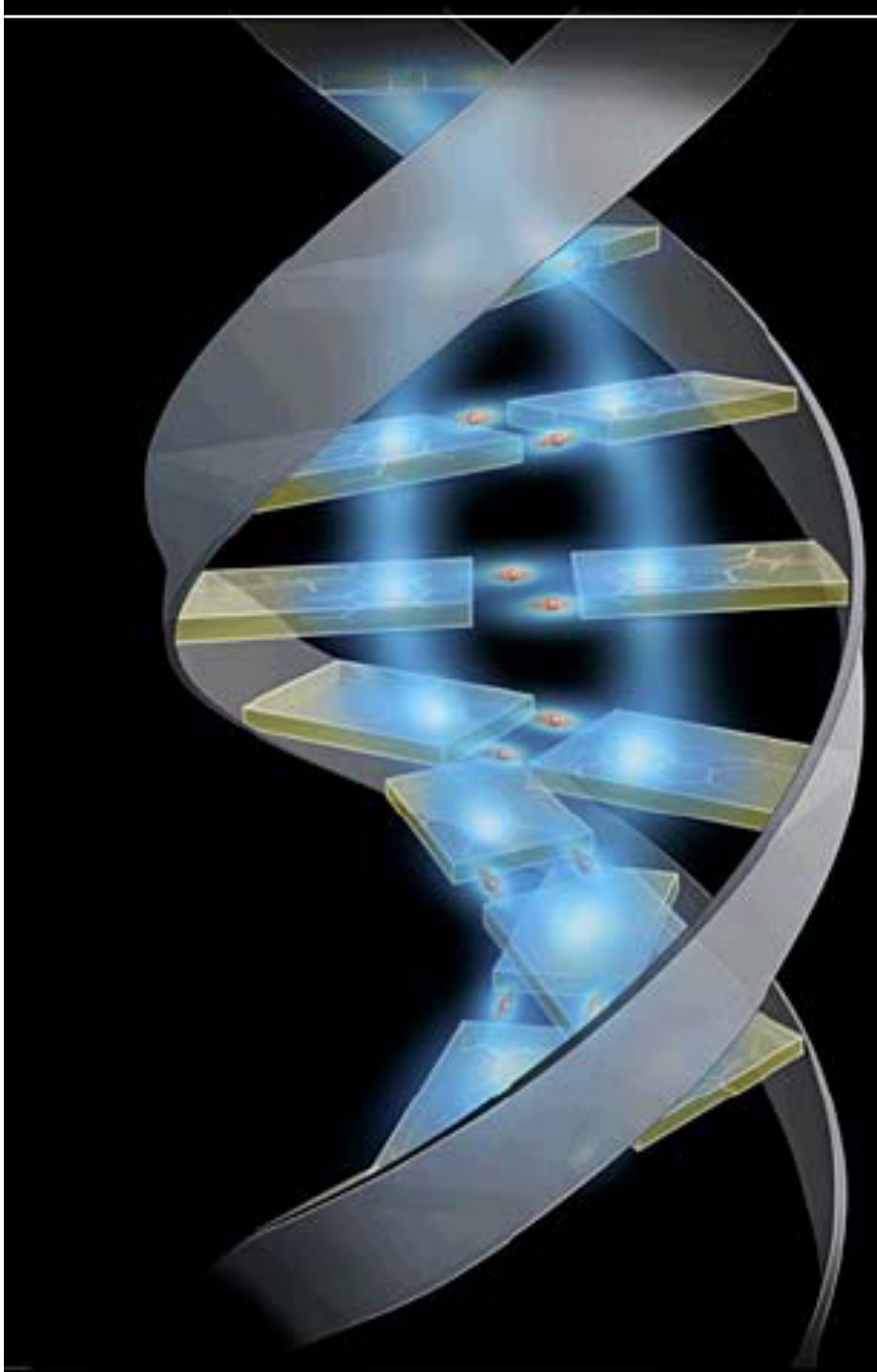
