Fidelity of DNA Replication

(DNA Repair)

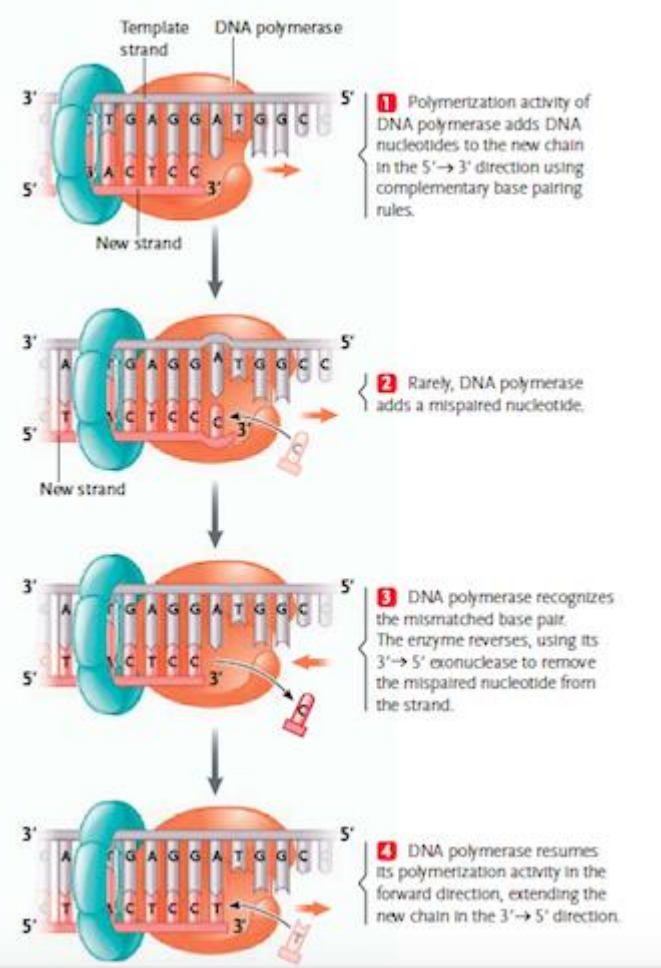
The DNA sequence can be changed as the result of copying errors introduced by DNA polymerases during replication and by environmental agents such as mutagenic chemicals and certain types of radiations. If such changed DNA sequences are left uncorrected in both growing and non-growing somatic cells, it will accumulate so many mutations which may even become lethal too.

DNA damage and repair is relevant to carcinogenesis as all the carcinogens are mutagens too that can cause a change in DNA SEQUENCE. All the effects of carcinogenic chemicals on tumour production can be accounted for by the DNA damage that they cause and by the error introduced into DNA during the cells effort to repair this damage.

Most damage to DNA is repaired by removal of the damaged bases followed by synthesis of the excised region. Some lesions of DNA, however, can be repaired by direct reversal of the damage. But, only a few types of DNA damage are repaired this way, particularly pyrimidine dimers resulting from exposure to UV lights and alkylating guanine residues resulting from addition of methyl/ethyl groups in purine rings. The mechanism of direct reversal of pyrimidine dimers is called photoreactivation. Energy derived from visible light is utilised to break the cyclobutane ring structure and restore the original pyrimidine bases. This process of repair of pyrimidine dimers is common in many prokaryotic and eukaryotic cells, though many other species, including humans, lack this mechanism of DNA repair.

