal of the damage, without the removal of any base or a nucleotide. For example, Photo-reactivation of pyrimidine-dimers, in which dimers are reversed by a light-dependent enzyme, **DNA photolyase**. This enzyme is present in almost all cells from bacteria to animals (Fig.12.3).

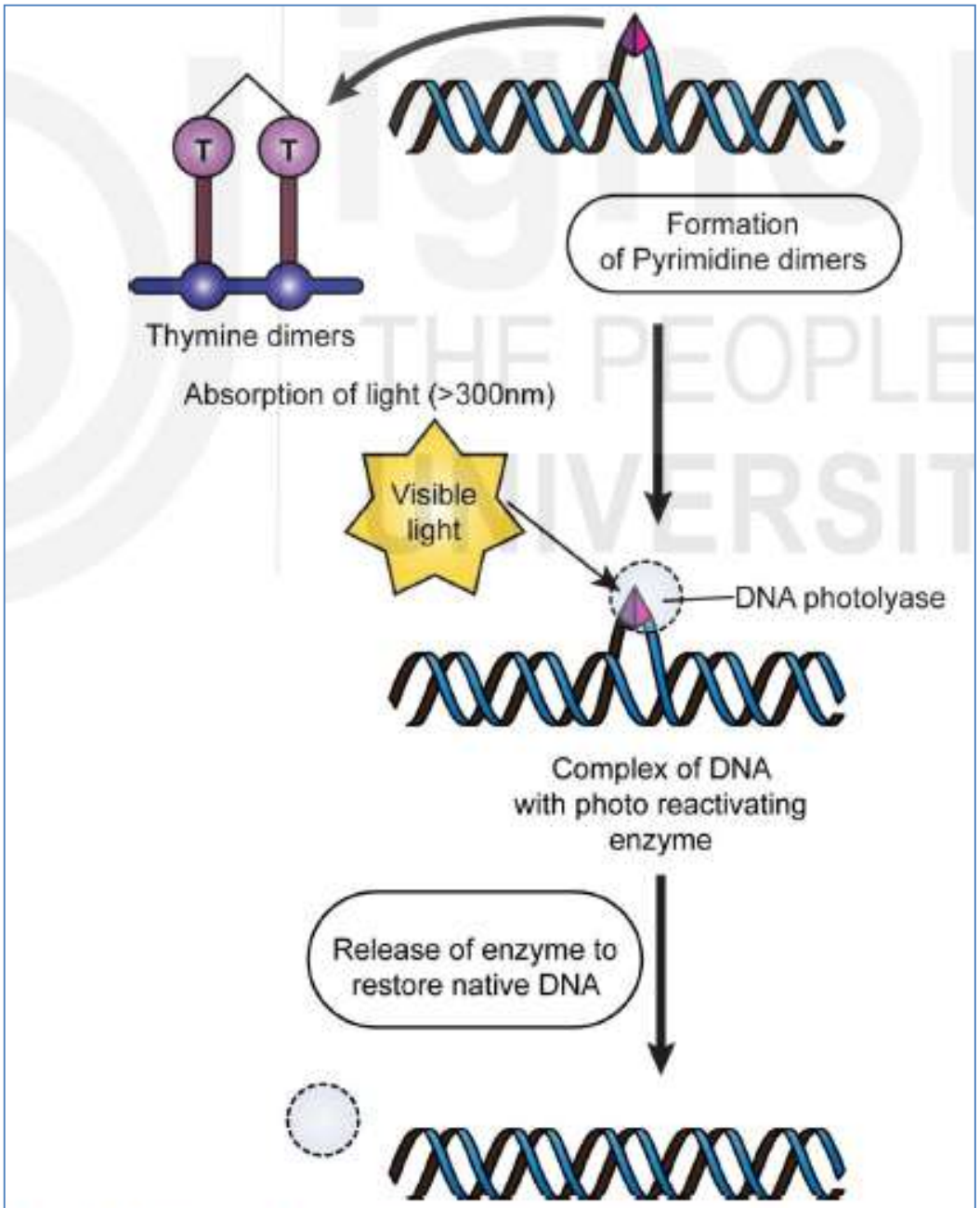

Fig. 12.3: Diagrammatic representation of direct repair mechanism using photolyase enzyme.

