DNA Repair

Types of repair: Direct repair; Excision repair: Base excision repair, Nucleotide excision repair in E. coli and eukaryotes; Mismatch repair: single and double-stranded- break repair;

Bypassing DNA damage during genome replication,

Non-homologous end-joining in humans,

SOS response in E. coli;

Defects in DNA repair causes human disease including cancer.

Mitesh Shrestha

Sources of DNA Damage

- Natural polymerase error
- Endogenous DNA damage
  - oxidative damage
  - Depurination
- Exogenous DNA damage
  - ultraviolet [UV 200-400 nm] radiation from the sun
  - other radiation frequencies, including x-rays and gamma rays
  - hydrolysis or thermal disruption
  - certain plant toxins
  - human-made mutagenic chemicals, especially aromatic compounds that act as DNA intercalating agents
  - viruses
- “Error-prone” DNA repair

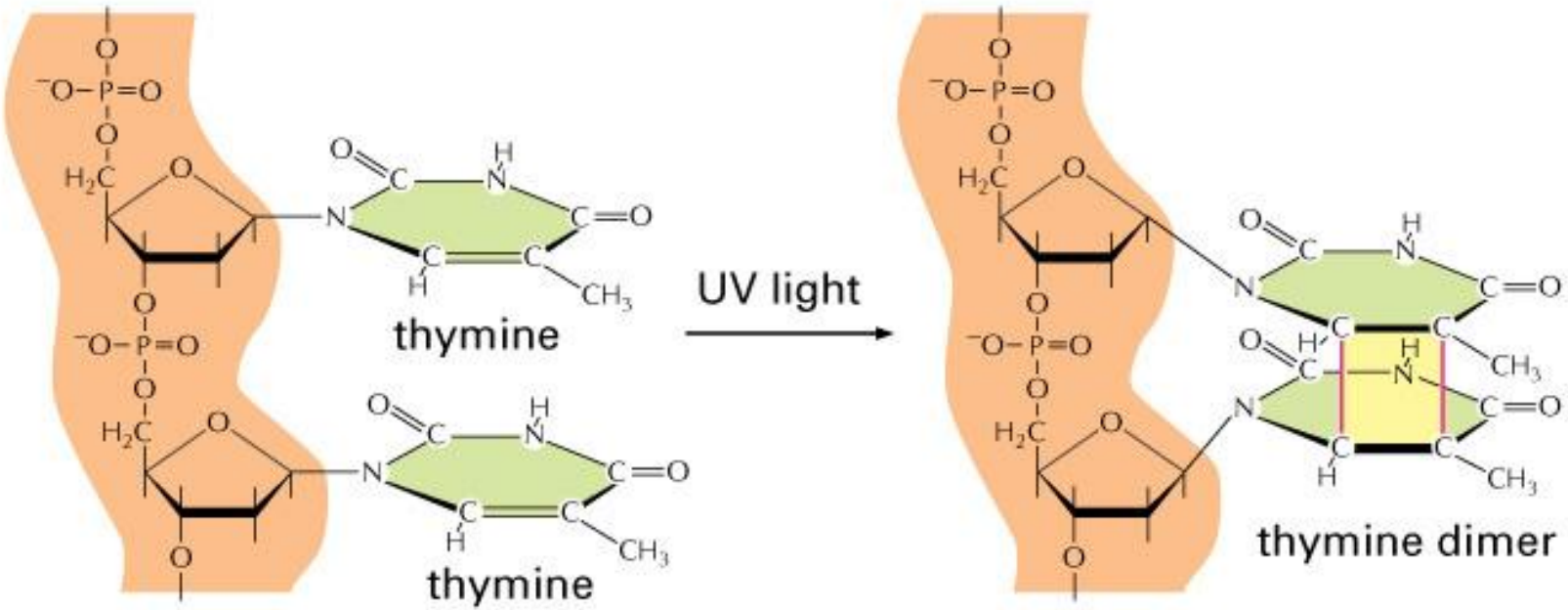
Types of repair

- Direct repair
- Excision repair:
  - Base excision repair,
  - Nucleotide excision repair in E. coli and eukaryotes;
  - Mismatch repair
- Post replication Repair
- Single and double-stranded- break repair

Direct repair

– Only a few types of DNA damage are repaired in this way, particularly pyrimidine dimers resulting from exposure to ultraviolet (UV) light and alkylated guanine residues that have been modified by the addition of methyl or ethyl groups at the O^6 position of the purine ring.

Random photons of ultraviolet (UV) light induce aberrant bonding between neighboring pyrimidines (thymine & cytosine) bases on the same strand of DNA. This will prevent the replication machine from duplicating the DNA. The cell will die!



This type of defect can be readily reversed by a process called photoreactivation. Visible light energy is used to reverse the defect (in bacteria, yeasts, protists, some plants, and some animals but NOT in humans)

Figure 6-24 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Direct repair for pyrimidine dimers

- UV light is one of the major sources of damage to DNA and is also the most thoroughly studied form of DNA damage in terms of repair mechanisms.

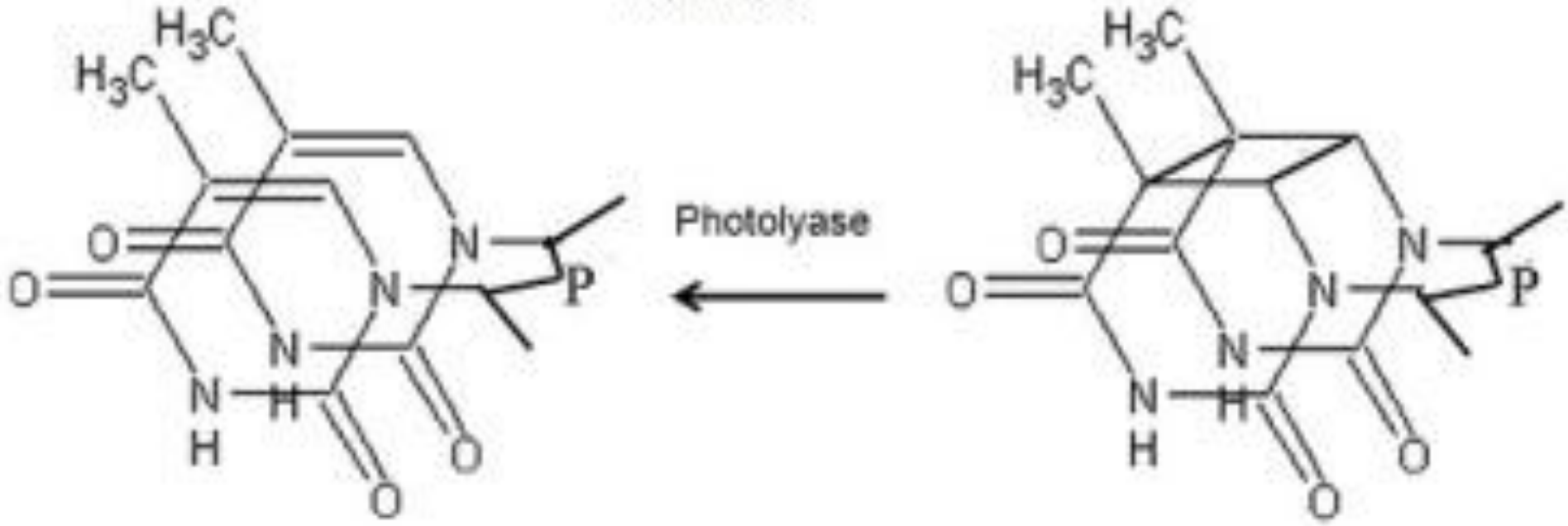
- Its importance is illustrated by the fact that exposure to solar UV irradiation is the cause of almost all skin cancer in humans. The major type of damage induced by UV light is the formation of pyrimidine dimers, in which adjacent pyrimidines on the same strand of DNA are joined by the formation of a cyclobutane ring resulting from saturation of the double bonds between carbons 5 and 6.

- The formation of such dimers distorts the structure of the DNA chain and blocks transcription or replication past the site of damage, so their repair is closely correlated with the ability of cells to survive UV irradiation.

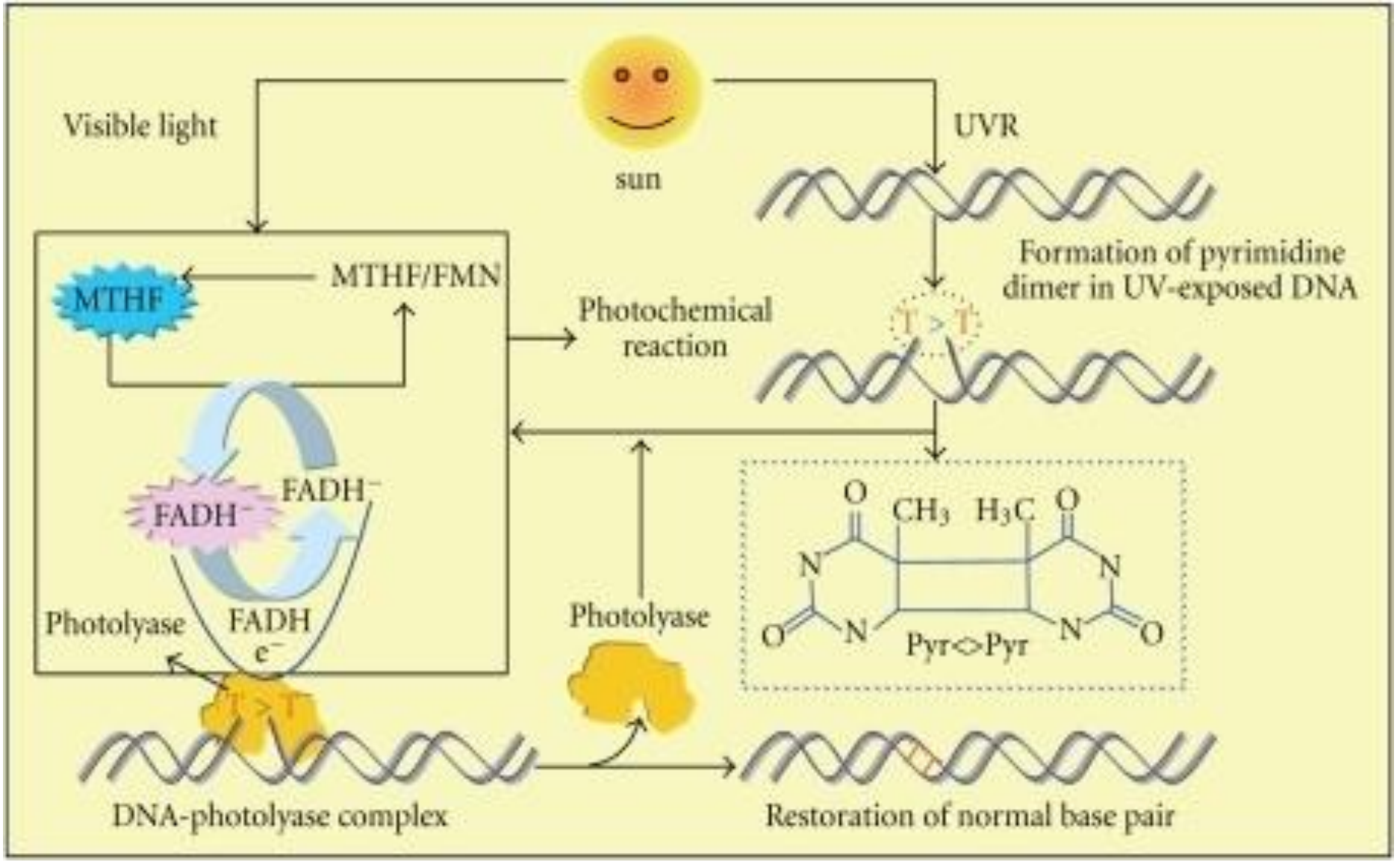
- One mechanism of repairing UV-induced pyrimidine dimers is direct reversal of the dimerization reaction. The process is called **Photoreactivation** because energy derived from visible light is utilized to break the cyclobutane ring structure. The original pyrimidine bases remain in DNA, now restored to their normal state.