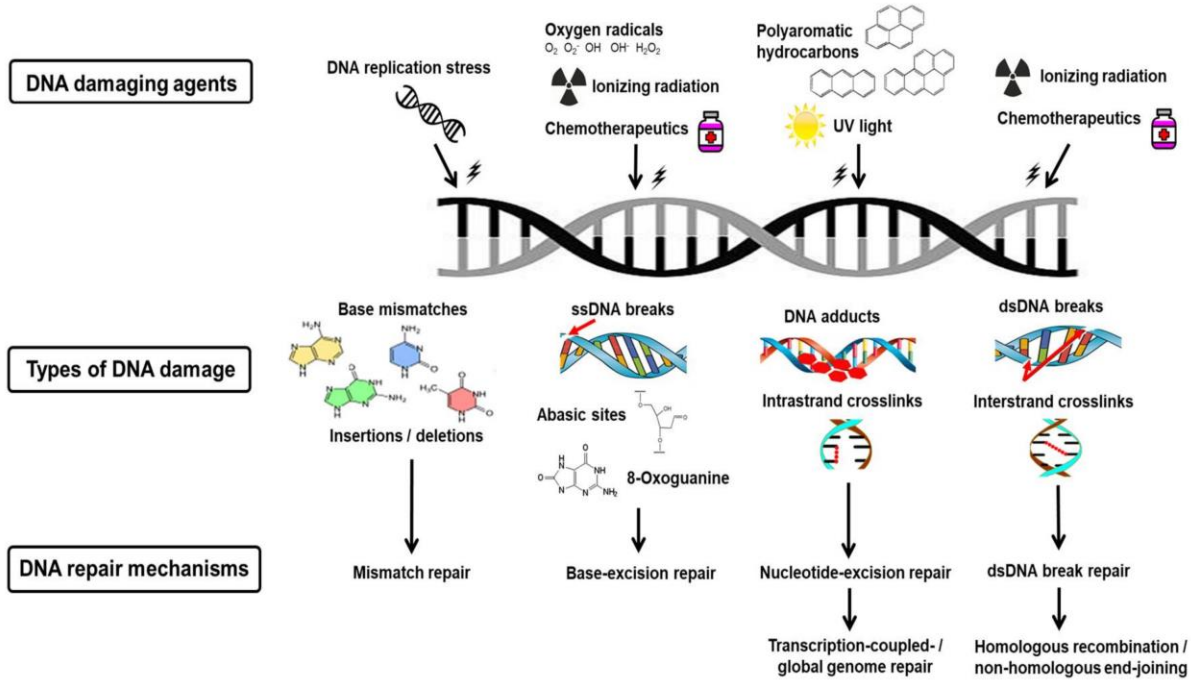
Alkylating guanine residues can be repaired by an enzyme called O^6methylguanine methyl transferase, which transfers the methyl group from O^6methylguanine to a cysteine residue in its active site and thus restores the original guanines.

It has been observed during DNA synthesis in *E. coli,* that the α subunit of DNA polymerase III introduces about 1 incorrect base in 10^4 internucleotide linkages. However, the measured mutation rate in bacterial cells is much less, about 1 mistake per 10^9 nucleotide polymerisation. This increased accuracy is largely due to the proofreading function of *E. coli* DNA polymerases. In DNA polymerase III, this proofreading function resides in the ε subunit of the core polymerase. When an incorrect base is incorporated during DNA synthesis, the polymerase pauses, then transfers the 3' end of growing chain to the exonuclease site, where the mispaired base is removed. Then the 3' end is transferred back to the polymerase site, where this region is copied correctly. Proofreading is a property of almost all bacterial DNA polymerases. Both the δ *and* ε DNA polymerases of animal cells possess proofreading activity, but not the α polymerases. Genetic studies in *E.coli* have shown that proofreading indeed play a critical role in maintaining sequencing fidelity during replication. It has been found that mutations in the gene encoding the ε subunit of polymerase III inactivate the proofreading function and lead to a thousand fold increase in the rate of spontaneous mutations.



In addition to proofreading activity of DNA polymerase, cells have evolved mechanisms for repairing DNA damaged by chemicals or radiation. DNA repair mechanisms have been studied most extensively in *E.coli*, using a combination of genetic and biochemical approaches. The diverse type of enzymatic repair mechanisms revealed by these studies can be divided into three broad categories:-

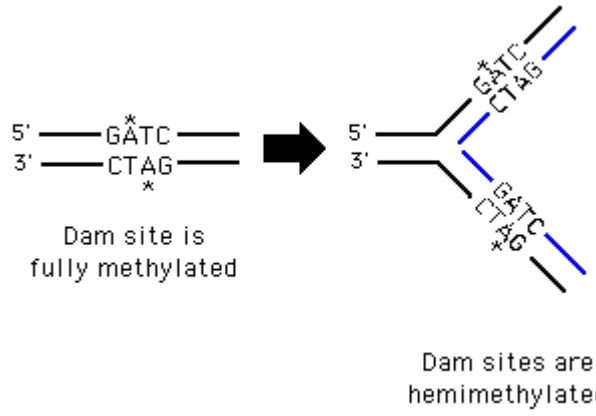
- Mismatch repair- that occurs immediately after DNA synthesis, uses the parental strand as a template to correct an incorrect nucleotide incorporated into the newly synthesised strand.
- Excision repair-that involves the repair of a damaged region by specialised nuclease systems and then DNA synthesis to fill the gap.
- End-joining repair- that repair double stranded DNA breaks by an end joining process.