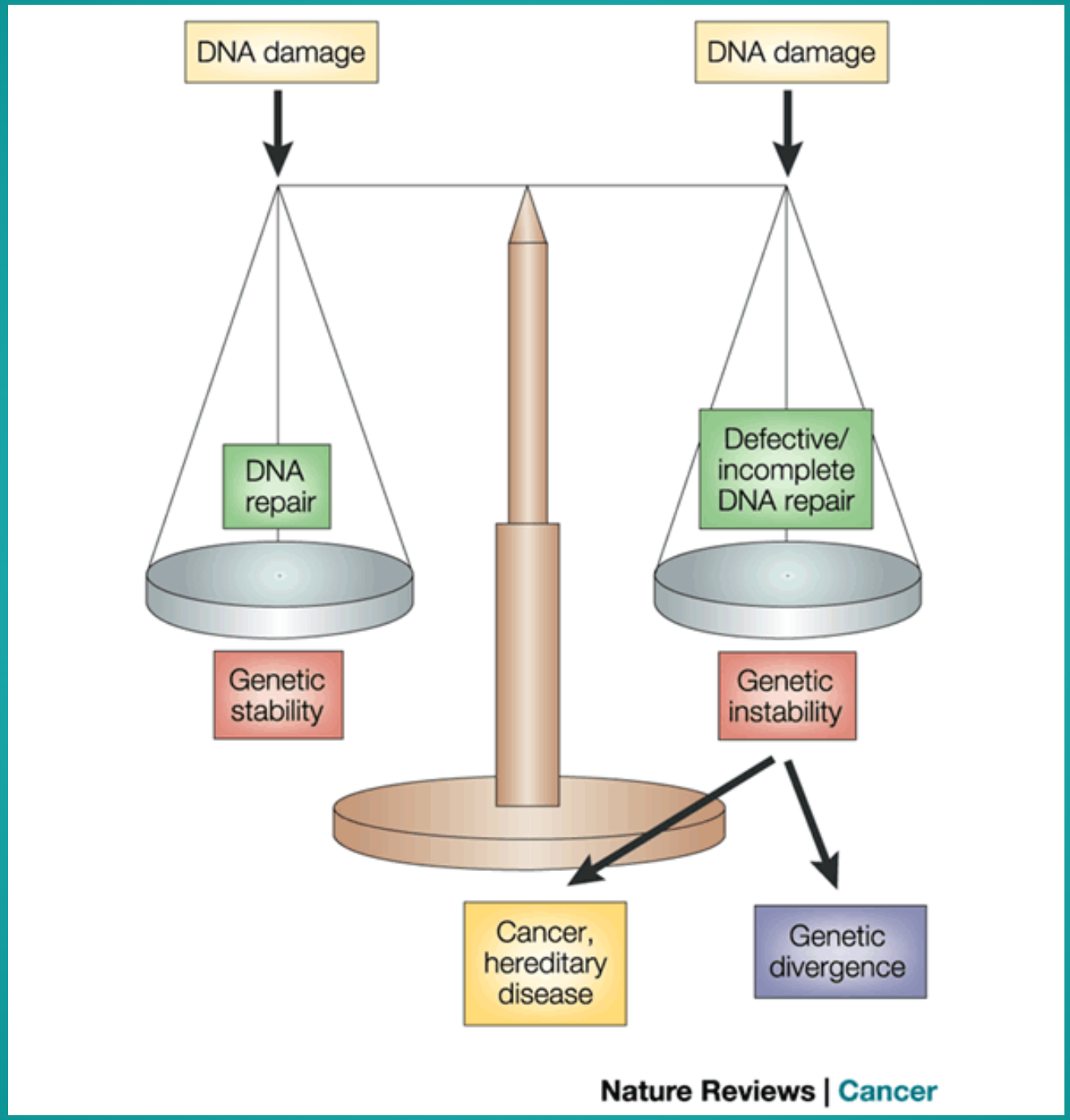