Direct Reversal

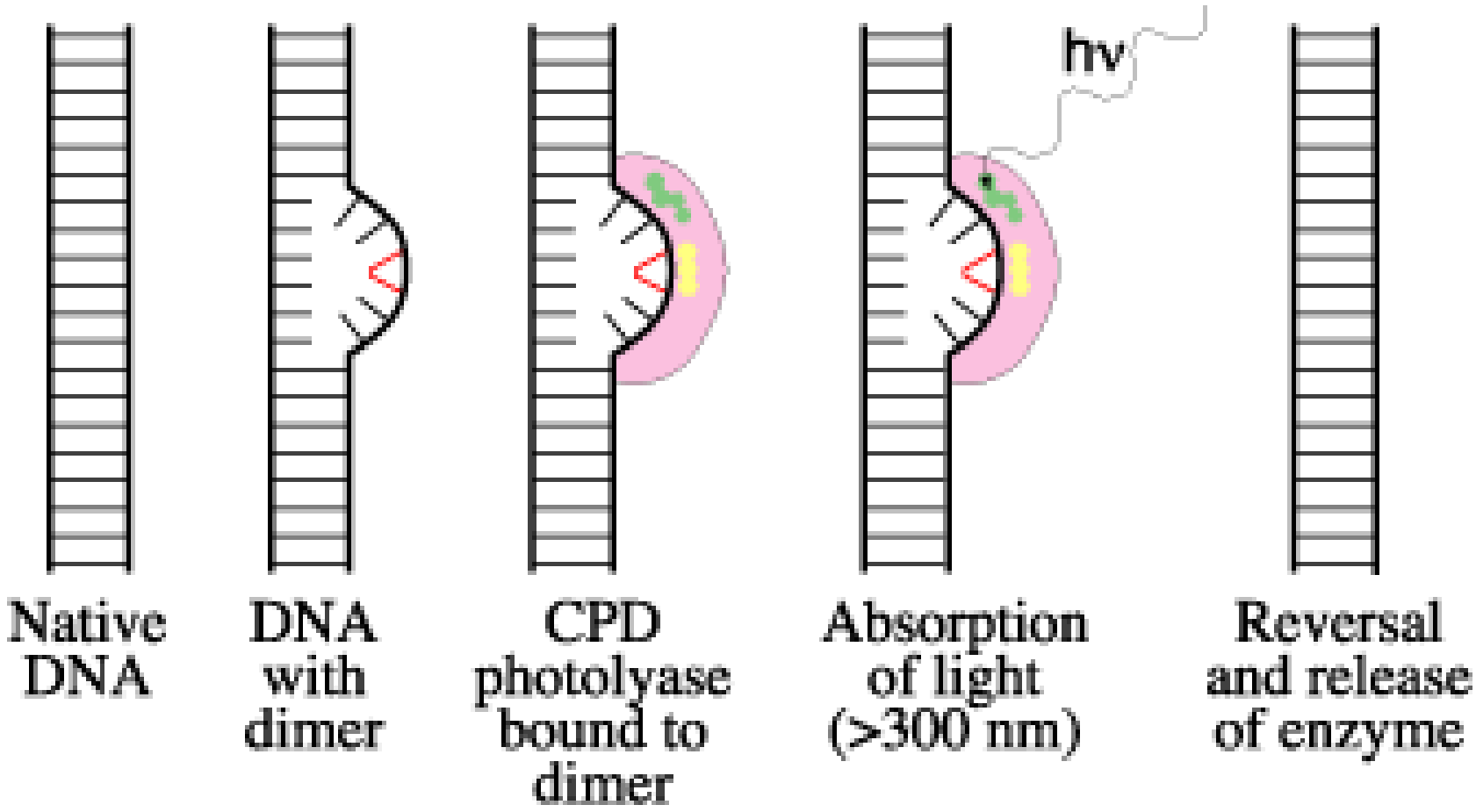
- **Photolyase** uses light energy to restore the original pyrimidines
- All the original atoms are still present – that's direct reversal
- Found in lots of organisms, but not placental mammals



Direct repair for pyrimidine dimers



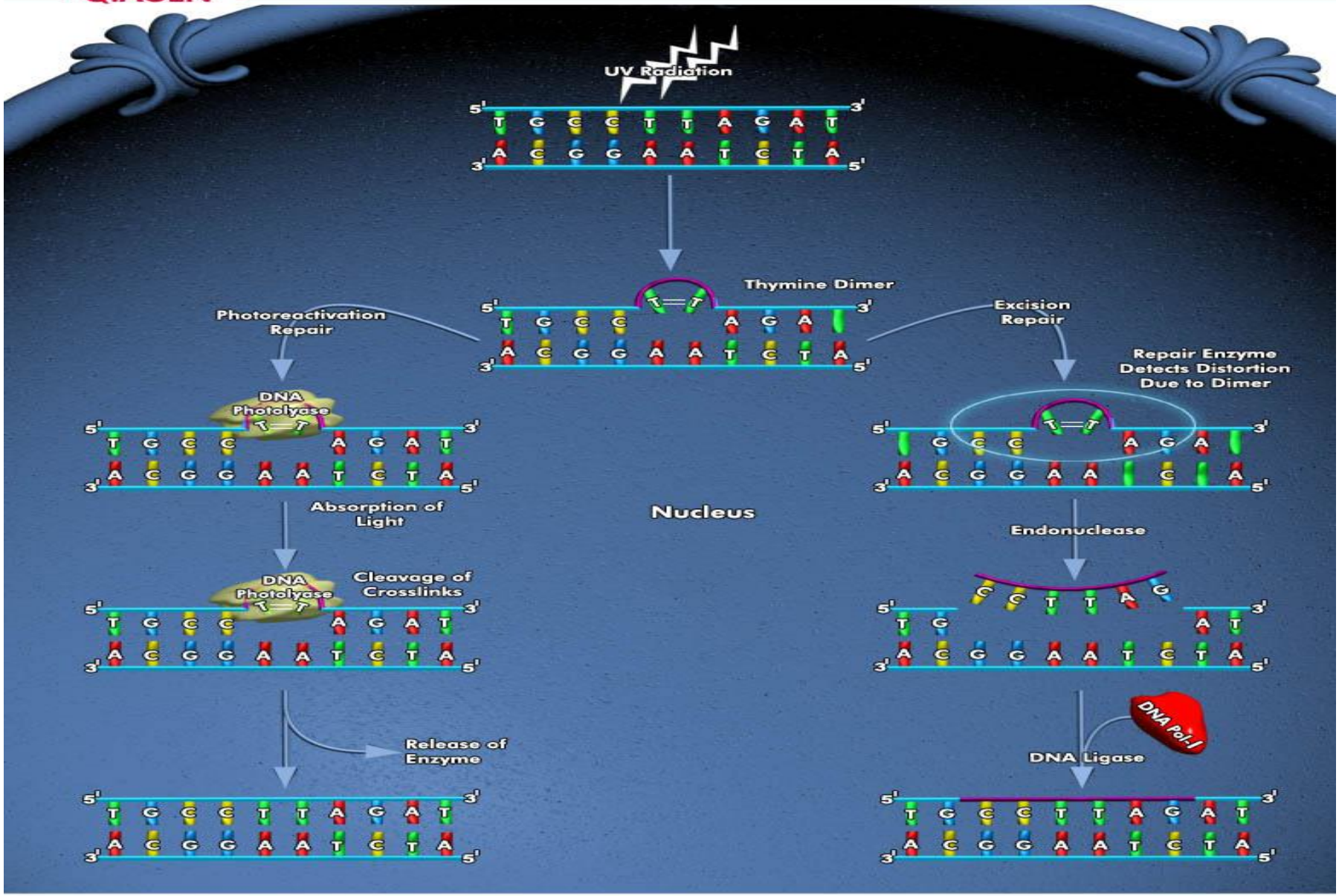
Direct repair for pyrimidine dimers



Direct repair for pyrimidine dimers

- The repair of pyrimidine dimers by photoreactivation is common to a variety of prokaryotic and eukaryotic cells, including E. coli, yeasts, and some species of plants and animals. Curiously, however, **photoreactivation is not universal; many species (including humans) lack this mechanism of DNA repair.**

Repair of Thymine Dimers



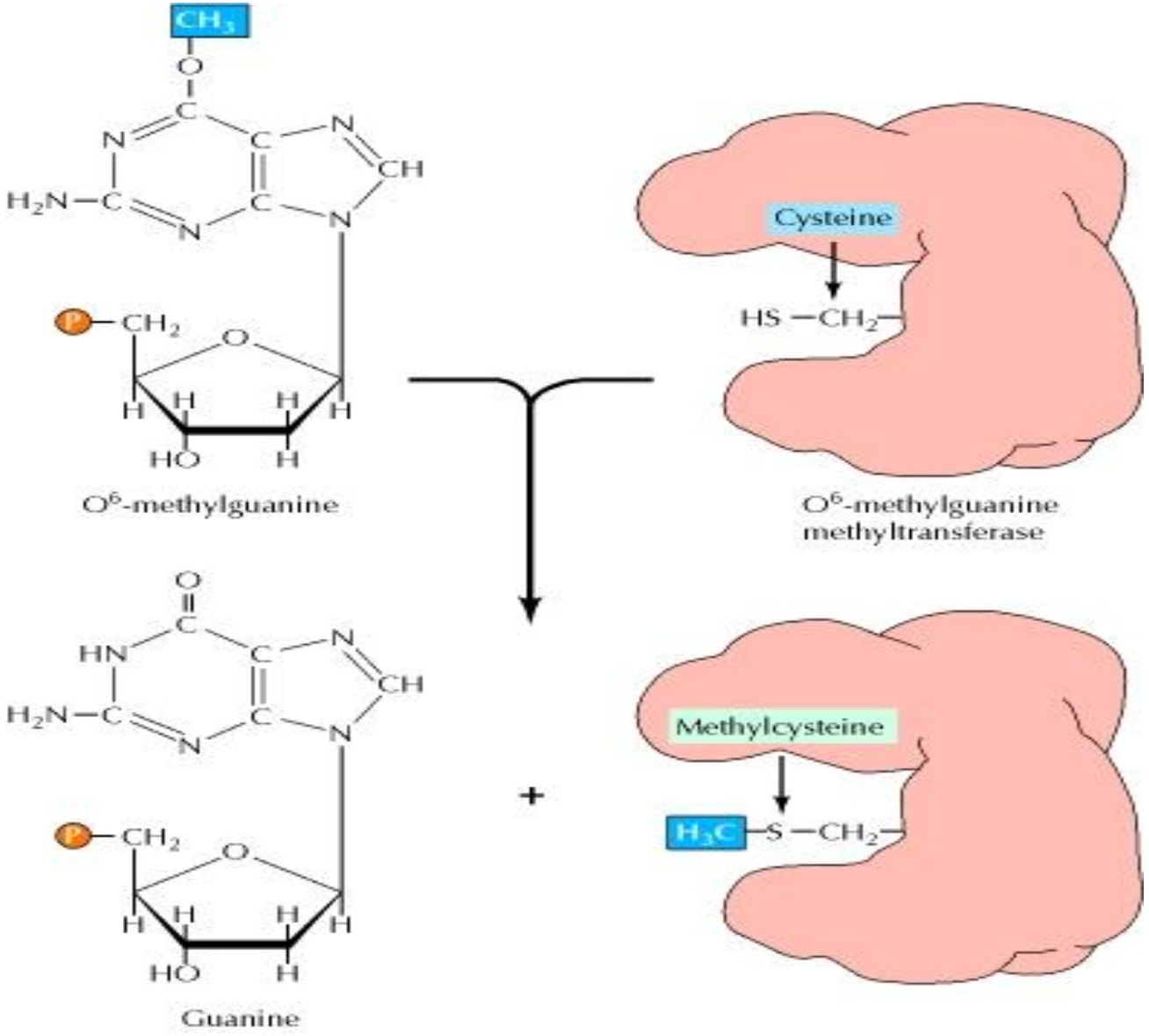
Direct repair for alkylated guanine

- Alkylating agents are reactive compounds that can transfer methyl or ethyl groups to a DNA base, thereby chemically modifying the base.

- A particularly important type of damage is methylation of the O^6 position of guanine, because the product, O^6-methylguanine, forms complementary base pairs with thymine instead of cytosine. This lesion can be repaired by an enzyme (called O^6-methylguanine methyltransferase) that transfers the methyl group from O^6-methylguanine to a cysteine residue in its active site.

- Enzymes that catalyze this direct repair reaction are widespread in both prokaryotes and eukaryotes, including humans.

Direct repair for alkylated guanine



Excision repair

- A **DNA repair endonuclease or endonuclease**-containing complex recognizes, binds to, and excised the damaged base or bases.

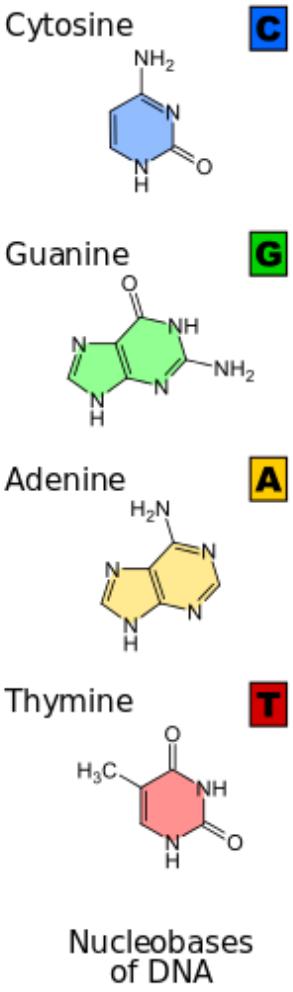
- A **DNA Polymerase** fills in the gap, using the undamaged complementary strand of DNA as a template.

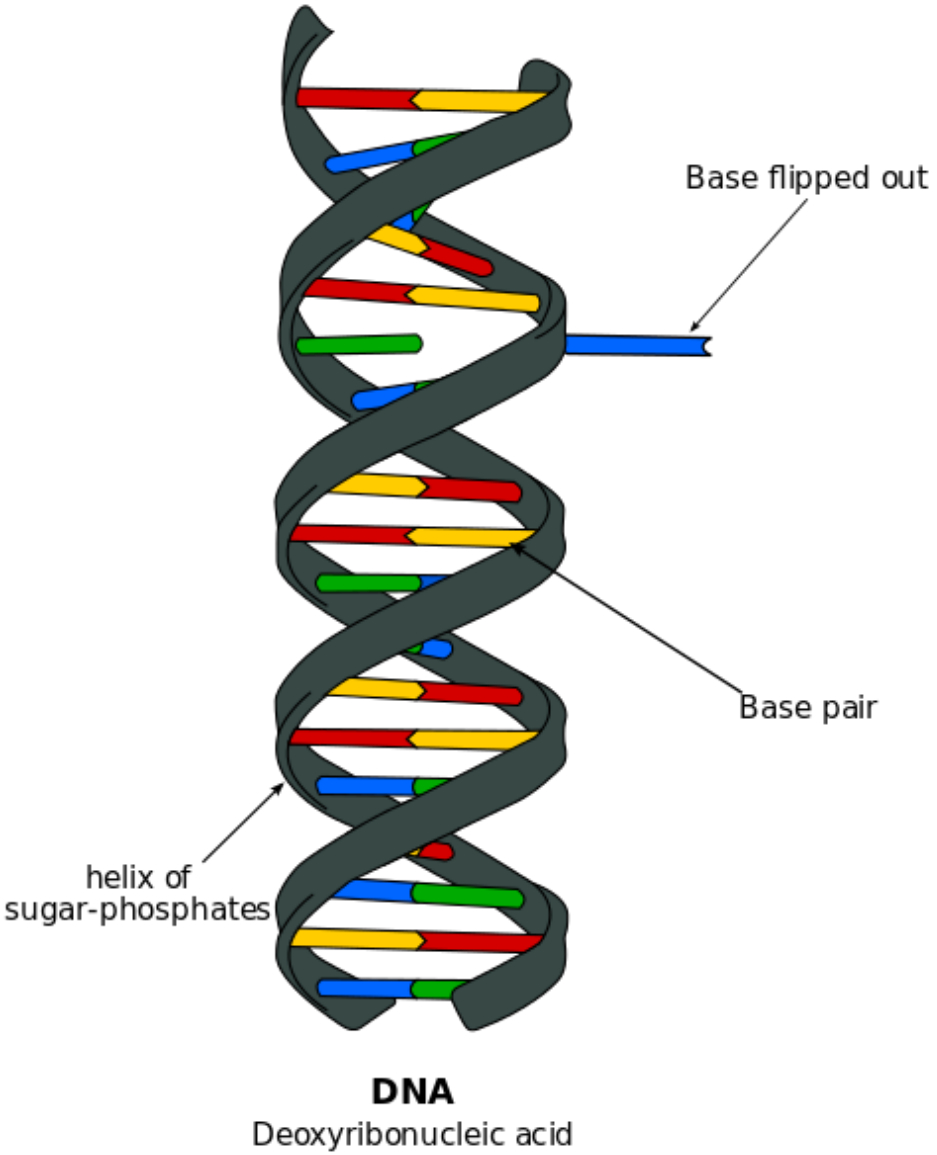
- **DNA ligase** seals the break left by DNA polymerase.