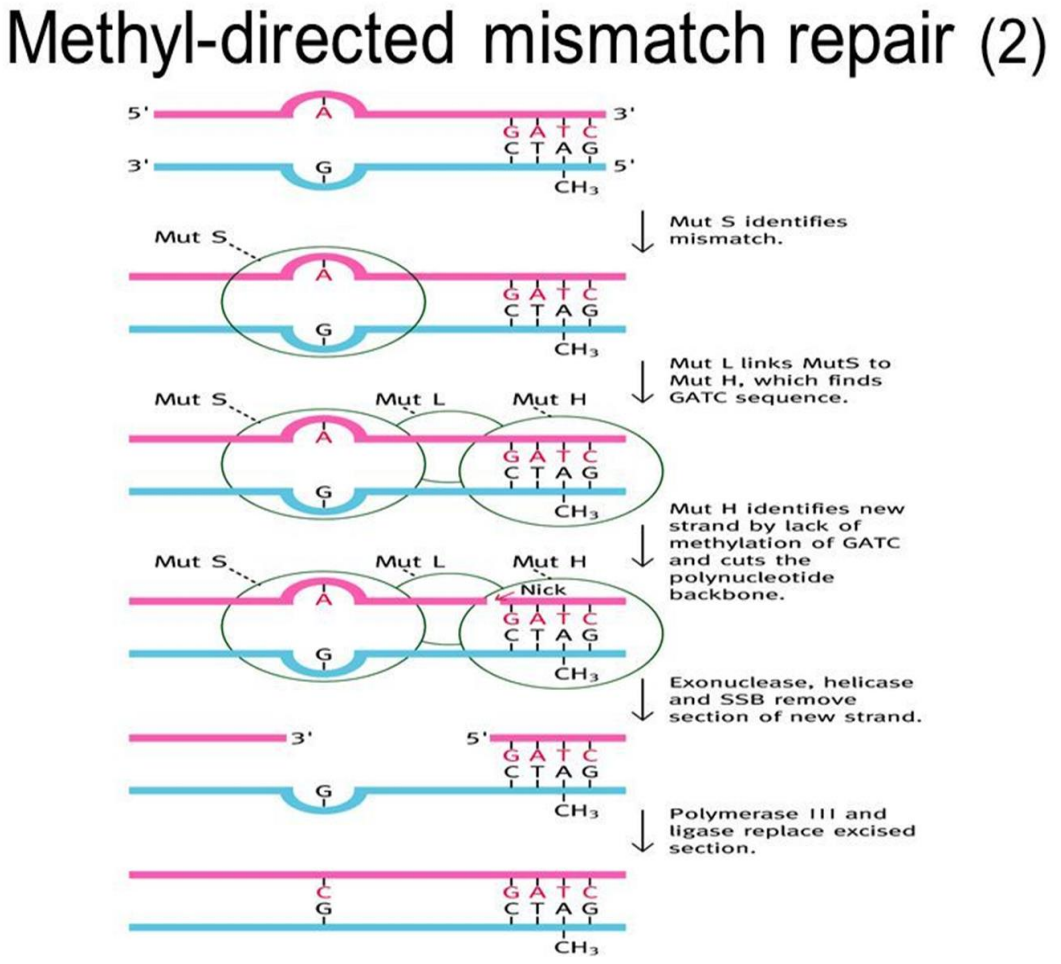
Mismatch Repair

Many spontaneous mutations are DNA sequence are point mutations, which involve a change in a single base pair in the DNA sequence. Bacterial and eukaryotic cells have a mismatch repair system that recognises and repairs all single-base mispairs, as well as insertions and deletions. The conceptual problem with mismatch 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.

In *E.coli* DNA, adenine residues in a GATC sequence are methylated at the 6 position. Since DNA polymerases incorporate adenine, not methyladenine, into DNA, adenine residues in newly replicated DNA are methylated only on the parental strand. The adenine in GATC sequences on the daughter strand are methylated by a specific enzyme called Dam Methyltransferase, only after a lag of several minutes. During this lag period, the newly replicated DNA contains hemimethylated GATC sequence.



A protein MutH, that binds specifically to hemimethylated sequences in *E.coli* distinguishes the methylated parental strand and unmethylated daughter strand. If an error occurs during DNA replication, resulting in a mismatched base repair near a GATC sequence, another protein, MutS, binds o this abnormally paired segment. Binding of MutS triggers binding of MutL, a linking protein that connects MutS with a hearby MutH. This cross-linking activates the latent MutH endonuclease, which cleaves specifically the unmethylated daughter strand. MutL and MutS then act together with an exonuclease and a helicase to excise the DNA between the strand break and the mismatch, with the resulting gap being filled by DNA polymerease and ligase.