12.3.3 Base Excision Repair

The lesions produced by mutagenic agents are recognized by a group of enzymes called **DNA glycosylases**. The enzyme detects and removes a specific kind of damaged base by cleaving N-glycosyl bond and plays a key role in base excision repair. For example, de-amination converts a cytosine base into uracil, a base typically found only in RNA. During DNA replication, uracil pairs with adenine because of uncorrected cytosine-to-uracil change. This process induces a mutation. To prevent such mutations, a glycosylase from the base excision repair pathway detects and removes de-aminated cytosine. Once the base has been removed, the "empty" piece of DNA backbone is also removed, and the gap is filled and sealed by enzyme DNA ligase (Fig. 12.4).

The DNA glycosylases also recognize and remove other abnormal bases, including hypoxanthine formed by the de-amination of adenine, pyrimidine dimers, alkylated purines other than O6-alkylguanine, and bases damaged by oxidation or ionizing radiation.

Fig. 12.4: Diagrammatic representation of base excision repair after DNA damage. De-amination of cytosine (C) leads to formation of uracil (U).

12.3.4 Nucleotide Excision Repair

Nucleotide excision repair is another pathway to remove and replace damaged nucleotides. It is the major repair system which operates on the bulky structure and large distortion of DNA. ***It is different from base-excision repair because DNA glycolyase in base excision repair only recognizes specific forms of damaged bases whereas nucleotide excision repair detects DNA damage that distorts the DNA molecule***. It gets activated when huge deformation is created in the helical structure of DNA by the DNA lesions. It detects bases that have been modified with bulky chemical groups and corrects types of damage that distort the DNA double helix, such as DNA methylation induced due to chemicals in cigarette smokers.