Base excision repair (BER)

- Major pathway for repair of modified bases, uracil misincorporation, oxidative damage
- Various DNA glycosylases recognize lesion and remove base at glycosidic bond, thereby producing an “abasic” or AP (apurinic/ apyrimidinic) site by base “**flipping out**”
- An AP endonuclease cleaves the phosphodiester backbone near the AP site.
- DNA polymerase I initiates repair synthesis from the free 3’ OH at the nick, removing a portion of the damaged strand (with its 5’ - 3’ exonuclease activity) and replacing it with undamaged DNA.
- The nick remaining after DNA polymerase I has dissociated is sealed by DNA ligase.

Flipping out





Base excision repair

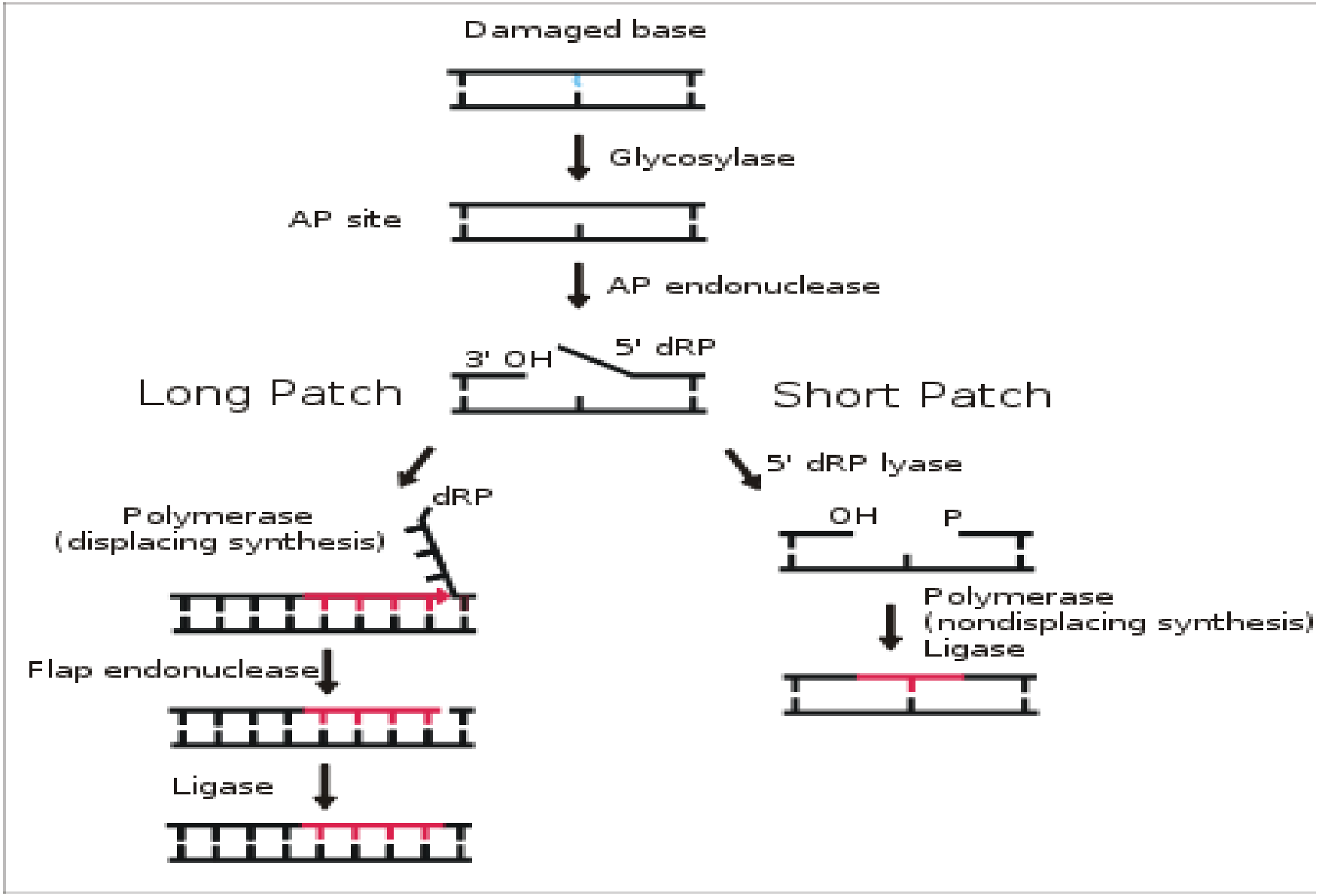
- Repair of uracil-containing DNA is a good example of base-excision repair, in which single damaged bases are recognized and removed from the DNA molecule.

- The excision of uracil in DNA is catalyzed by DNA glycosylase, an enzyme that cleaves the bond linking the base (uracil) to the deoxyribose of the DNA backbone. This reaction yields free uracil and an apyrimidinic site—a sugar with no base attached.

- DNA glycosylases also recognize and remove other abnormal bases, including hypoxanthine formed by the deamination of adenine, pyrimidine dimers, alkylated purines other than O^6-alkylguanine, and bases damaged by oxidation or ionizing radiation.

- Examples of bases cleaved by DNA glycosylases: Uracil (deamination of C); 8-oxoG paired with C (oxidation of G); Adenine across from 8-oxoG (misincorporation) ; Thymine across from G (5-meC deamination); Alkyl-adenine (3-meA, 7-meG, hypoxanthine)

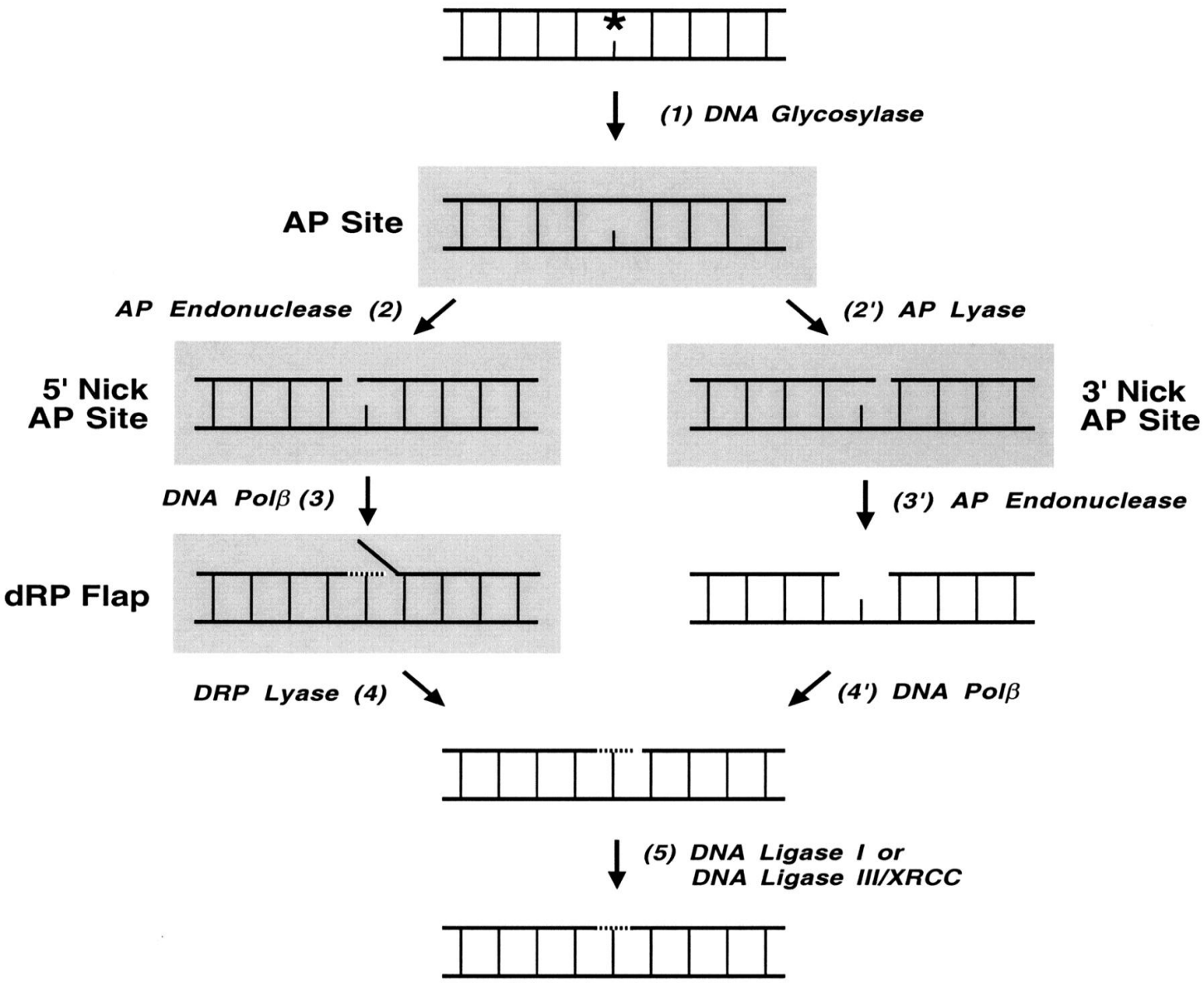
Base excision repair



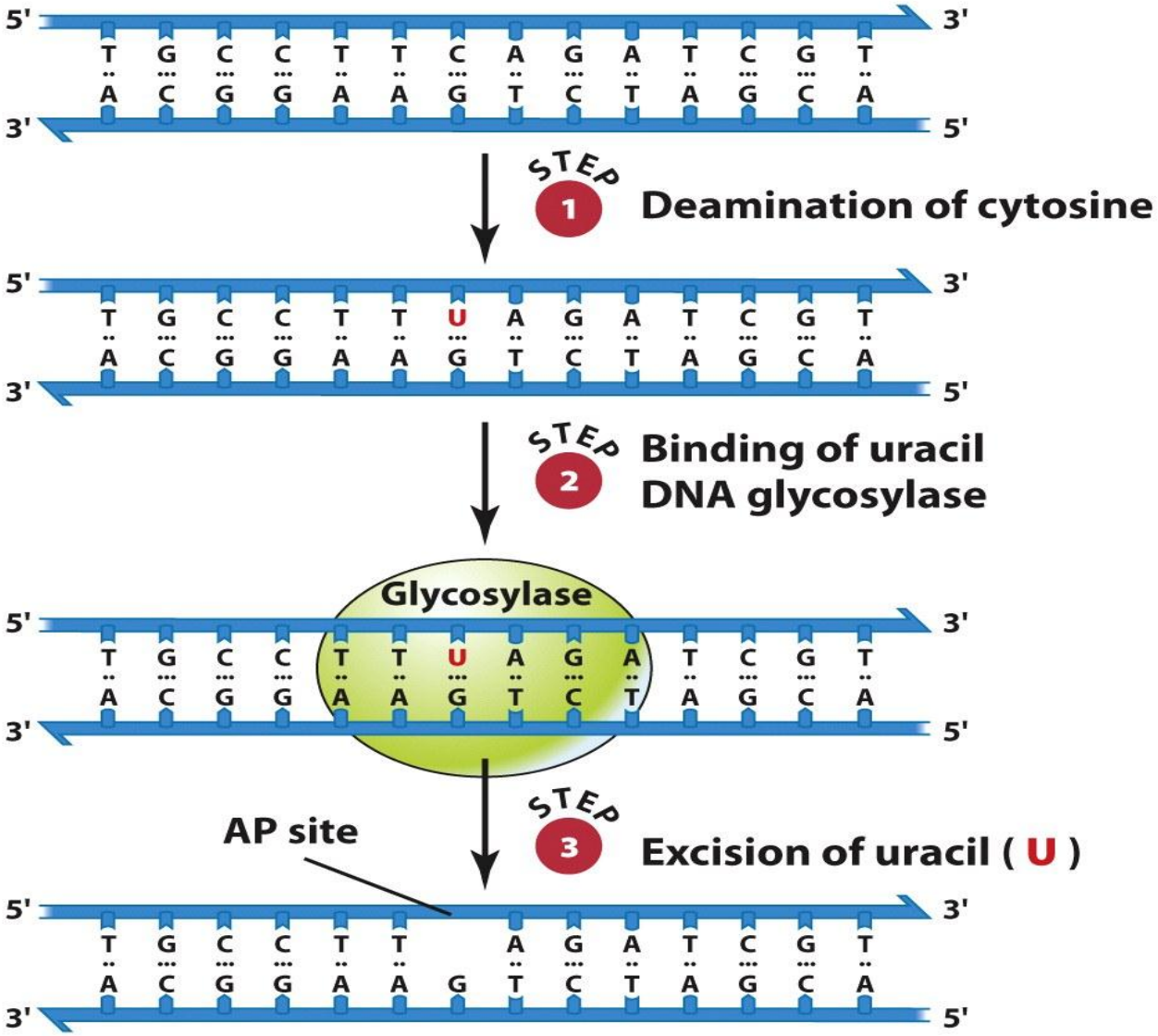
Types of lesions repaired by BER

- Oxidative lesions; 8-oxo-G, highly mutagenic, mispairs with A, producing GC --> TA transversions example MutY, MutM=Fpg from E. coli
- Deoxyuracil: from misincorporation of dU or deamination of dC-->dU, example Ung, uracil N-glycosylase
- Various alkylation products e. g. 3-meA
- These lesions are not distorting and do not block DNA polymerases
- Spontaneous depurination (esp. G) yield abasic sites that are repaired by second half of BER pathway