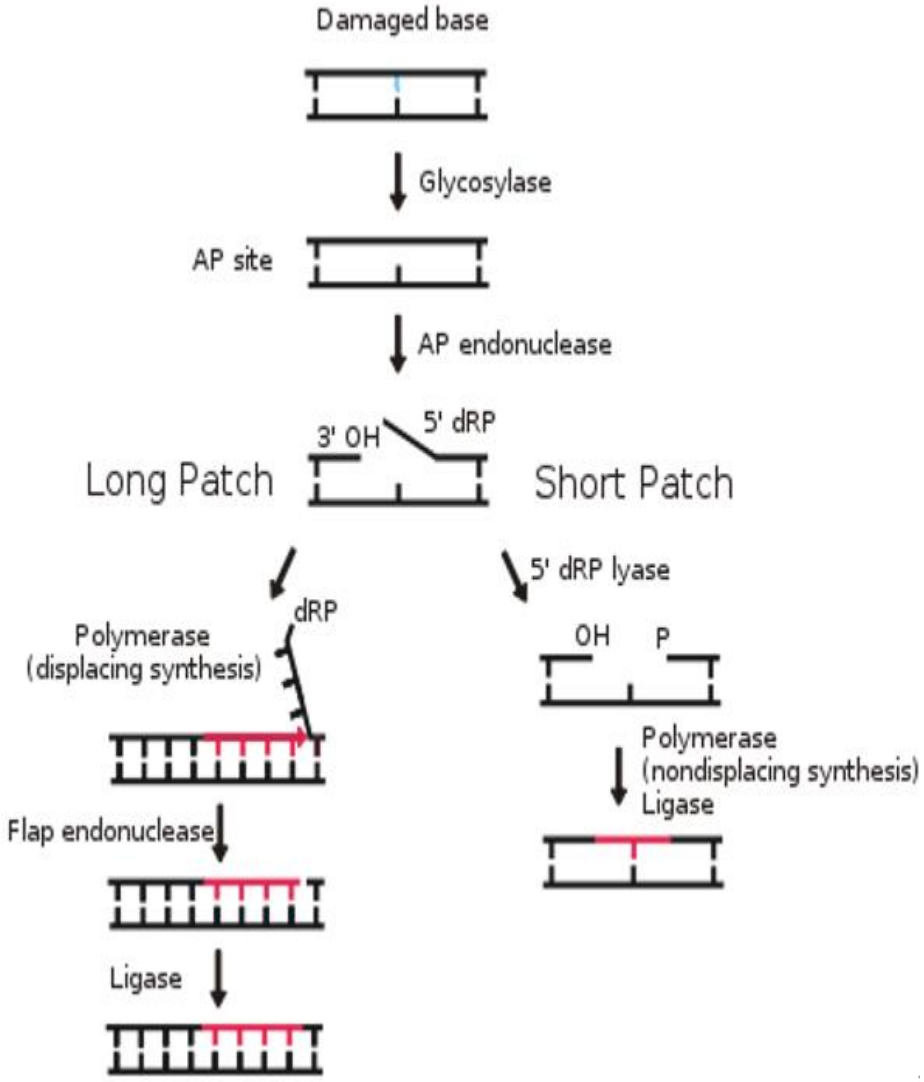


Excision Repair

Excision repair is a more general means of repairing a wide variety of chemical alterations to DNA. Thus it is the most important type of DNA repair mechanism of both prokaryote and eukaryote. In excision repair, the damaged DNA is recognised and removed, either as free bases or as nucleotides. The resulting gap is then filled by synthesis of new strand, using the

undamaged complementary strand as template. Two different types of excision repairs are-base excision repair and nucleotide excision repair.