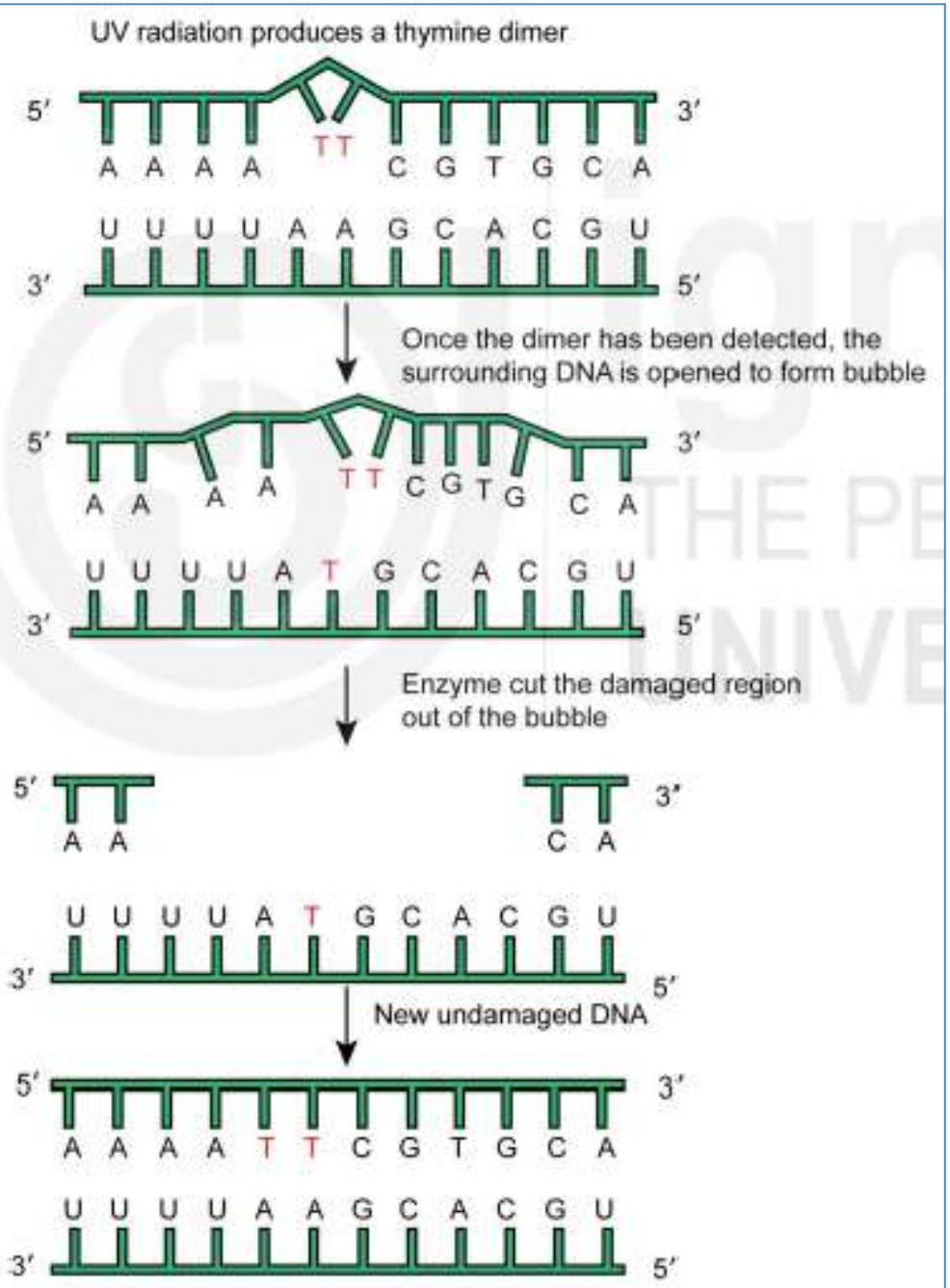

Fig.12.5: Diagrammatic representation of nucleotide excision repair after DNA damage.

12.3.5 Mismatch Repair

During DNA replication sometimes mismatched bases get incorporated. Generally, proofreading activity of DNA polymerase removes such mismatched bases. If escaped by proofreading, mismatch repair system remove and replace mispaired bases. In mismatch repair mechanism, a protein complex (MutS, MutL, and MutH) recognizes and binds to the mispaired base. A second complex composed of Helicases and Exonucleases cuts the DNA near the mismatch, removes incorrect nucleotide and a surrounding patch of DNA. A DNA polymerase then replaces the missing section with correct nucleotides, and an enzyme called a DNA ligase seals the gap (Fig.12.6).

The eukaryotic homologs of MutS and MutL bind to the mismatched bases and correct them like *E.coli*. In mammalian cells, presence of single strand breaks in the newly replicated DNA provides strand-specificity of mismatch repair. Mutations in homologs of MutS, and MutL are responsible for inherited colon cancer.

MutS binds to the mismatched base, followed by MutL which activates MutH. The MutH then cleaves the unmodified strand. MutS, MutL, helicase and an exonuclease remove part of the unmodified strand that contains the mismatch. The DNA polymerase and ligase complete the process of repair.