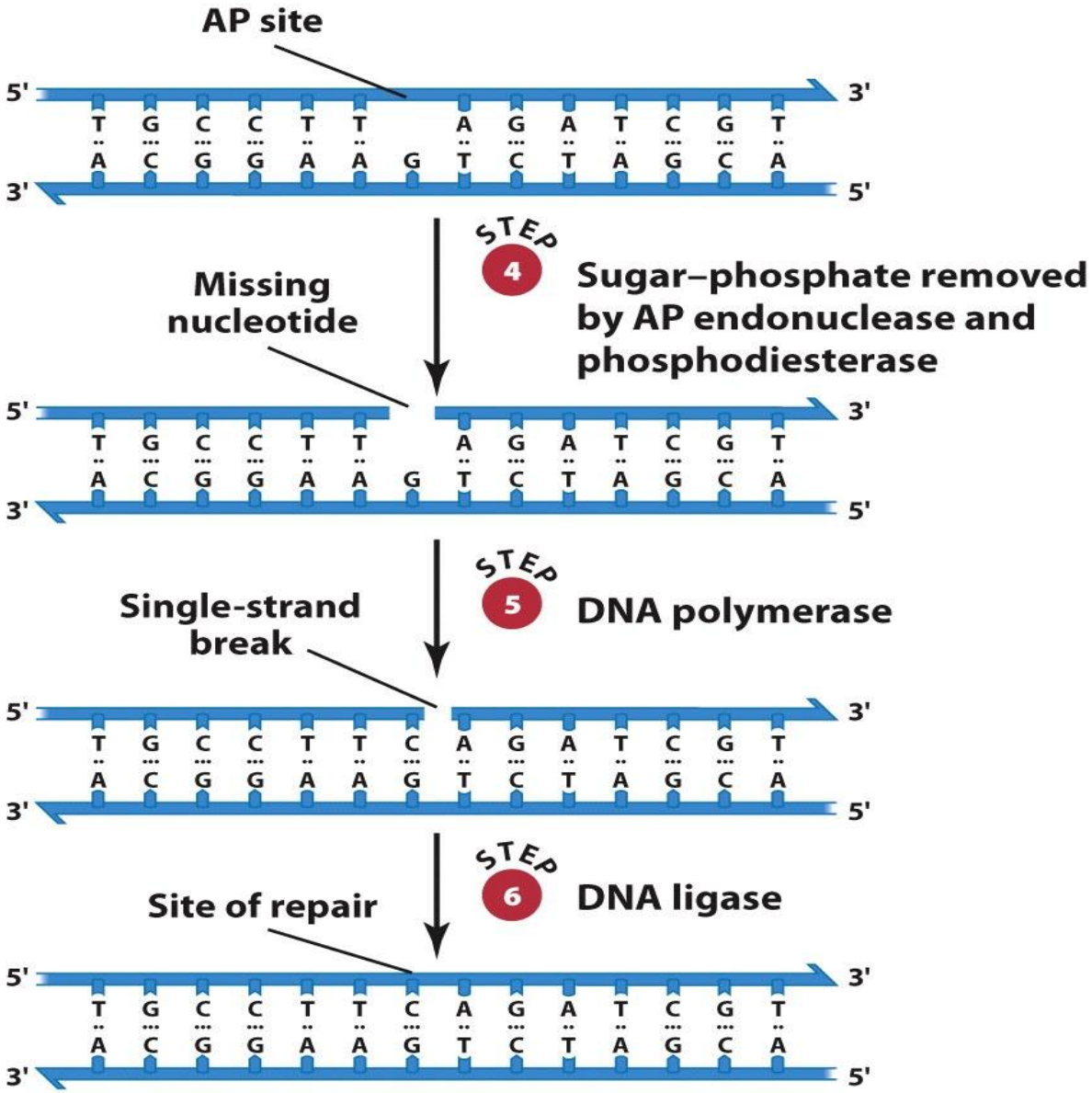
Mechanism of BER



Mechanism of BER



Mechanism of BER



Nucleotide Excision Repair

- Nucleotide excision repair (NER) is a particularly important excision mechanism that removes **DNA damage induced by ultraviolet light (UV).**

- UV DNA damage results in bulky DNA adducts - these adducts are mostly thymine dimers and 6,4-photoproducts. Recognition of the damage leads to removal of a short single-stranded DNA segment that contains the lesion. The undamaged single-stranded DNA remains and DNA polymerase uses it as a template to synthesize a short complementary sequence. Final ligation to complete NER and form a double stranded DNA is carried out by DNA ligase.

- NER can be divided into two sub pathways:
  - Global Genomic NER (GG-NER or GGR) and
  - Transcription Coupled NER (TC-NER or TCR).

- The two sub pathways differ in how they recognize DNA damage but they share the same process for lesion incision, repair, and ligation.

Nucleotide Excision Repair

Global Genomic NER (GG-NER or GGR)

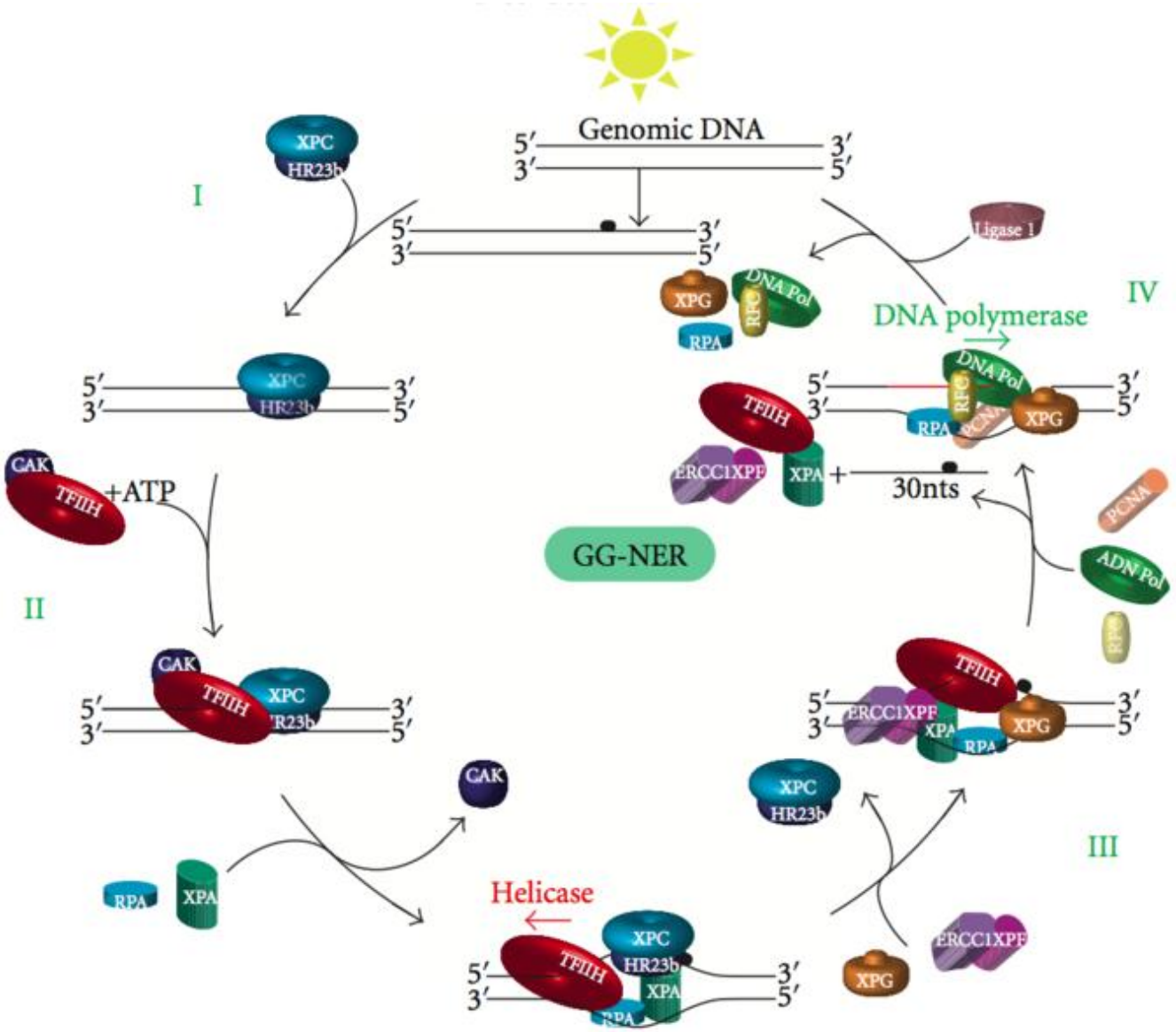
- Global genomic NER **repairs damage in both transcribed and untranscribed** DNA strands in active and inactive genes throughout the genome. - this process is **not dependent on transcription**.

- This pathway **employs several "damage sensing" proteins** including the DNA-damage binding (DDB) and XPC-Rad23B complexes that constantly scan the genome and recognize helix distortions: the XPC-Rad23B complex is responsible for distortion recognition, while DDB1 and DDB2 (XPE) can also recognize some types of damage caused by UV light.

- Upon identification of a damaged site, subsequent repair proteins are then recruited to the damaged DNA to verify presence of DNA damage, excise the damaged DNA surrounding the lesion then fill in the repair patch.

- Mutations in GG-NER machinery are responsible for multiple genetic disorders including:
  - **Xeroderma pigmentosum (XP)**: severe photosensitivity, high cancer rates in areas of the body exposed to the sun (e.g. skin)

Global Genomic NER (GG-NER or GGR)

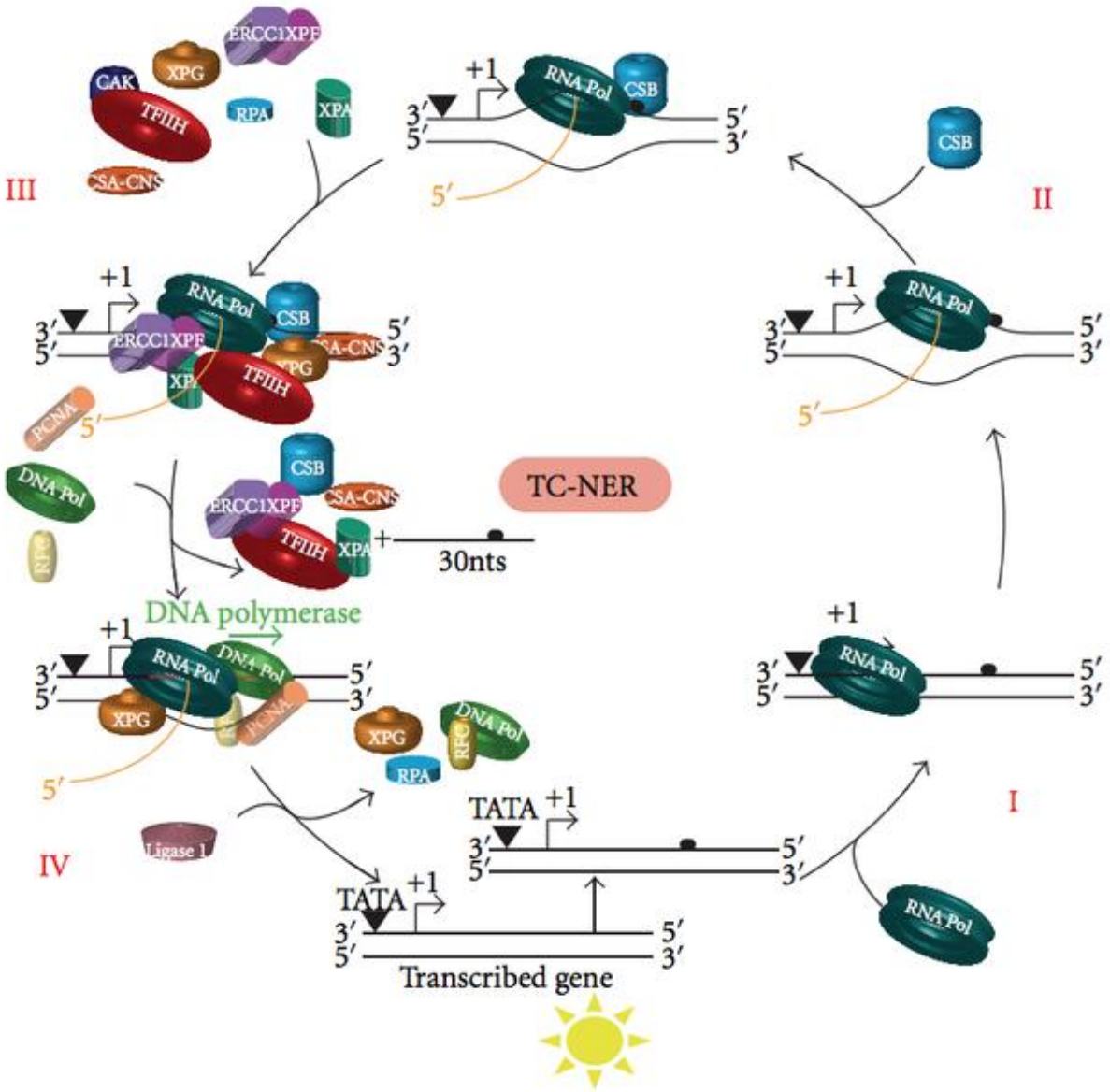


Transcription Coupled NER (TC-NER or TCR)

- At any given time, most of the genome in an organism is not undergoing transcription; there is a difference in NER efficiency between transcriptionally silent and transcriptionally active regions of the genome. For many types of lesions, NER repairs the transcribed strands of transcriptionally active genes faster than it repairs nontranscribed strands and transcriptionally silent DNA.