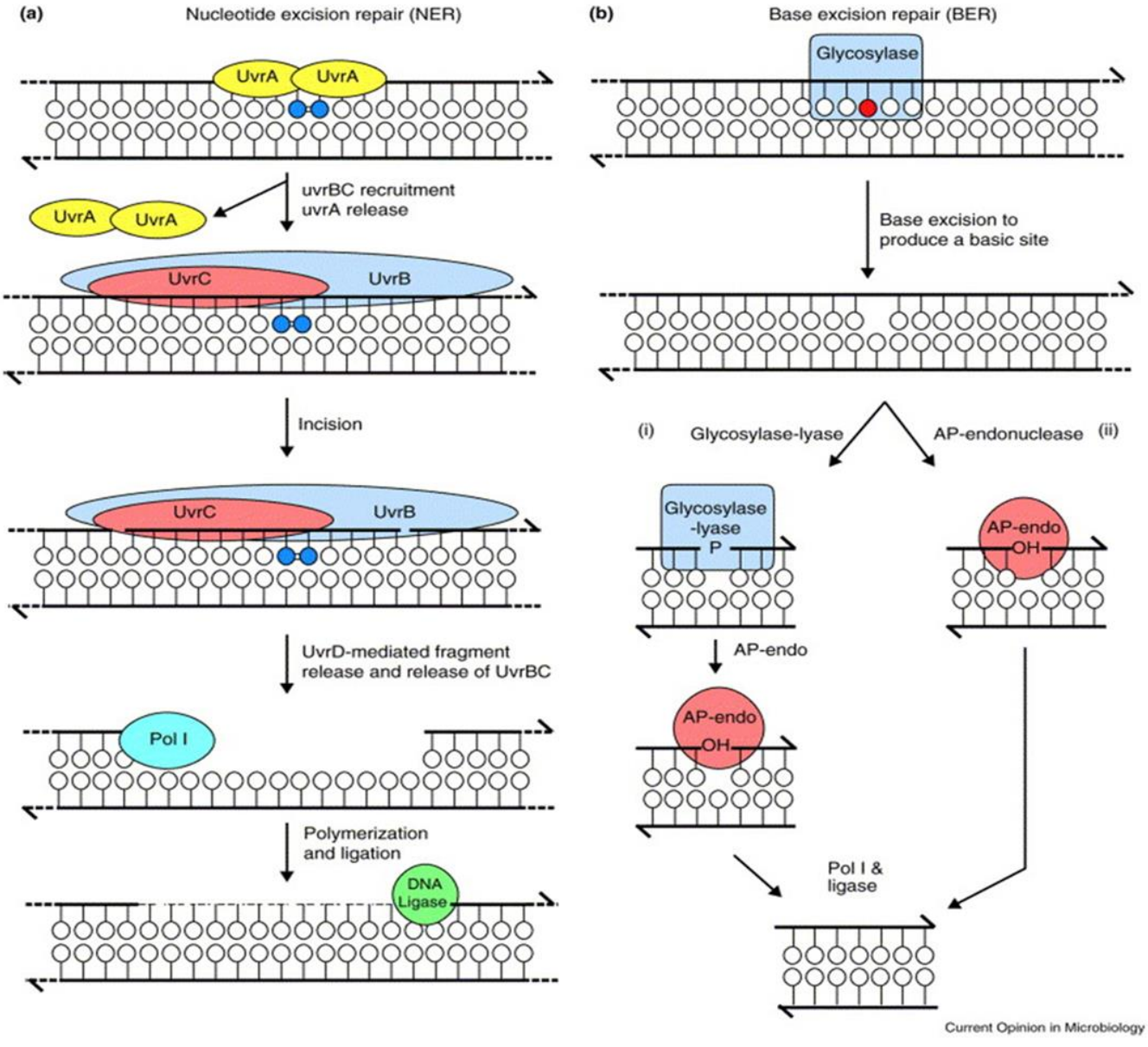
In base excision repair, single damaged bases are recognised and removed from the DNA molecule. Uracil can appear in DNA occasionally either being incorporated in place of thymine during DNA synthesis or by deamination of cytosine. The excision of uracil in DNA is catalysed by DNA glycosylase, an enzyme that cleaves the bond linking uracil to the deoxyribose of the DNA backbone. This reaction yields free base and an apyrimidine or apurinic site (AP site)- a sugar with no bases attached. Such AP sites are repaired by AP endonucleases, which cleaves adjacent to the APsites. The remaining deoxyribose moity is then removed, and the resulting single base gap is filled by DNA polymerase and ligase.



Nucleotide excision repair recognises a wide variety of damaged bases that distort the DNA molecule, including UV induced pyrimidine dimers and large chemical groups attached to DNA bases as a result of reaction of many carcinogens with DNA.

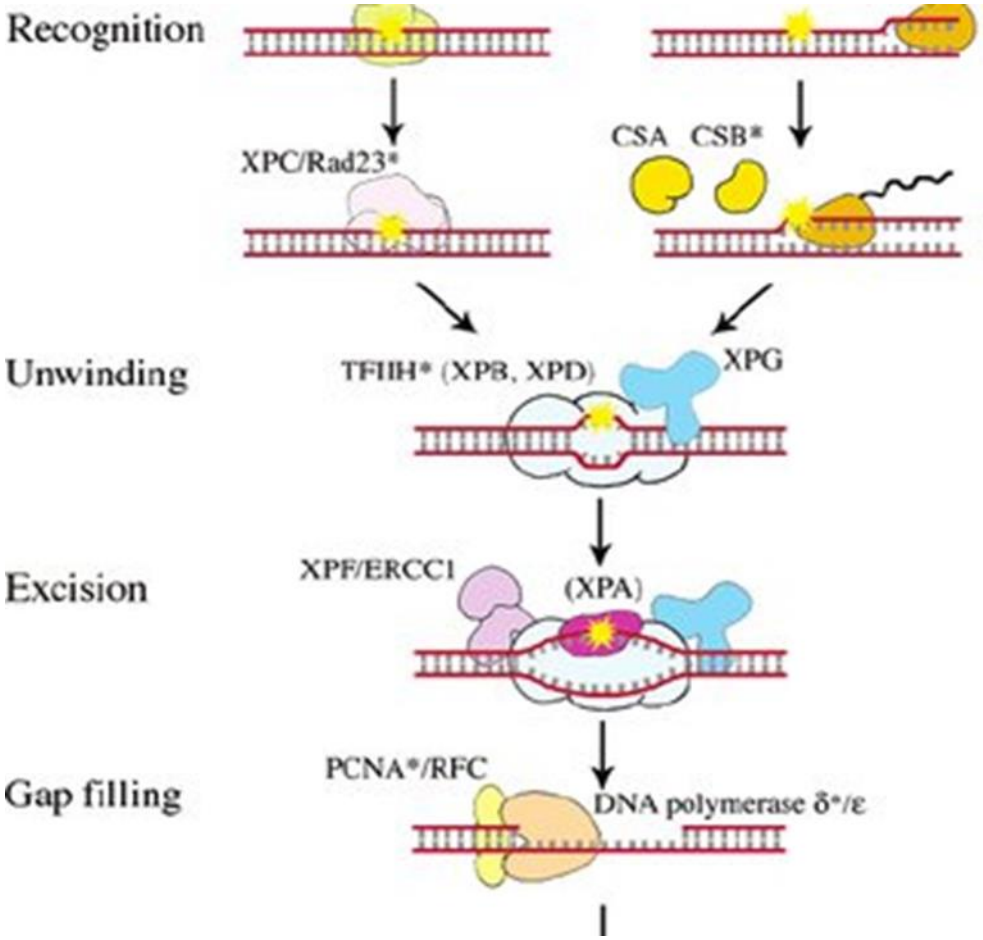
In *E.coli*, nucleotide excision repair is catalysed by the product of three genes (uvr A, B and C), that were identified because mutations at these loci result in extreme sensitivity to UV light.