Mechanism of

DNA REPAIR


DNA damage
DNA damage
DNA repair
Defective/ incomplete DNA repair
Genetic stability
Genetic instability
Cancer, hereditary disease
Genetic divergence
Nature Reviews | Cancer

Overview

Life has faced the fundamental problem that the form in which genetic information is stored is not chemically inert

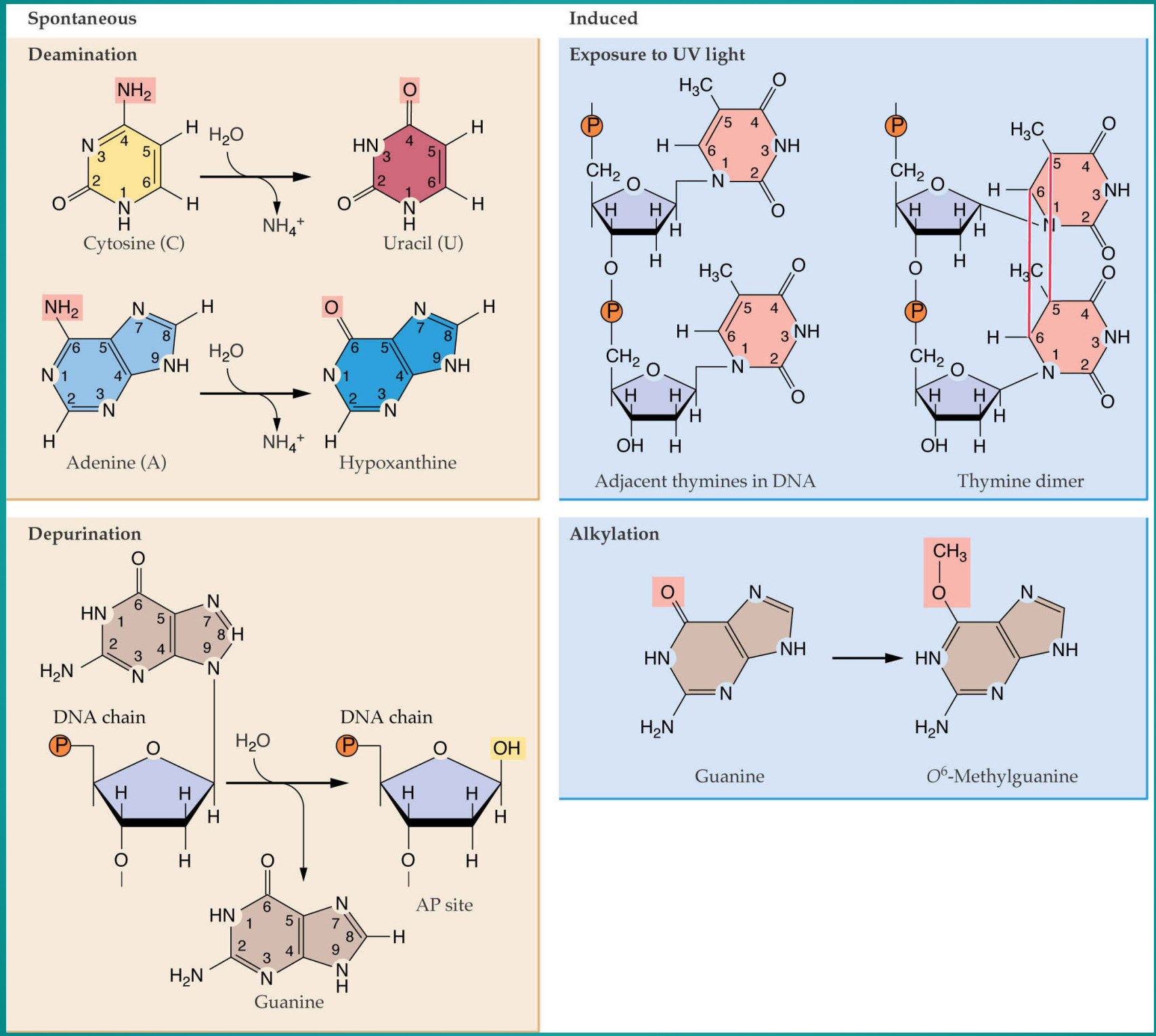
DNA integrity is challenged by the damaging effect of numerous chemical and physical agents, compromising its function.

To protect The DNA integrity, an intricate network of DNA repair systems has evolved early in evolution