- TC-NER initiates when RNA polymerase stalls at a lesion in DNA: the blocked **RNA polymerase serves as a damage recognition signal**, which replaces the need for the distortion recognition properties of the XPC-RAD23B and DDB complexes. **CS proteins (CSA and CSB) bind some types of DNA damage instead of XPC-Rad23B.**
- Mutations in TC-NER machinery are responsible for multiple genetic disorders including:
  - **Trichothiodystrophy (TTD):** some individuals are photosensitive, ichthyosis, mental/physical retardation
  - **Cockayne syndrome (CS):** photosensitivity, mental retardation, progeria-like features, microcephaly

Transcription Coupled NER (TC-NER or TCR)



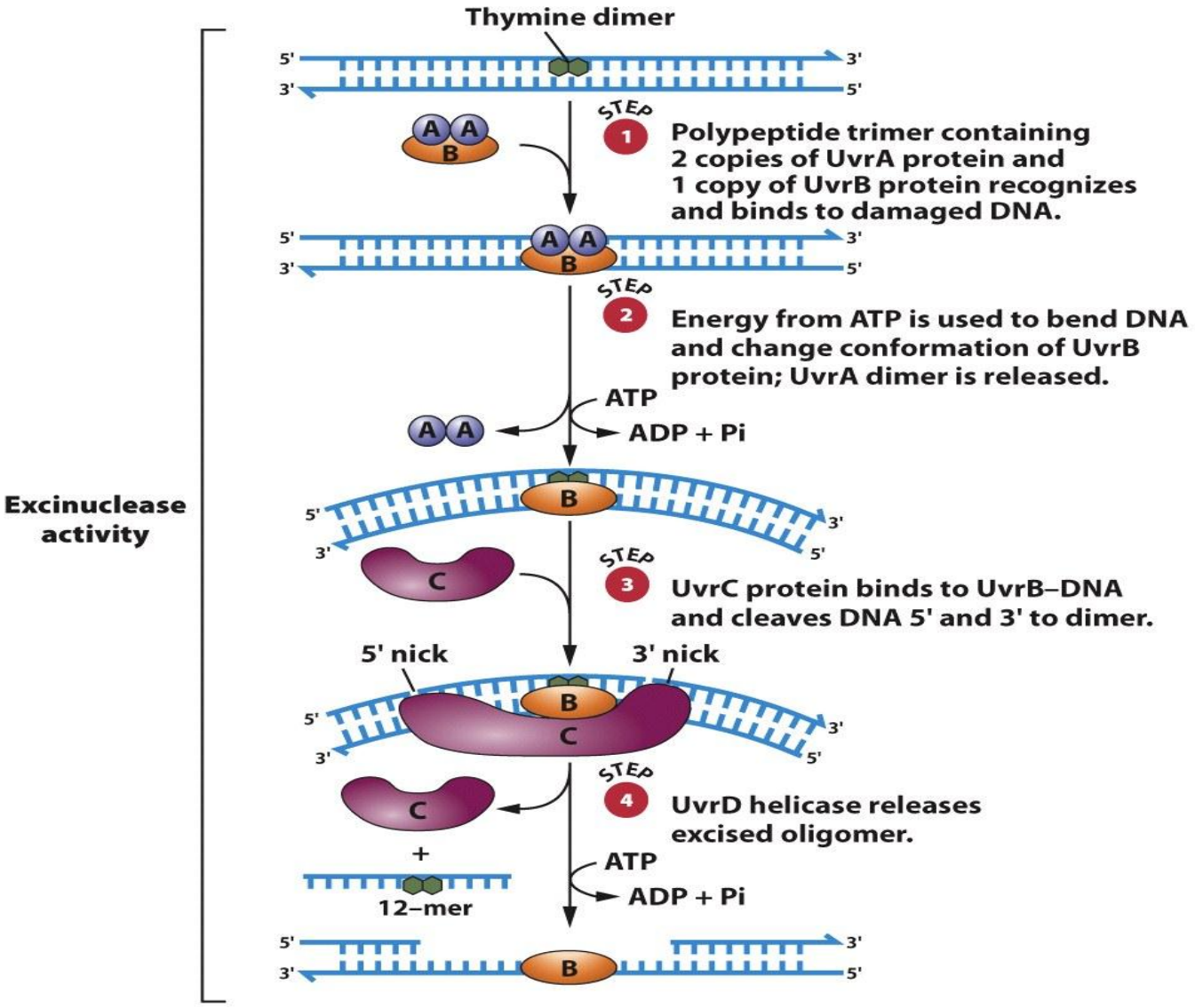
Nucleotide Excision Repair in *E. coli*

- In E. coli, nucleotide-excision repair is catalyzed by the products of three genes (uvrA, B, and C) that were identified because mutations at these loci result in extreme sensitivity to UV light.

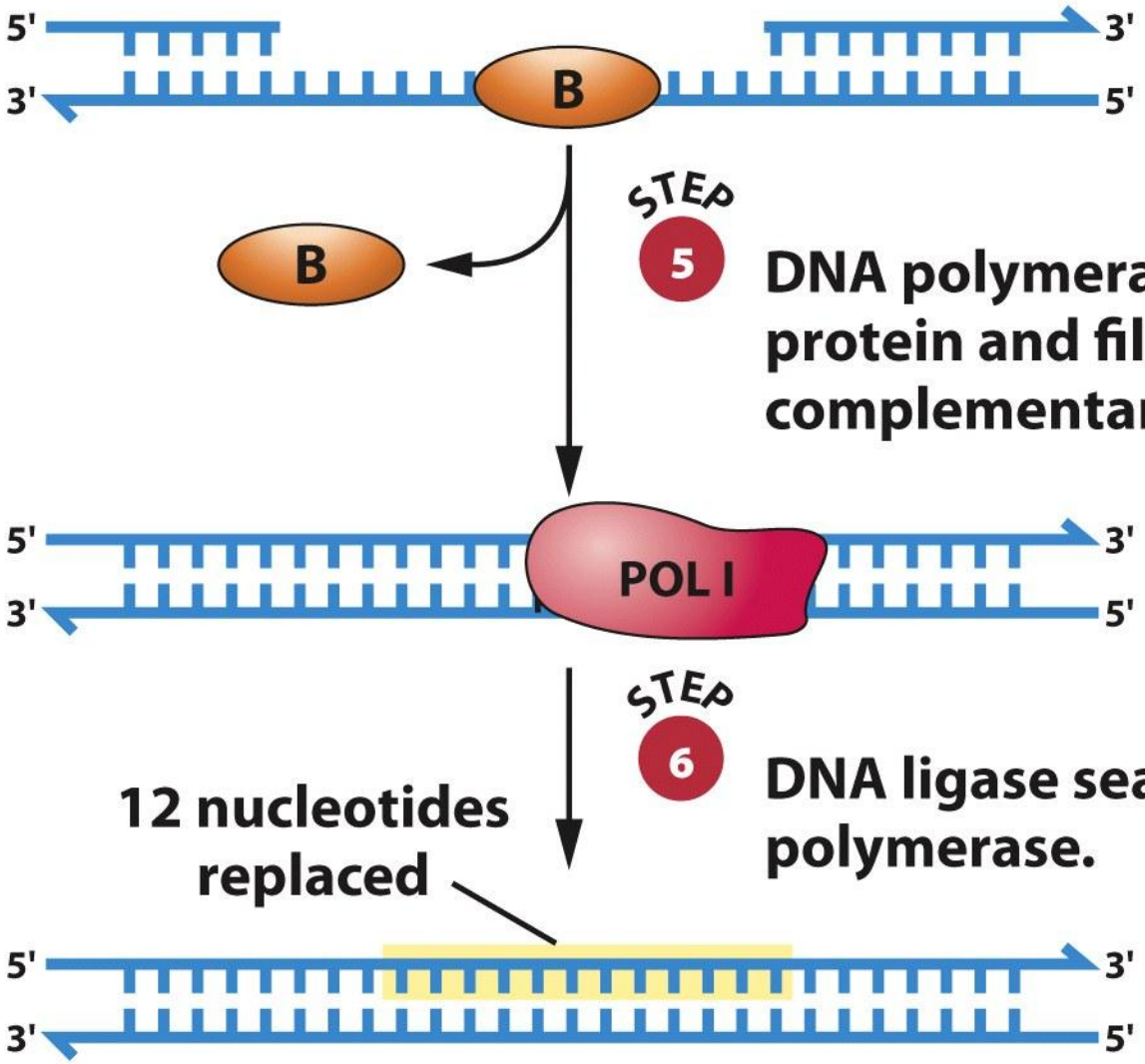
- The protein UvrA recognizes damaged DNA and recruits UvrB and UvrC to the site of the lesion. UvrB and UvrC then cleave on the 3′ and 5′ sides of the damaged site, respectively, thus excising an oligonucleotide consisting of 12 or 13 bases.

- The UvrABC complex is frequently called an **excinuclease**, a name that reflects 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, and the resulting gap is filled by DNA polymerase I and sealed by ligase.

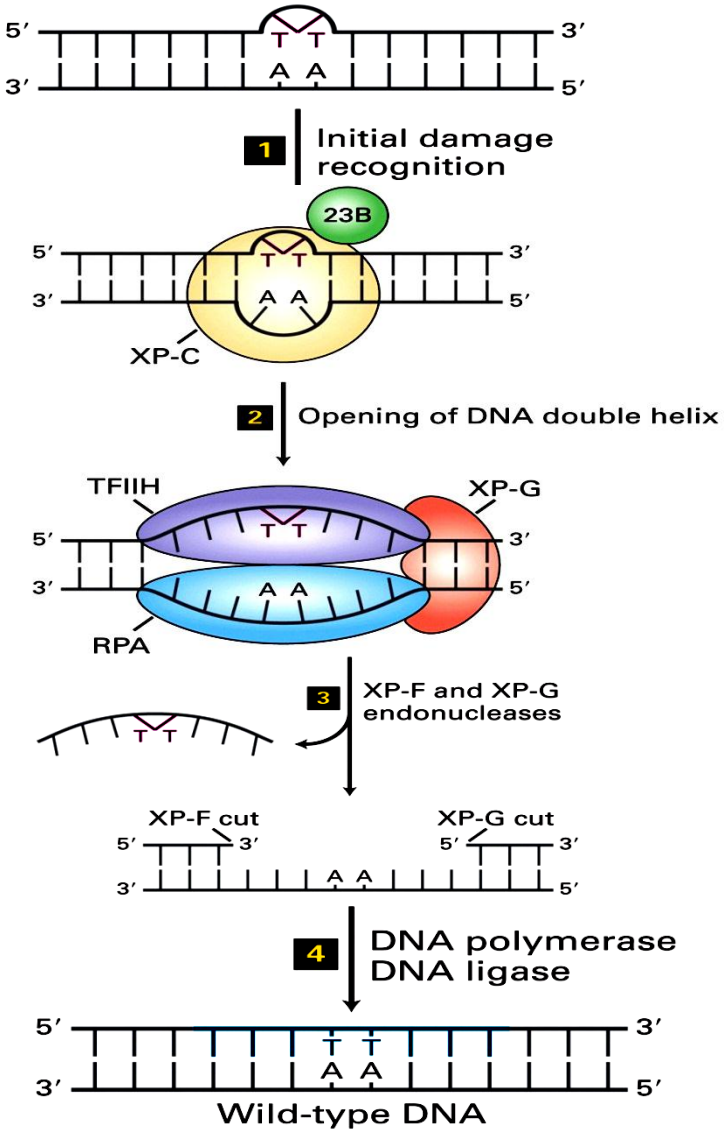
Nucleotide Excision Repair in *E. coli*



Nucleotide Excision Repair in *E. coli*



Nucleotide Excision Repair in Eukaryotes



Mutations in any of at least seven genes, *XP-A* through *XP-G*, cause an inherited sensitivity to UV-induced skin cancer called xeroderma pigmentosum. The XP proteins are among >30 required for nucleotide excision repair.

Mismatch repair (MMR)

- Despite extraordinary fidelity of DNA synthesis, errors do persist
- Such errors can be detected and repaired by the post-replication mismatch repair system
- Prokaryotes and eukaryotes use a similar mechanism with common structural features
- Defects in MMR elevate spontaneous mutation rates 10-1000x
- Defects in MMR underlie human predisposition to colon and other cancers (“HNPCC”)
- MMR also processes mispairs that result from heteroduplex DNA formed during genetic recombination: act to exclude “homologous” recombination

Basis of MMR recognition

- MutS dimer (in yeast, Msh2/Msh3 or Msh2/Msh6 heterodimer)
- By DNA binding expts in vitro and DNA heteroduplex repair expts in vivo: MMR can recognize all base substitutions except C:C and short frameshift loops <4 bp
- Transition mispairs G:T and A:C and one base loops are particularly well-recognized (these are also the most common polymerase errors)

Mismatch repair (MMR)

- In E. coli, the ability of the mismatch repair system to distinguish between parental DNA and newly synthesized DNA is based on the fact that DNA of this bacterium is modified by the methylation of adenine residues within the sequence GATC to form 6-methyladenine.
- Since methylation occurs after replication, newly synthesized DNA strands are not methylated and thus can be specifically recognized by the mismatch repair enzymes.
- Mismatch repair is initiated by the protein MutS, which recognizes the mismatch and forms a complex with two other proteins called MutL and MutH. The MutH endonuclease then cleaves the unmethylated DNA strand at a GATC sequence. 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 polymerase and ligase.