The protein UvrA recognises damaged DNA and recruits UvrB and UvrC to the site of the lesion. UvrB and C then cleaves on the 3' and 5' sides of the damaged site, respectively, thus excising an oligonucleotide consisting of 12-15 bases. The Uvr ABC complex is frequently called an exonuclease, indicating its ability to directly excise an oligonucleotide. The action of a helicase is then required to remove the damage containing oligonucleotide from the double stranded DNA molecule. Lastly, the resulting gap is filled by DNA polymerase and sealed by ligase.



Nucleotide excision repair system have also been studied extensively in eukaryotes, particularly in yeasts and in humans. In yeasts, several genes involved in DNA repair (Rad genes for radiation sensitivity) have been identified by isolation of mutants with increased sensitivity to UV light. In humans, this system is most extensively studied in inherited diseases like xeroderma pigmentosum (XP), cockayne's syndrome and trichothiodystrophy. Molecular cloning has now identified seven different repair genes (XPA through XPG) that are mutated

in case of XP disease. In mammalian cells XPA proteins initiates repair along with XPC by recognising damaged DNA and forming complex with other proteins involved in the repair process (XPB and XPD). They act as helicases that unwind the damaged DNA. The binding of XPA to damaged DNA recruits XPF and XPG to the repair complex, which act as endonuclease cleaving an oligonucleotide of approximately 29 bases. The resulting gap is filled by pol δ *and* ε in association with RFC and PCNA and finally sealed by ligase.