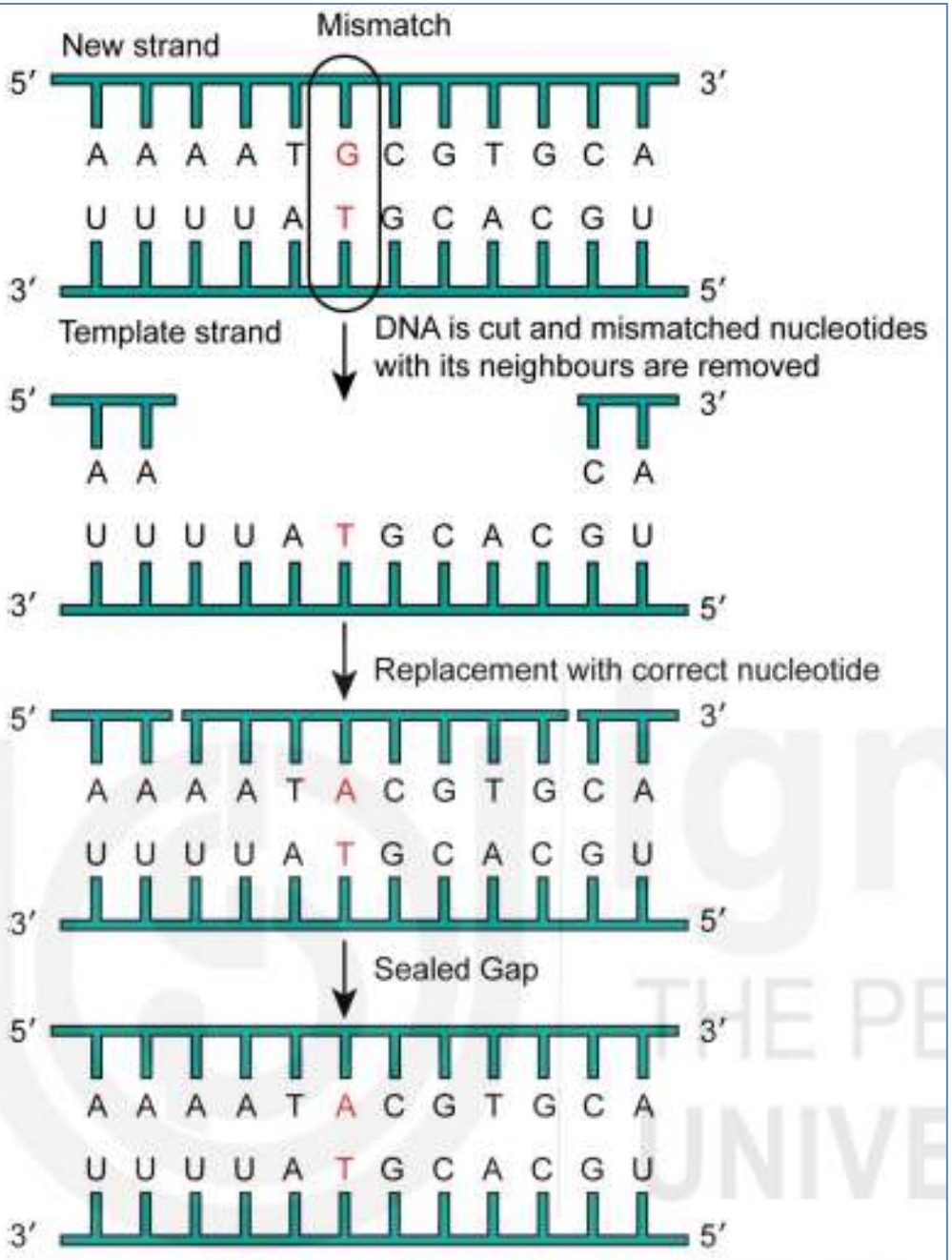

Fig. 12.6: Diagrammatic representation of mismatch repair mechanism after DNA damage.

12.3.6 Recombination Repair

Some types of environmental factors or high-energy radiation causes double-stranded breaks in DNA that may lead to loss of large segments of chromosomes and the hundreds of genes (Fig.12.7). In principle, a recombination event occurs on either side of the damaged site. It allows undamaged single strand to pair with the damaged strand which facilitated reconstruction and continuation of the replication fork bypassing the damaged site. The recombination-repair pathways help to restore the fork after completion of the damage has been repaired. This is post replication repair system. The pathway for handling a stalled replication fork requires RecA and the RecBC system in *E. coli*. In one possible pathway, RecA binds to single-stranded DNA at the stalled replication fork, stabilizes it, and possibly acts as the sensor that detects the stalling event. The RecBC is involved in excision-repair of the damage. After the damage has been repaired, replication can resume. Stalled replication forks can be rescued by recombination-repair.