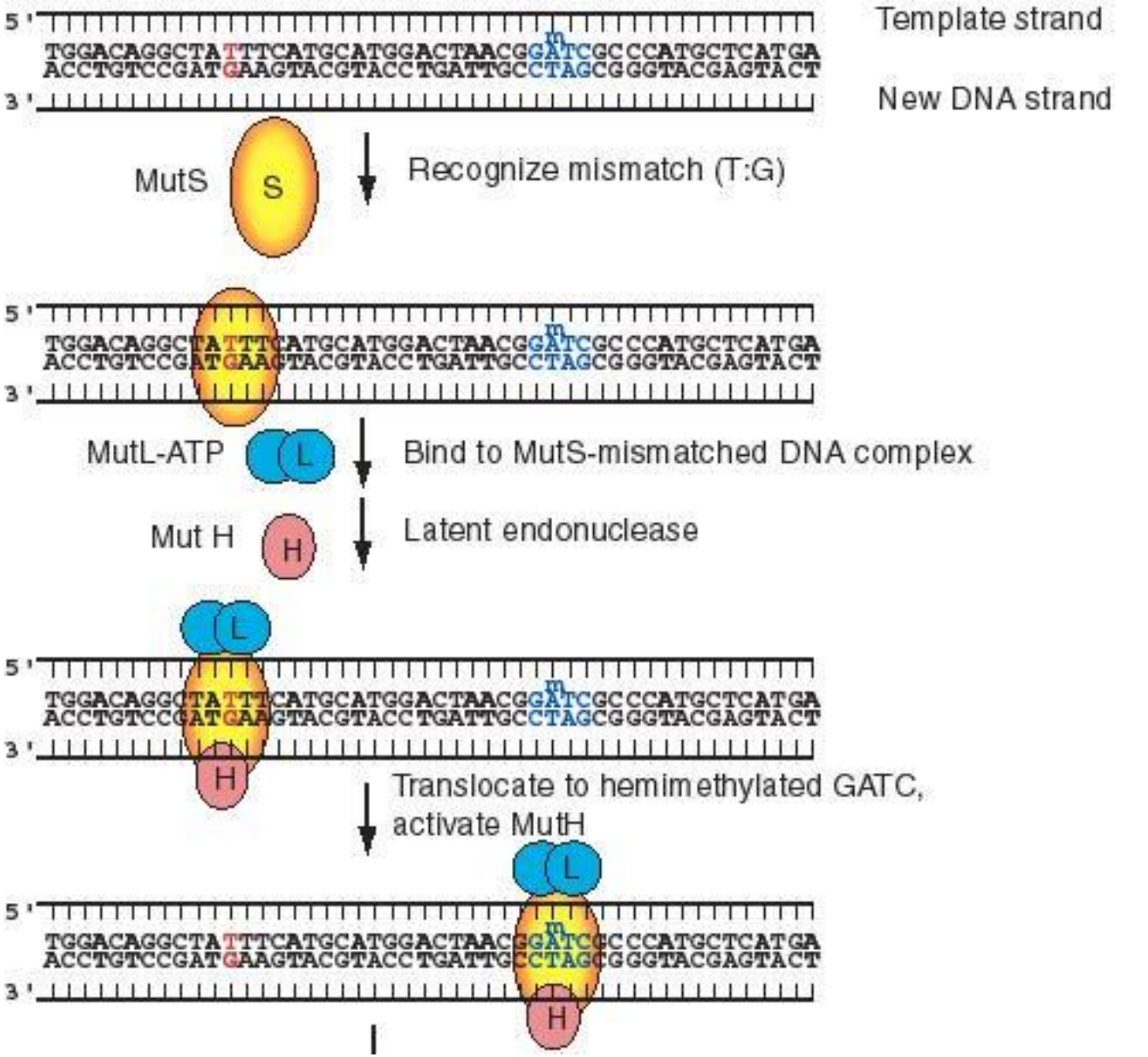
Role of methylation in discriminating parental and progeny strands

- *dam* methylase acts on the A of GATC (note that this sequence is symmetical or pseudopalindromic).

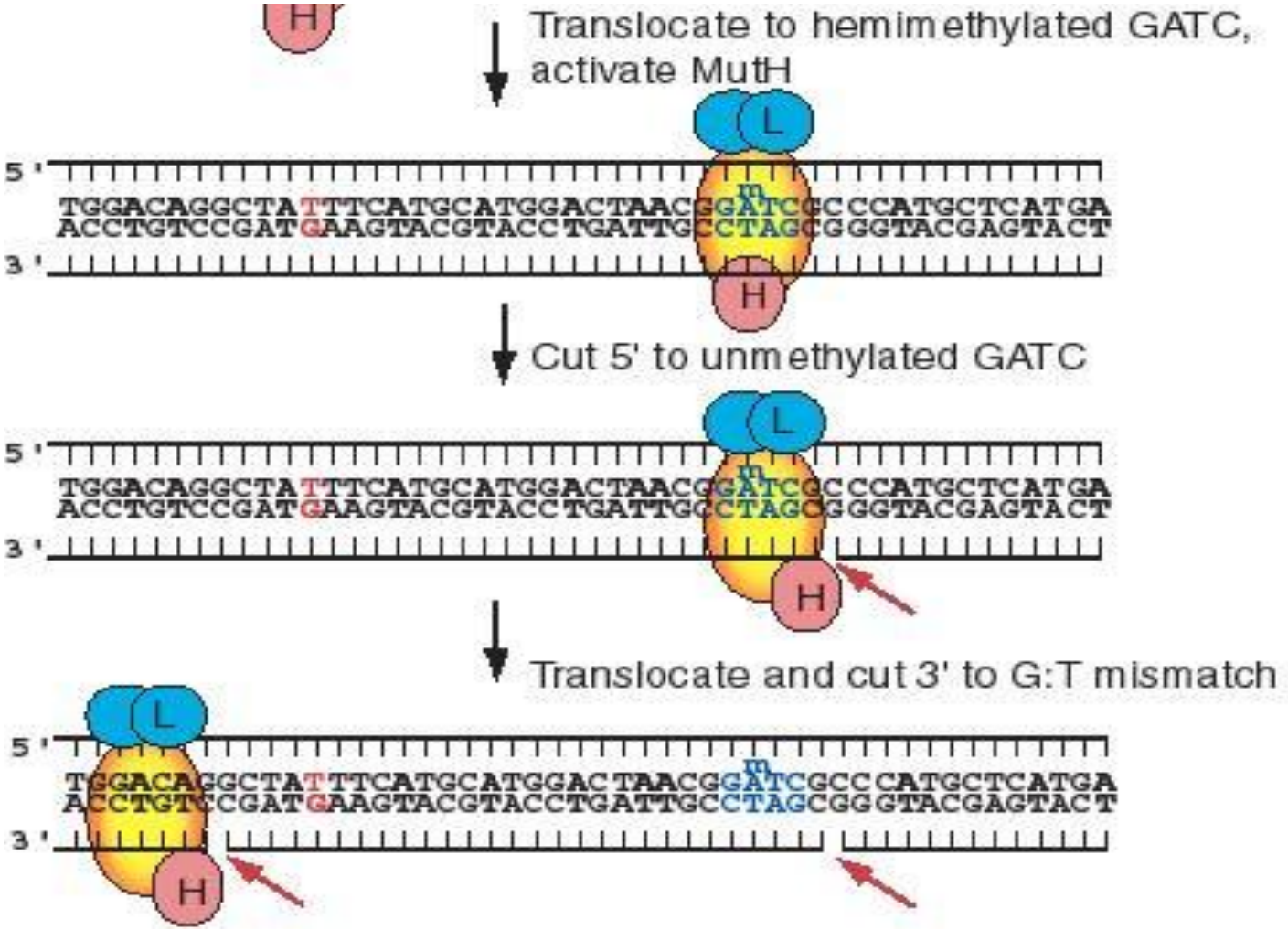
- Methylation is delayed for several minutes after replication.

- Mismatch repair works on the un-methylated strand (which is newly replicated) so that replication errors are removed preferentially.

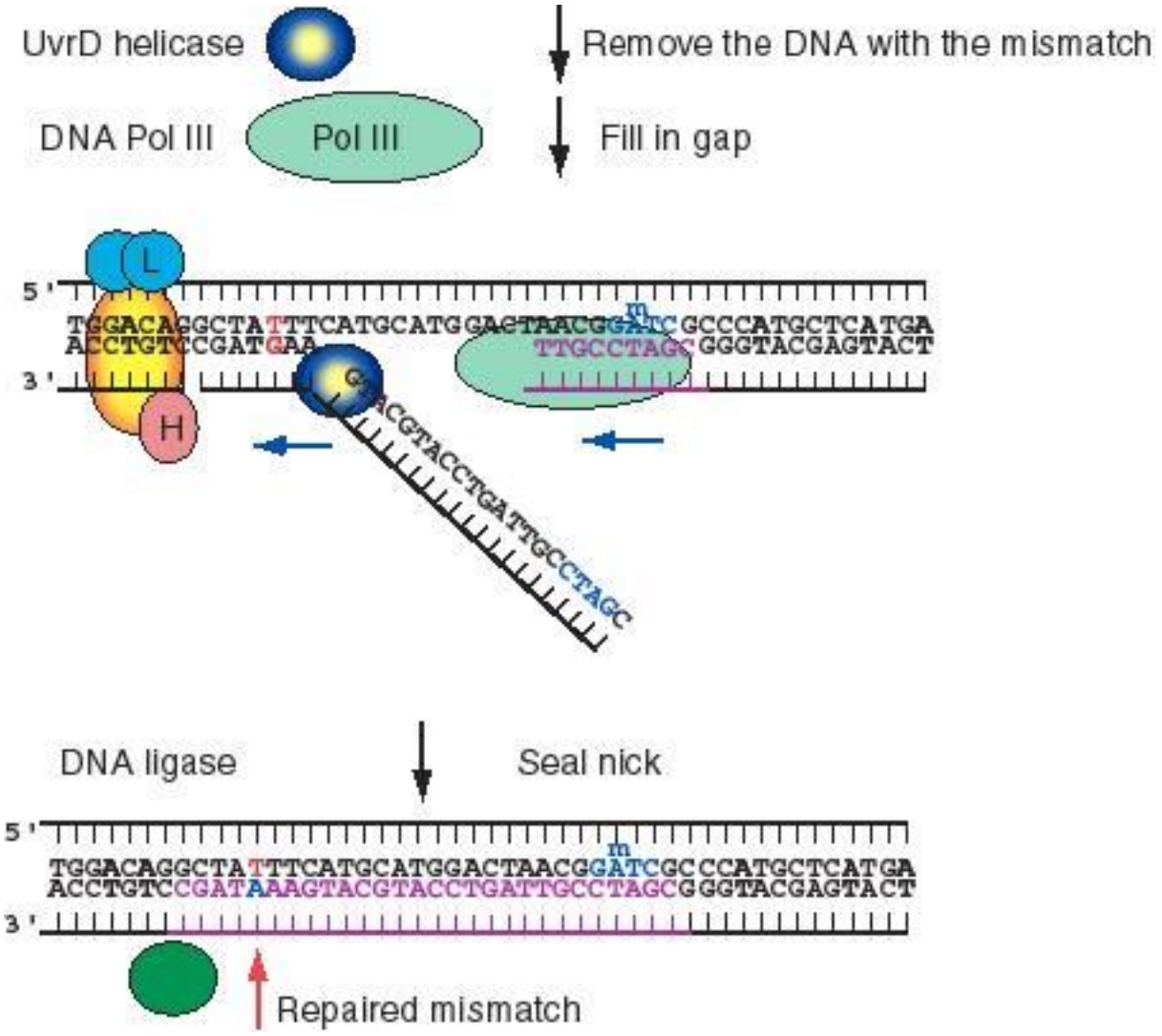
MutH, L, S action in mismatch repair #1



MutH, L, S action in mismatch repair #2



Mismatch repair: Excision of the misincorporated nucleotide

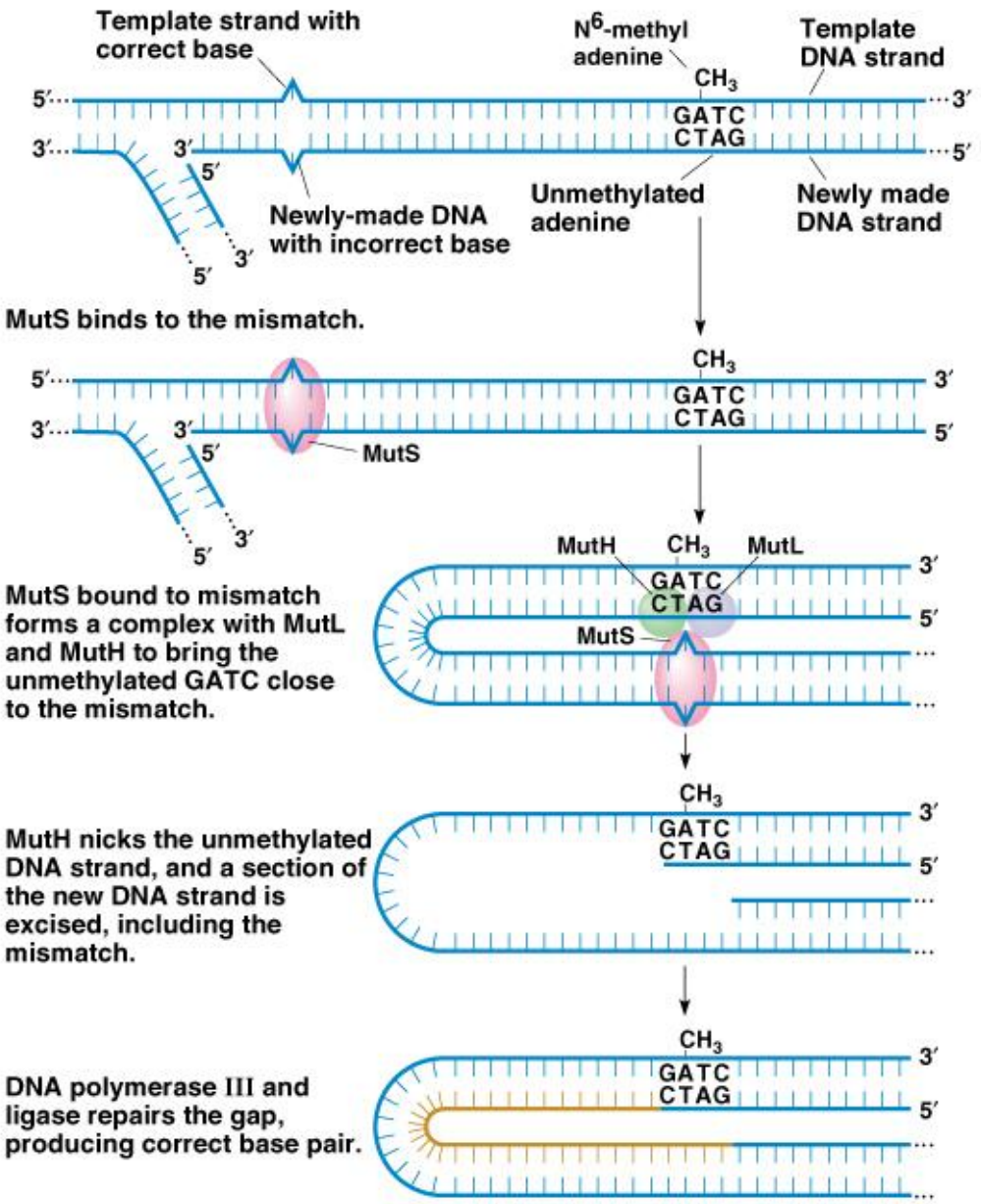


The problem of strand discrimination

- MMR can only aid replication fidelity if repair is targeted to newly synthesized strand
- In *E. coli*, this is accomplished by the transient lack of methylation of adenines in GA*TC motifs (by the “Dam” methylase)
- MutH endonuclease cleaves only unmethylated GATC sites, allowing entry on newly synthesized strand
- *dam* mutants are “mutators” and show random repair of either DNA strand
- In other bacteria and in eukaryotes, the basis of strand discrimination is not understood, although entry at nicks in discontinuously synthesized DNA has been proposed