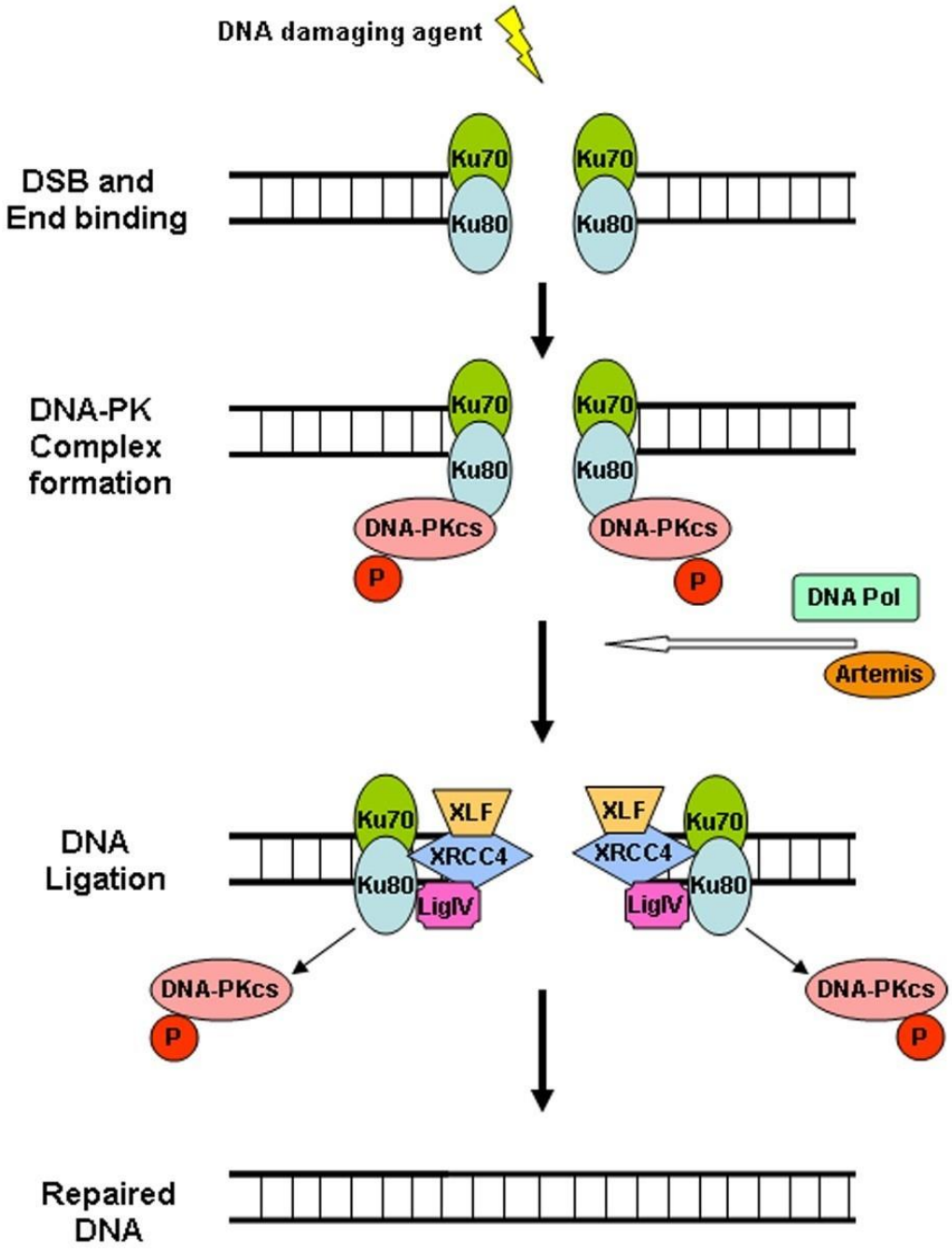
End-Joining Repair

A cell that has suffered a particularly double strand break usually contains other breaks too, that can be repaired by joining the free DNA ends. Double strand breaks are caused by ionizing radiation and by anticancer drugs. Such breaks can be correctly repaired only if the free ends of the DNA re-join exactly. Such repair is complicated by the absence of single strand regions that can direct base pairing during the re-joining process. Double strand breaks can be repaired either by homologous recombination or by re-joining the ends of the two DNA molecules.

In case of homologous recombination, the double strand break on one chromosome is repaired using the information on the homologous intact chromosome.

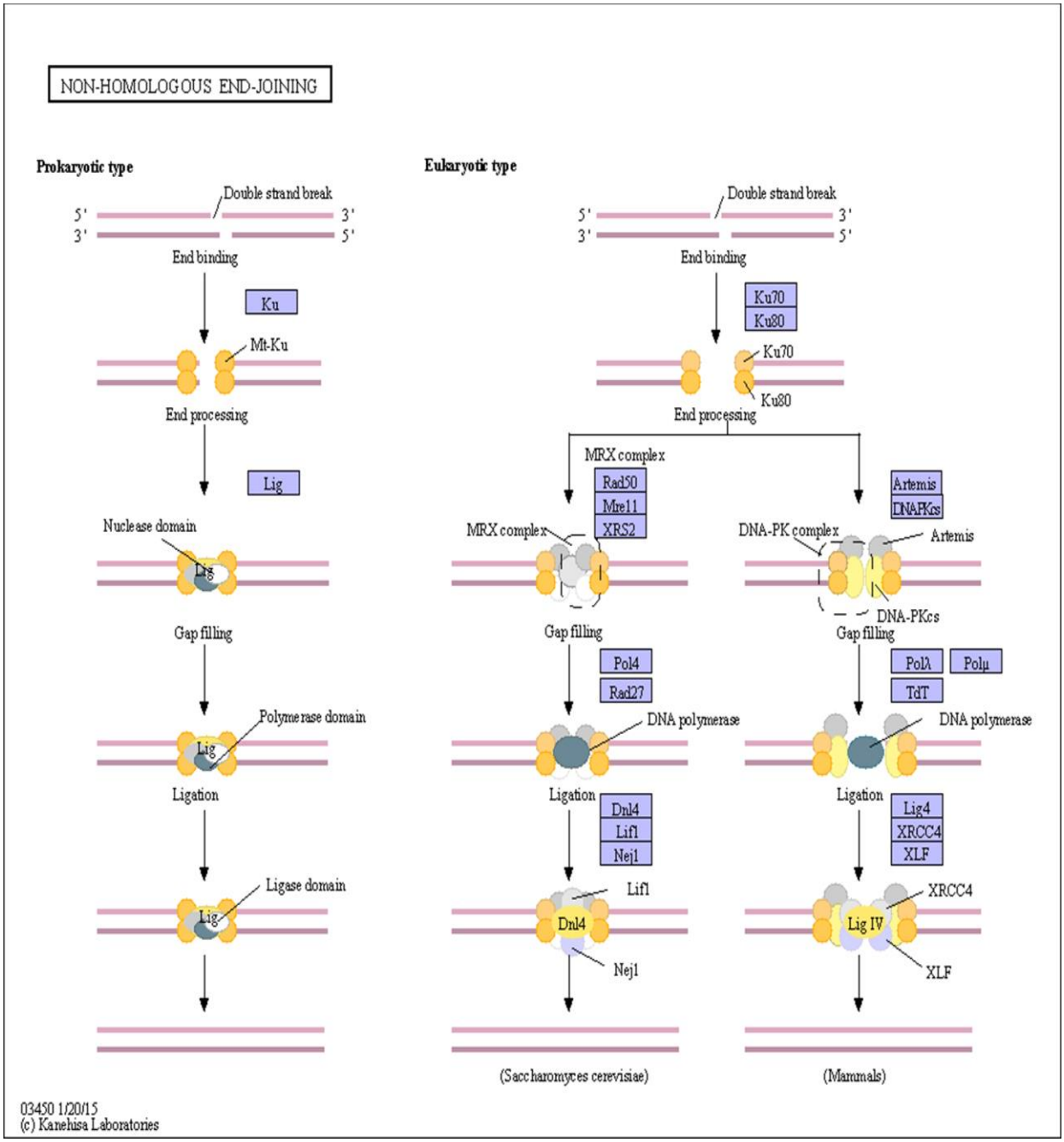
In case of end-joining of two non-homologous DNAs, a complex of two protein, Ku and a DNA dependent protein kinase, binds to the ends of a double strand break. After formation of