Another pathway i.e. Non-homologous end joining (NHEJ) is a repair mechanism which does not involve homologous recombination. It facilitates joining of two ends of the broken DNA double strand using a heterodimeric enzyme complex consisting of proteins Ku. The Ku, a member of highly conserved family protein found in bacteria, yeast and humans.

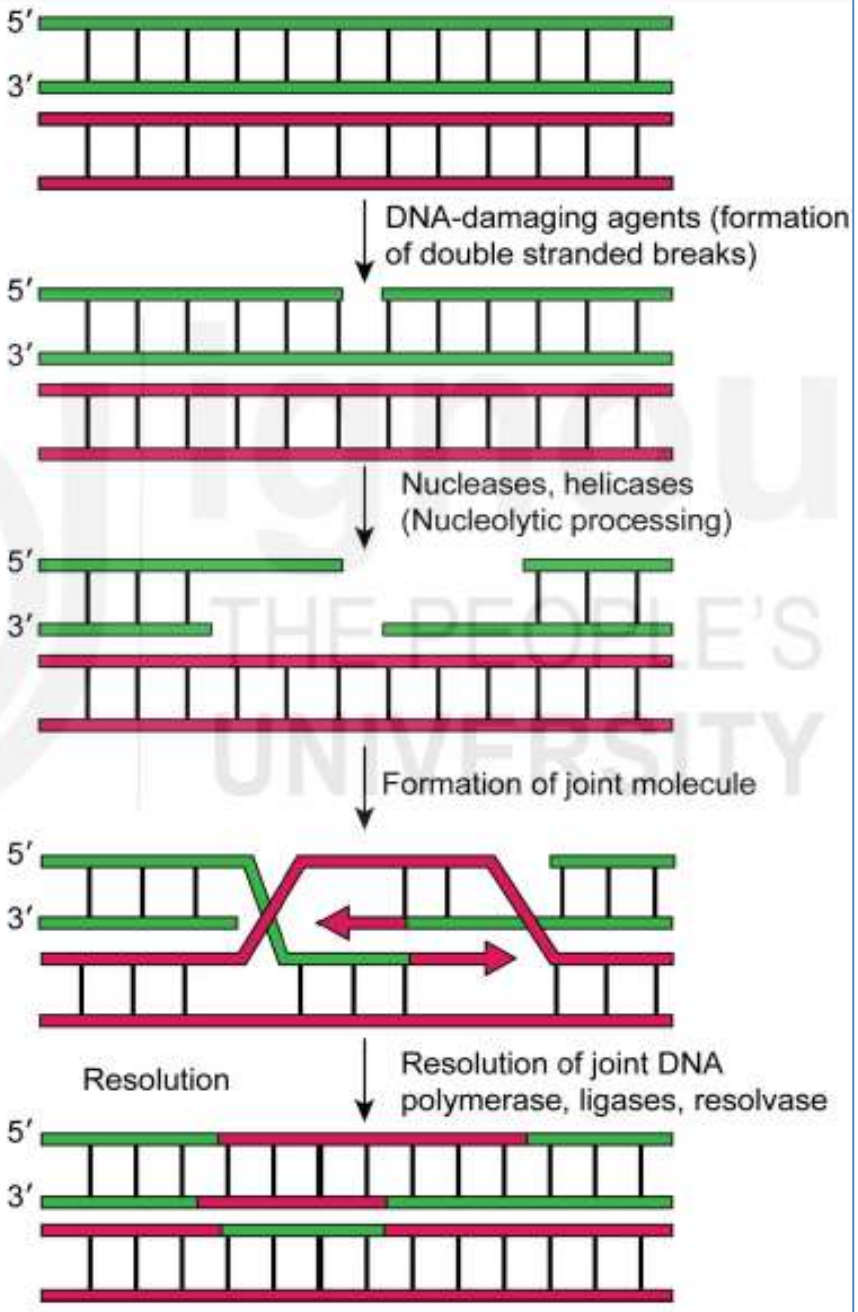

Fig. 12.7: Diagrammatic representation of recombination repair mechanism of Double stranded break in DNA.