Mechanism of mismatch correction repair

MutH - Binds 7-meGATC
MutS - Binds mismatch
MutL - links MutH and MutS



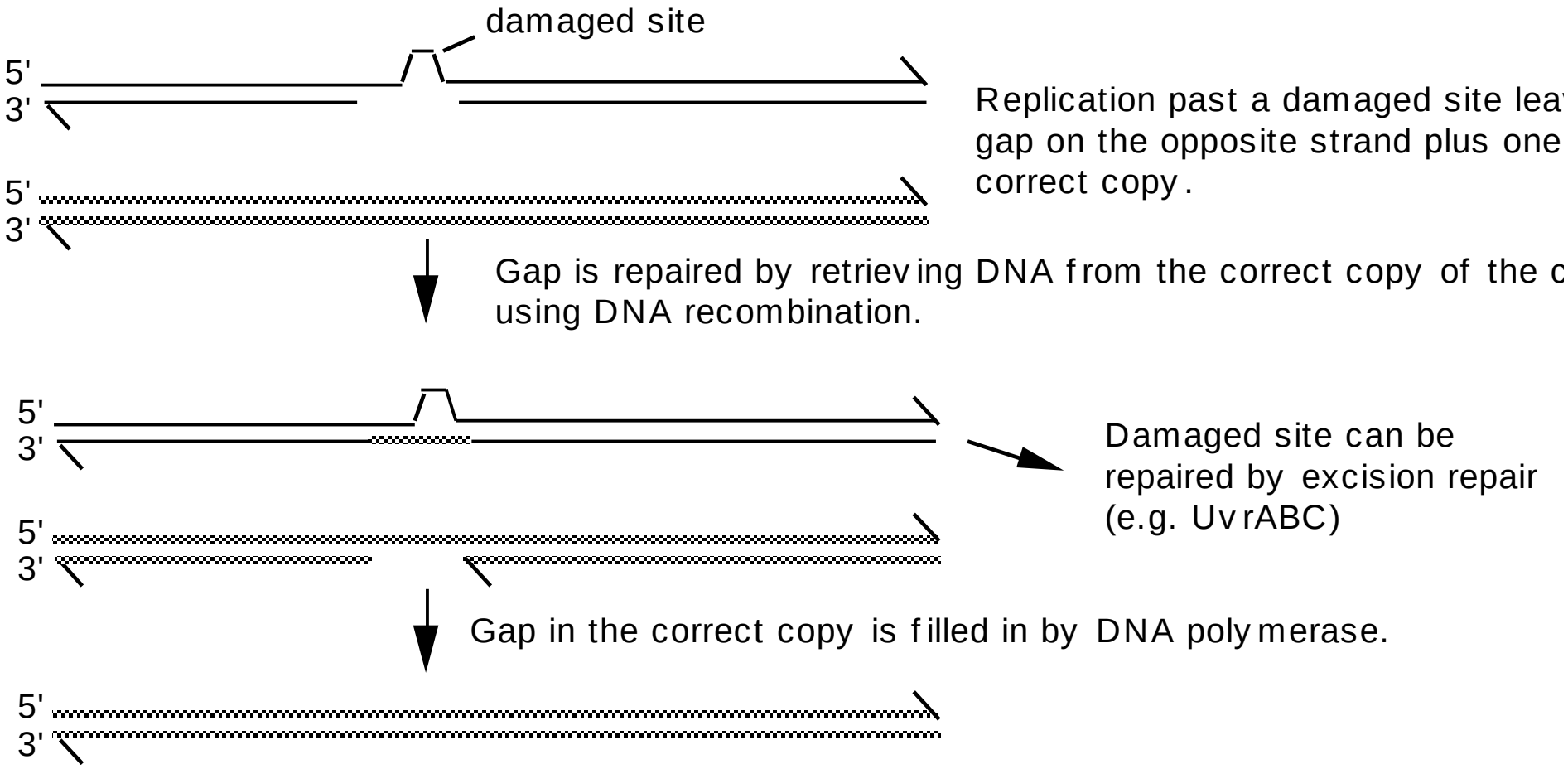
Post replication error

- The direct reversal and excision repair systems act to correct DNA damage before replication, so that replicative DNA synthesis can proceed using an undamaged DNA strand as a template.
- Pyrimidine dimers and many other types of lesions cannot be copied by the normal action of DNA polymerases, so replication is blocked at the sites of such damage.
- Downstream of the damaged site, however, replication can be initiated again by the synthesis of an Okazaki fragment and can proceed along the damaged template strand. The result is a daughter strand that has a gap opposite the site of damage to the parental strand.
- One of two types of mechanisms may be used to repair such gaps in newly synthesized DNA:
  - Recombinational repair
  - Error-prone repair.

Recombinational repair

- Depends on the fact that **one strand of the parental DNA was undamaged** and therefore was copied during replication to yield a normal daughter molecule. The undamaged parental strand can be used to fill the gap opposite the site of damage in the other daughter molecule by recombination between homologous DNA sequences.
- Because the resulting gap in the previously intact parental strand is opposite an undamaged strand, it can be filled in by DNA polymerase. Although the other parent molecule still retains the original damage (e.g., a pyrimidine dimer), the damage now lies opposite a normal strand and can be dealt with later by excision repair.
- By a similar mechanism, recombination with an intact DNA molecule can be used to repair double strand breaks, which are frequently introduced into DNA by radiation and other damaging agents.

Retrieval of information from a homologous chromosome



Error-prone repair

- Last resort for DNA repair
- A gap opposite a site of DNA damage is filled by newly synthesized DNA.
- Since the new DNA is synthesized from a damaged template strand, this form of DNA synthesis is very inaccurate and leads to frequent mutations.
- It is used only in bacteria that have been subjected to potentially lethal conditions, such as extensive UV irradiation. Such treatments induce the SOS response, which may be viewed as a mechanism for dealing with extreme environmental stress.
- The SOS response includes inhibition of cell division and induction of repair systems to cope with a high level of DNA damage.
- Under these conditions, error-prone repair mechanisms are used, presumably as a way of dealing with damage so extensive that cell death is the only alternative.

Role of *umuC* and *umuD* genes in error-prone repair

- Named for the UV ***non*** mutable phenotype of mutants with defects in these genes.

- Needed for bypass synthesis; mechanism is under investigation. E.g. these proteins may reduce the template requirement for the polymerase.

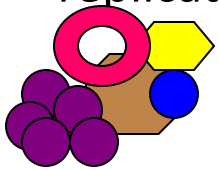
- UmuD protein is proteolytically activated by LexA.

UmuC, UmuD in error-prone repair

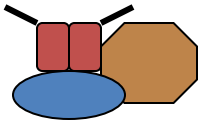
UV damage

DNA replication

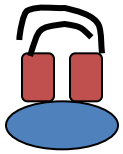
DNA Pol III



Translesional synthesis (error-prone)



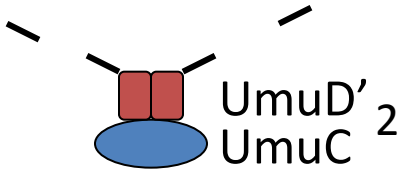
UmuD 2
UmuC



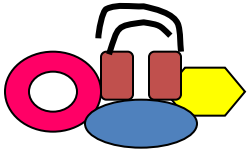
UV damage, increase RecA:ssDNA

Activate protease
Induce *umuC*$^{+}$, *umuD*$^{+}$

UmuD' 2
UmuC

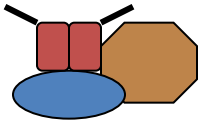


beta



epsilon

Pol III alpha



DNA damage checkpoint control

Polymerase for translesional synthesis

Graham Walker

Bypassing DNA damage during genome replication

- Unrepaired DNA lesions in the template strand block synthesis by replicative DNA polymerases.

- In eukaryotes, the Rad6–Rad18 ubiquitin-conjugating enzyme complex regulates replication through DNA lesions via **Translesion Synthesis (TLS)** by specialized DNA polymerases (Pols) and by means that use **Template Switching.**

- Genetic studies in the yeast Saccharomyces cerevisiae have indicated that Rad6–Rad18-dependent replication through UV-induced DNA lesions can occur by Polη- or Polζ-mediated TLS, or by a Rad5–Mms2–Ubc13 pathway that promotes lesion bypass by template switching.

- A Rad6–Rad18-independent template-switching pathway dependent on Rad51, Rad52, and Rad54 proteins can also restore the continuity of newly synthesized DNA from UV-damaged DNA templates.

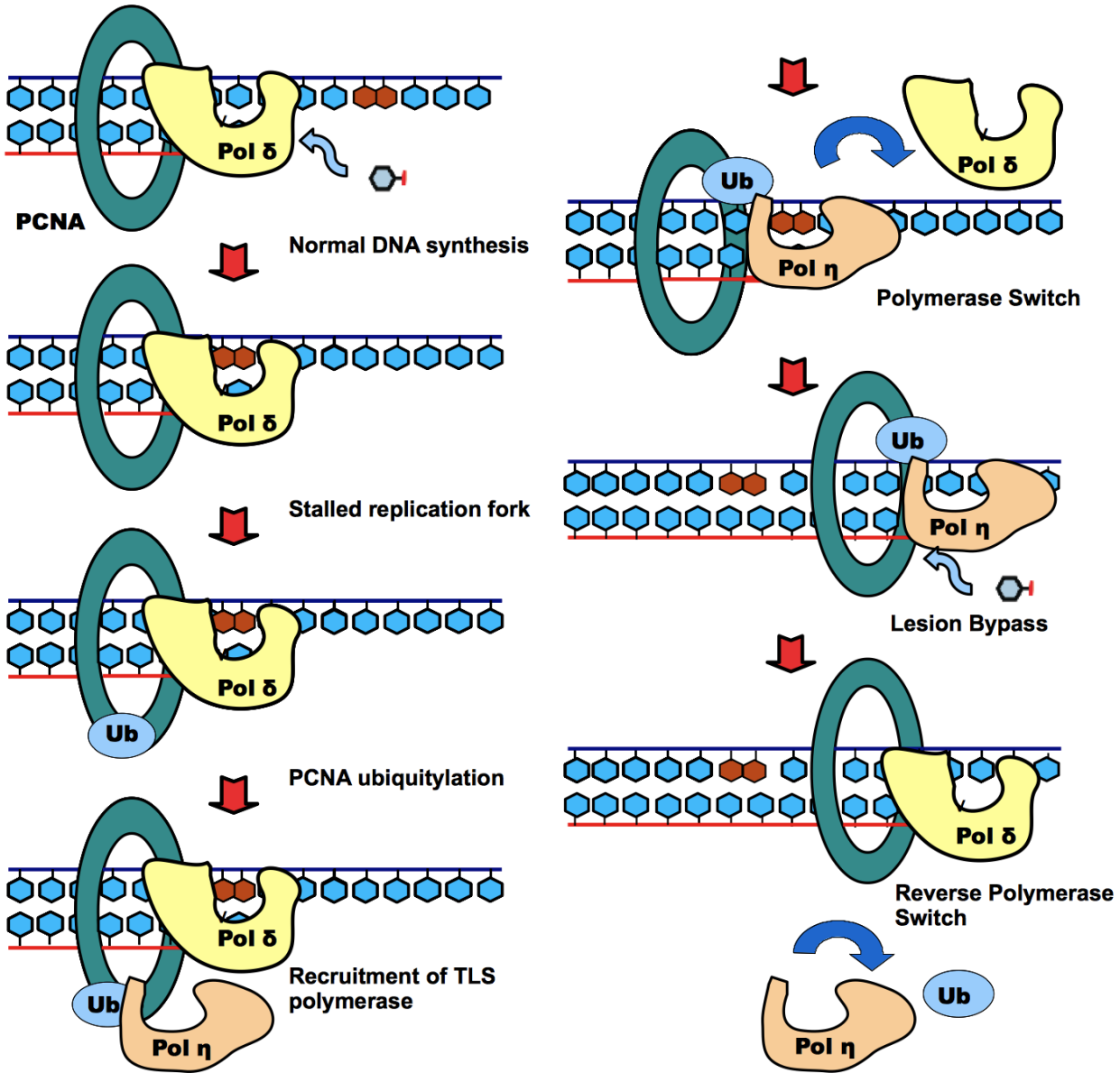
Translesion Synthesis (TLS)

- Translesion synthesis (TLS) is a DNA damage tolerance process that allows the DNA replication machinery to replicate past DNA lesions such as thymine dimers or AP sites.