a synapse in which the broken ends overlap, Ku unwinds the ends, revealing short homologous sequences. The unpaired single stranded 5' ends are removed by mechanisms that are not well understood, and the two double stranded molecules ligated together. As a result, the double strand break is repaired, but several base pairs at the site of the break are removed.



Higher eukaryotes have DNA repair mechanisms analogous to those of *E.coli.* Human mismatch repair process can be initiated by the human MutSα protein, homologous to bacterial MutS. It binds to both mismatched base pairs and to small insertions and deletions. Another protein MutSβ mainly binds to insertions or deletions. The MutI protein is recruited to the

DNA by MutSα or MutSβ, but the identity of the nuclease that actually cuts the DNA is unknown. Following cleavage at either 3' or 5' end, an exonuclease removes 100 to 200 nucleotides from the cut strand. DNA pol δ is principally responsible for filling the gap and the strands are sealed afterwards by the action of a DNA ligase.



Error-Prone Repair

Direct reversals and excision repairs can correct only pre-replication damages. But, the gap formed post-replication due to damaged parental strand can be repaired either by recombinational repair or by error prone repair.

When the presence of a thymine dimer blocks replication, The DNA polymerase can bypass the lesion and reinitiate replication at a new site downstream. The result is a gap opposite the dimer in the newly synthesised DNA STRAND. In recombinational repair, this gap is filled by recombination with the undamaged parental strand. Although this leaves a gap in the previously intact parental strand, it can be filled by the action of polymerase and ligase, using the intact daughter strand as a template. Two intact DNA molecules are thus formed and the remaining thymine dimer eventually can be removed by excision repair.

In error-prone repair, also called as SOS-repair system, a gap opposite gap site of DNA damage is filled by newly synthesised DNA. Since the new strand is synthesized from a damaged template, it is very inaccurate and leads to frequent mutations, and thus, referred to as error-prone. It repairs UV induced extensive damage and other potentially lethal conditions. This type of repair is not dealt by Uvr ABC system, but by the activity of Rec a proteins, which also participate in homologous recombination. The SOS response includes inhibition of cell division and induction of repair systems to cope with a high level of DNA damage.

The Error-Prone (SOS) Repair Mechanism

The error-prone repair mechanism involves DNA pol. III and 2 other gene products encoded by *umuCD*.

The UmuCD proteins are produced in times of dire emergency and instruct DNA pol. III to insert any bases opposite the tymine dimers, as the DNA damage would otherwise be lethal.

The risk of several mutations is worth the risk as measured against threat of death.

- SOS repair is error-prone. This is why UV is a mutagen. May be due to RecA binding ssDNA in lesions, which could then bind to DNA Pol III complex passing through this area of the DNA and inhibit 3'>5' exonuclease (proofreading) ability. This makes replication faster but also results in more mutations.
- This affect on proofreading seems to involve UmuD'-UmuC complex as well. RecA facilitates proteolytic cleavage of UmuD to form UmuD'. The UmuD'-UmuC complex may bind to the RecA-Pol III complex and promote error-prone replication.