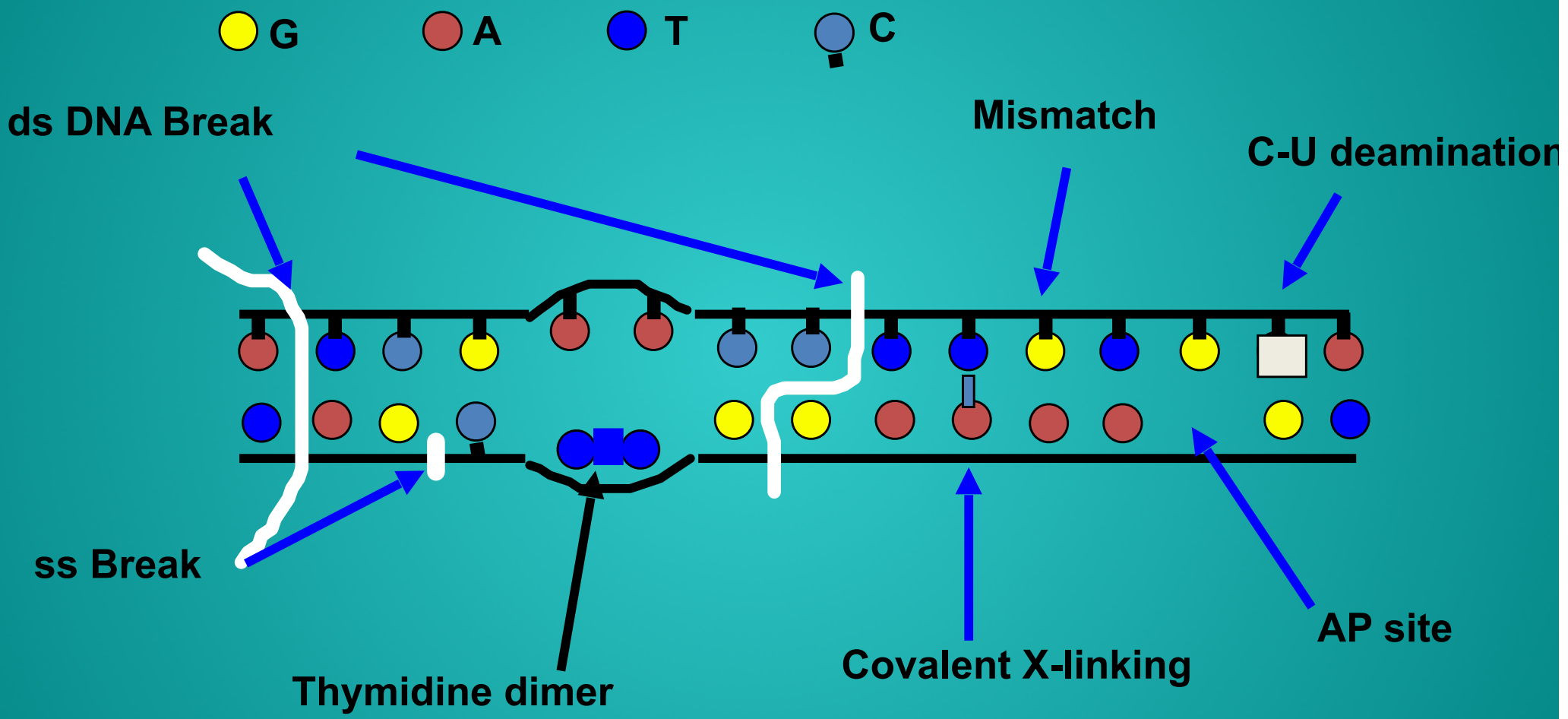
DNA is the only macromolecule which is repaired by the cell

Types of DNA Damage

1. Deamination: (C → U and A → hypoxanthine)
2. Depurination: purine base (A or G) lost
3. T-T and T-C dimers: bases become cross-linked, T-T more prominent, caused by UV light
4. Alkylation: an alkyl group (e.g., CH_3) gets added to bases; chemical induced; some harmless, some cause mutations by mispairing during replication or stop polymerase altogether
5. Oxidative damage: guanine oxidizes to 8-oxo-guanine, also cause SS and DS breaks, very important for organelles
6. Replication errors: wrong nucleotide (or modified) inserted
7. Double-strand breaks (DSB): induced by ionizing radiation, transposons, topoisomerases, homing endonucleases, mechanical stress on chromosomes, or a single-strand nick in a single-stranded region (e.g., during replication and transcription)


Spontaneous
Induced
Deamination
Cytosine (C)
Uracil (U)
Adenine (A)
Hypoxanthine
Exposure to UV light
Adjacent thymines in DNA
Thymine dimer
Depurination
DNA chain
DNA chain
AP site
Guanine
Alkylation
Guanine
O6-Methylguanine