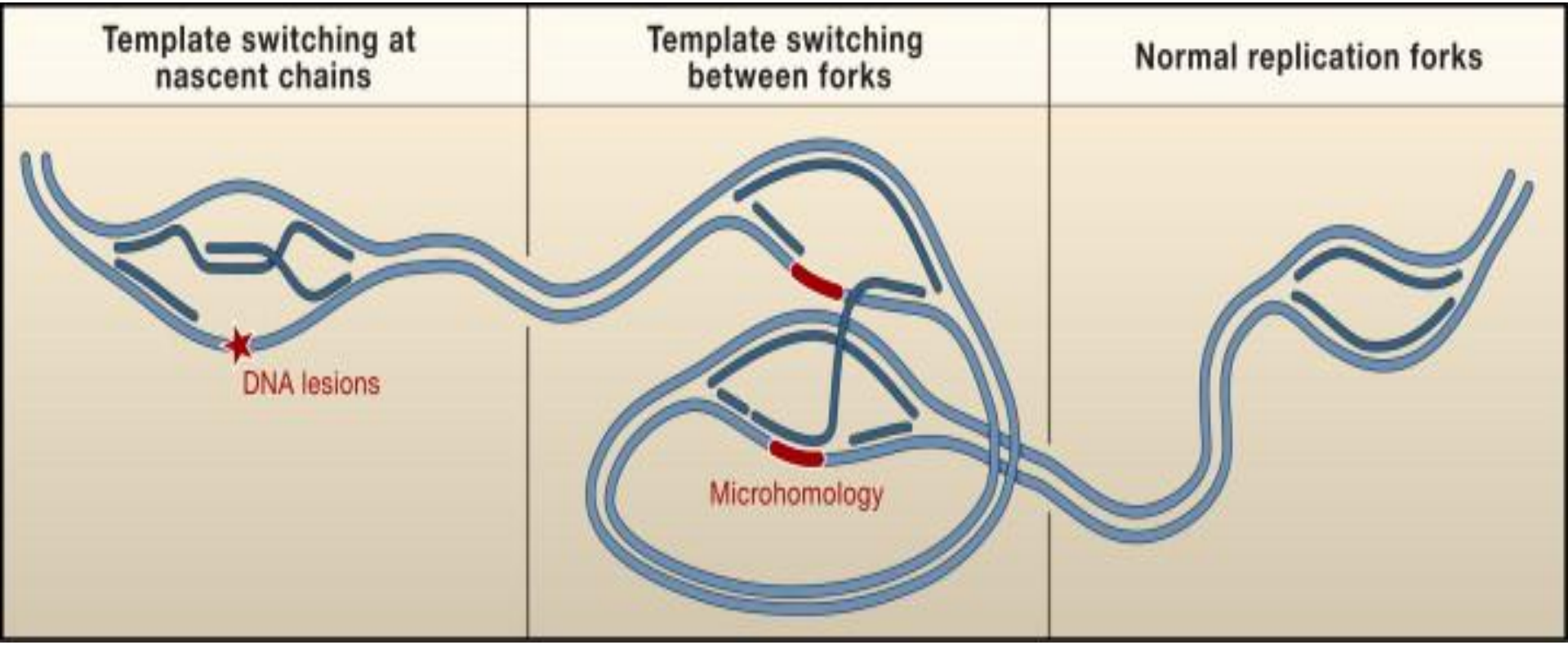
- It involves switching out regular DNA polymerases for specialized translesion polymerases (i.e. DNA polymerase IV or V, from the Y Polymerase family), often with larger active sites that can facilitate the insertion of bases opposite damaged nucleotides.

- When a lesion is encountered, the replication fork will stall, PCNA will switch from a processive polymerase to a TLS polymerase such as Pol ι (Iota) to fix the lesion, then PCNA may switch to Pol ζ (Zeta) to extend the mismatch, and last PCNA will switch to the processive polymerase to continue replication.

Translesion Synthesis (TLS)



Template Switching

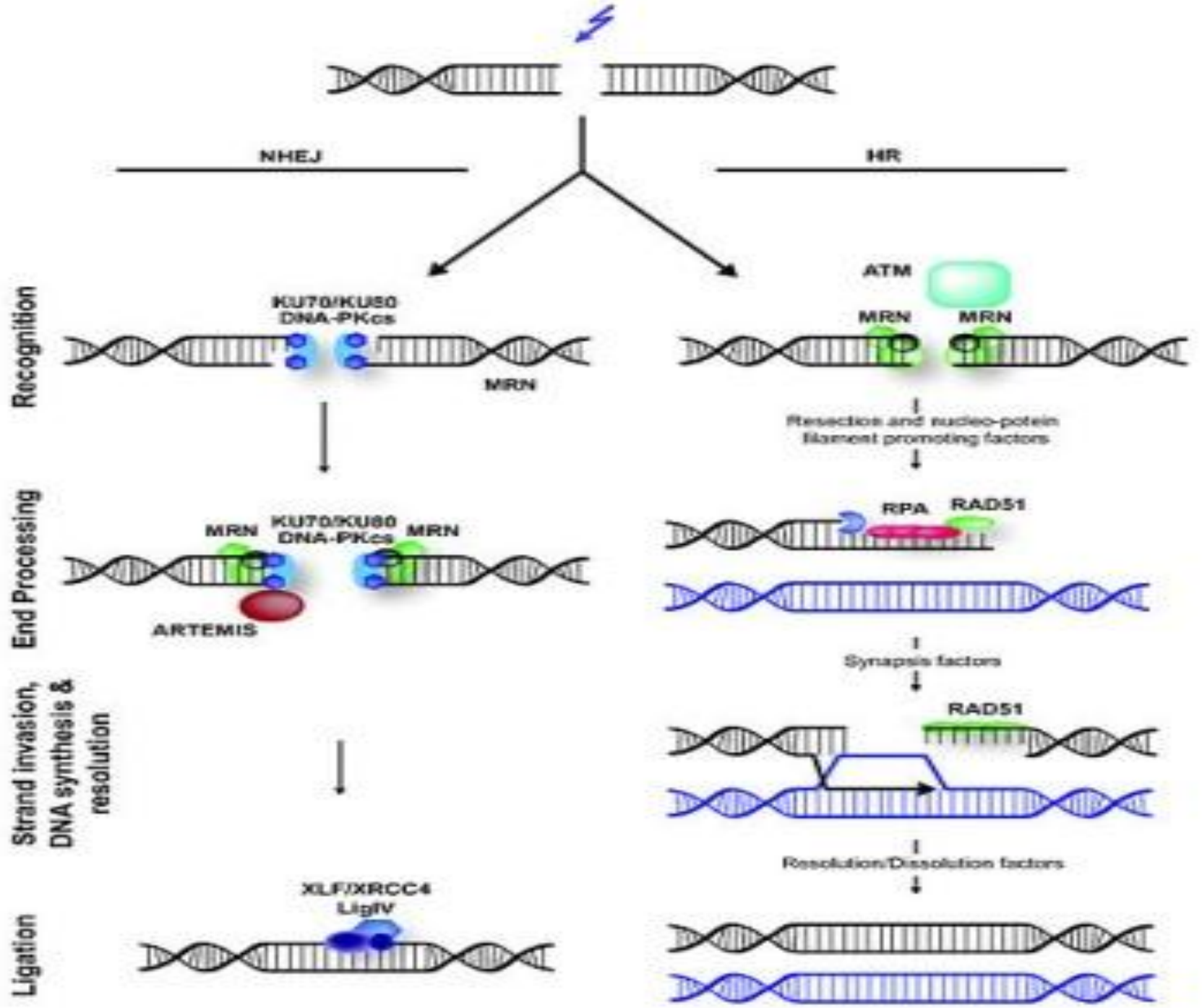


Non-homologous end-joining in humans

- Double-strand breaks (DSBs) arise in dividing cells about ten times per cell per day. Causes include replication across a nick, free radicals of oxidative metabolism, ionizing radiation, and inadvertent action by enzymes of DNA metabolism (such as failures of type II topoisomerases or cleavage by recombinases at off-target sites).
- There are two major double-strand break repair pathways.
  - Homologous recombination (HR) can repair double-strand breaks, but only during S phase and typically only if there are hundreds of base pairs of homology.
  - The more commonly used pathway is non - homologous DNA end joining, abbreviated NHEJ. NHEJ can repair a DSB at any time during the cell cycle and does not require any homology, although a few nucleotides of terminal microhomology are often utilized by the NHEJ enzymes, if present.

Non-homologous end joining (NHEJ)

- Non-homologous end joining (NHEJ) is a pathway that repairs double-strand breaks in DNA. NHEJ is referred to as "non-homologous" because the break ends are directly ligated without the need for a homologous template, in contrast to homology directed repair, which requires a homologous sequence to guide repair.


NHEJ
HR
Recognition
KU70/KU80
DNA-PKcs
MRN
ATM
MRN
MRN
Resection and nucleo-potein
filament promoting factors
End Processing
MRN
KU70/KU80
DNA-PKcs
MRN
ARTEMIS
RPA
RAD51
Synapsis factors
Strand invasion,
DNA synthesis &
resolution
RAD51
Resolution/Dissolution factors
Ligation
XLF/XRCC4
LigIV

SOS response in E. coli

- The SOS response is a global response to DNA damage in which the cell cycle is arrested and DNA repair and mutagenesis are 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 LexA repressor thereby inducing the response. It is an error-prone repair system that is attributed to mutagenesis.

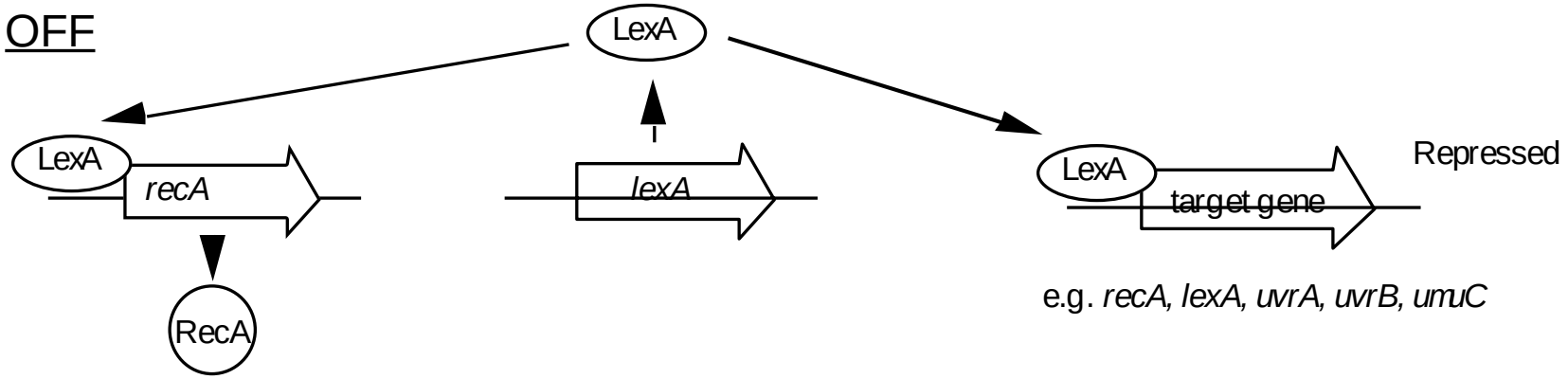
Mechanism for SOS response in E. coli

- 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.

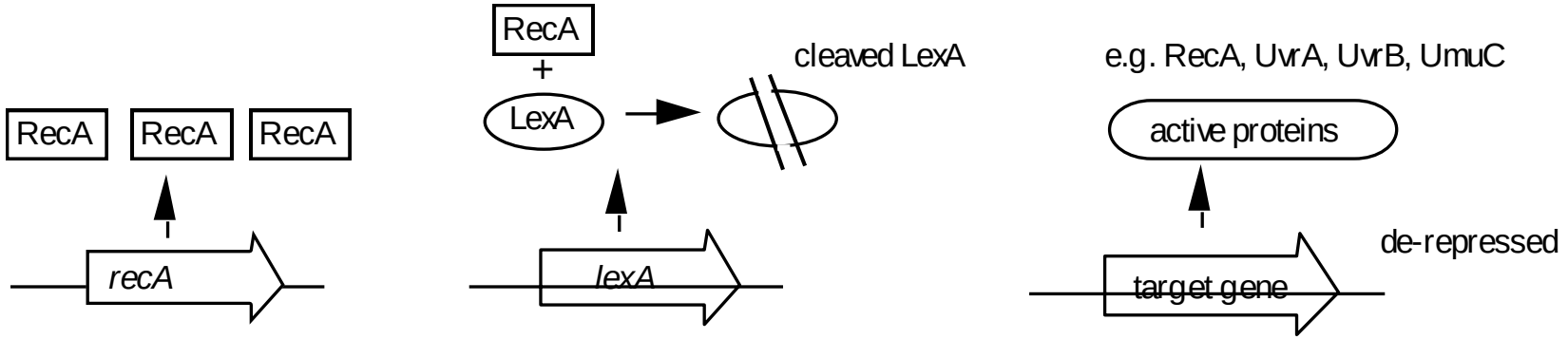
Mechanism for SOS response in E. coli

- 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.
- Genes having a weak SOS box (such as lexA, recA, uvrA, uvrB, and uvrD) are fully induced in response to even weak SOS-inducing treatments. Thus the **first SOS repair mechanism to be induced is nucleotide excision repair** (NER), whose aim is to fix DNA damage without commitment to a full-fledged SOS response.
- If, however, NER does not suffice to fix the damage, the LexA concentration is further reduced, so the expression of genes with stronger LexA boxes (such as sulA, umuD, umuC - these are expressed late) is induced. SulA stops cell division by binding to FtsZ, the initiating protein in this process. This causes filamentation, and the induction of UmuDC-dependent mutagenic repair.
- As a result of these properties, some genes may be partially induced in response to even endogenous levels of DNA damage, while other genes appear to be induced only when high or persistent DNA damage is present in the cell.

SOS response is controlled by LexA and RecA



ON RecA is activated in the presence of damaged DNA. It serves as a co-protease to activate a latent, self-cleaving proteolytic activity in LexA, thereby removing the repressor from SOS inducible genes.



Diseases due to defects in DNA repair Mechanism

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.