Types of DNA Damage Summarized

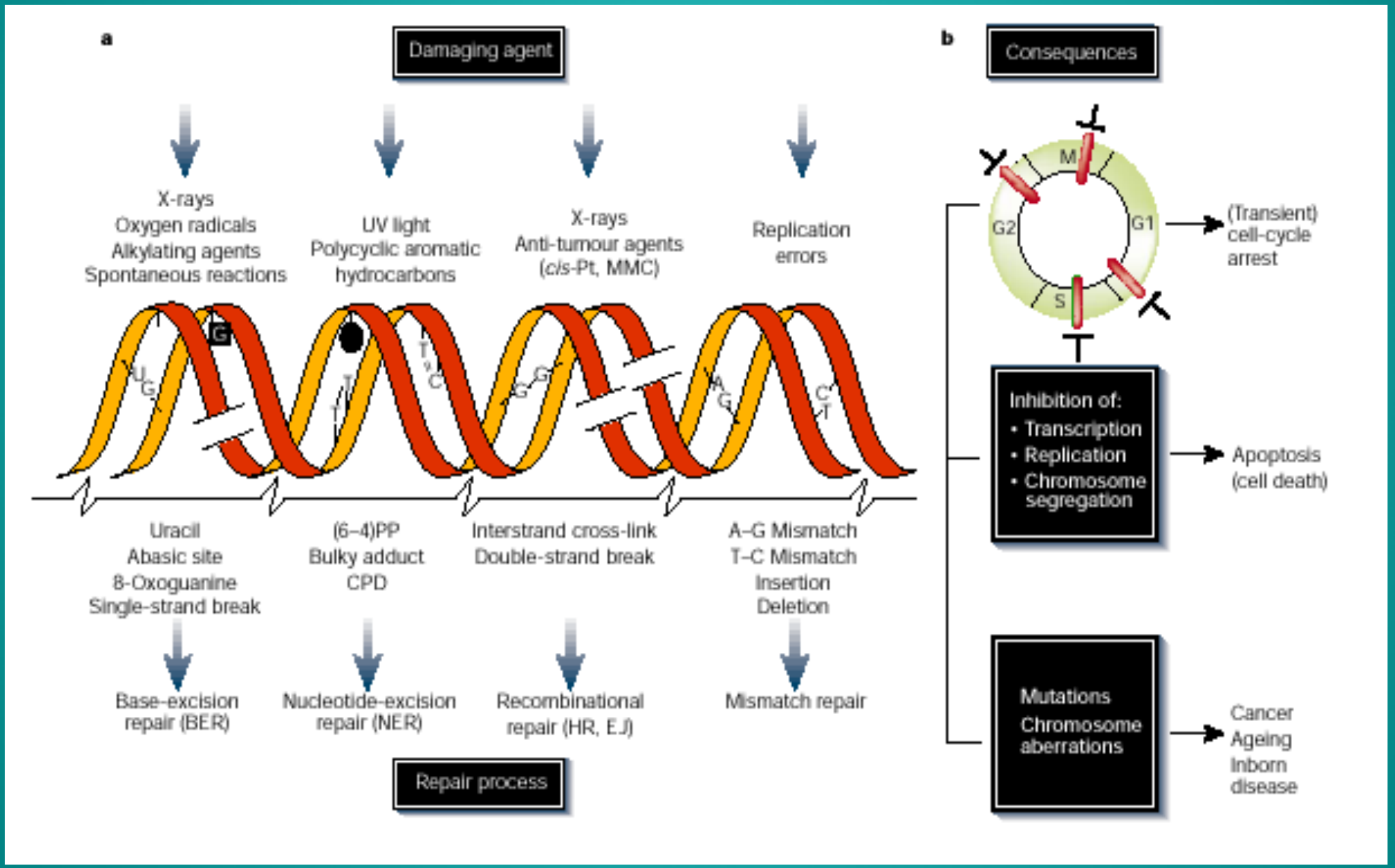


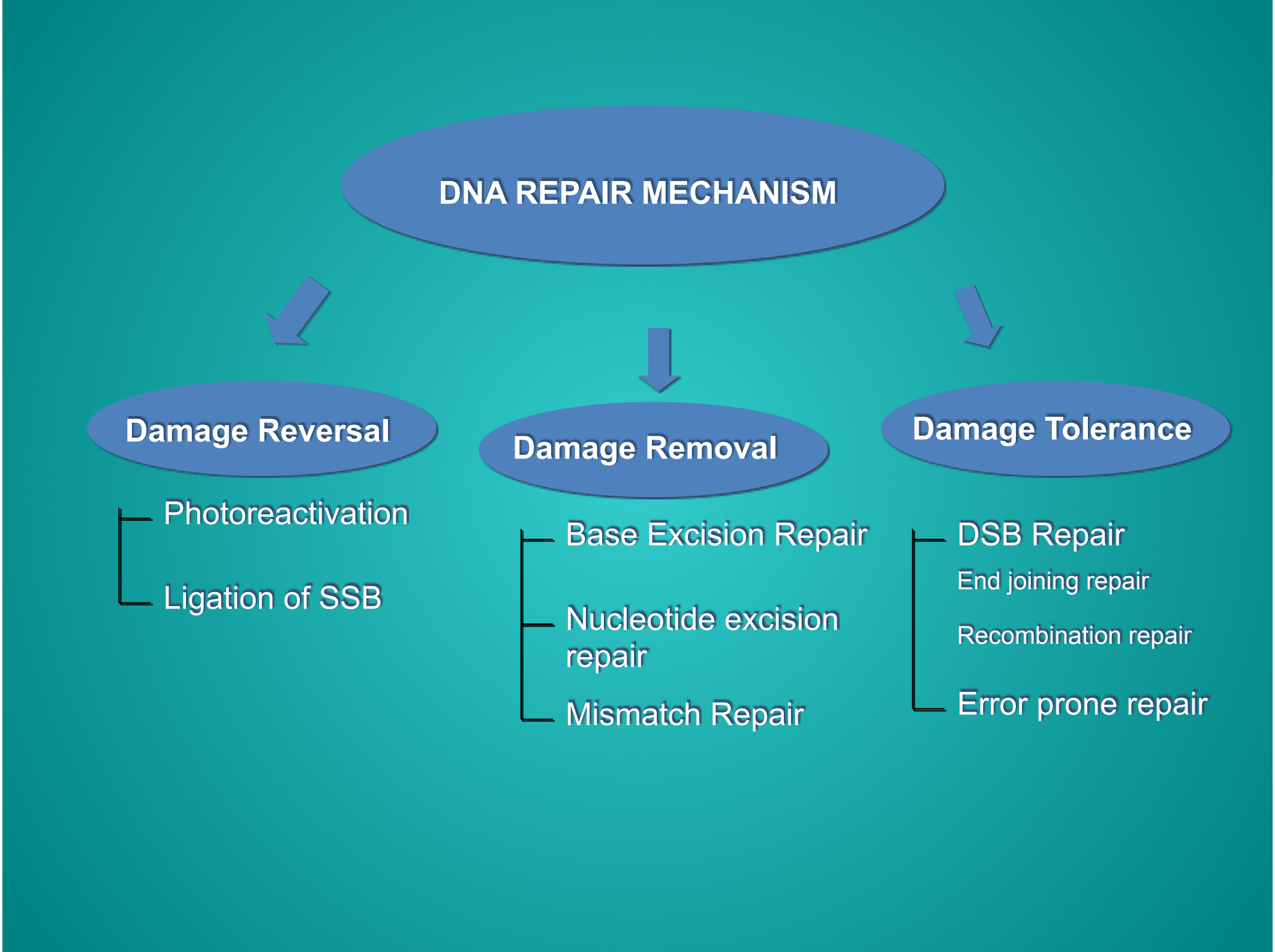
Total damage from all mechanisms: 10^4 - 10^6 lesions/day/cell

DNA Repair

- DNA damage may arise: (i) spontaneously, (ii) environmental exposure to mutagens, or (iii) cellular metabolism.
- DNA damage may be classified as: (I) strand breaks, (ii) base loss (AP site), (iii) base damages, (iv) adducts, (v) cross-links, (vi) sugar damages, (vii) DNA-protein cross links.
- DNA damage, if not repaired, may affect replication and transcription, leading to mutation or cell death.

IMPORTANCE OF DNA REPAIR




DNA REPAIR MECHANISM
Damage Reversal
Photoreactivation
Ligation of SSB
Damage Removal
Base Excision Repair
Nucleotide excision repair
Mismatch Repair
Damage Tolerance
DSB Repair
End joining repair
Recombination repair
Error prone repair

Damage reversal

Photo reactivation: most simple way for DNA repair : a single step reaction.
Photolyase enzyme can split pyrimidine dimers: breaks the covalent bond

Existence in mammalian not yet proved.

Most of damaged bases are repaired by one enzyme, removes mismatched base restoring the correct one. Without the need to break the DNA backbone:
- Uracil, product of cytosine deamination, is detected, removed by uracil N-glycosylase, and replaced by cytosine.

Hypoxanthine, product of adenine deamination, is recognized, removed by hypoxanthine N-glycosylase then replaced by adenine

-Alkylation is repaired by enzymatic transfer of methyl group by Methyl Guanine-DNA MethylTransferase (M G M T), and Guanine is formed

-Demethylation is used to repair 1-methyl adenine and 3-methyl cytosine.

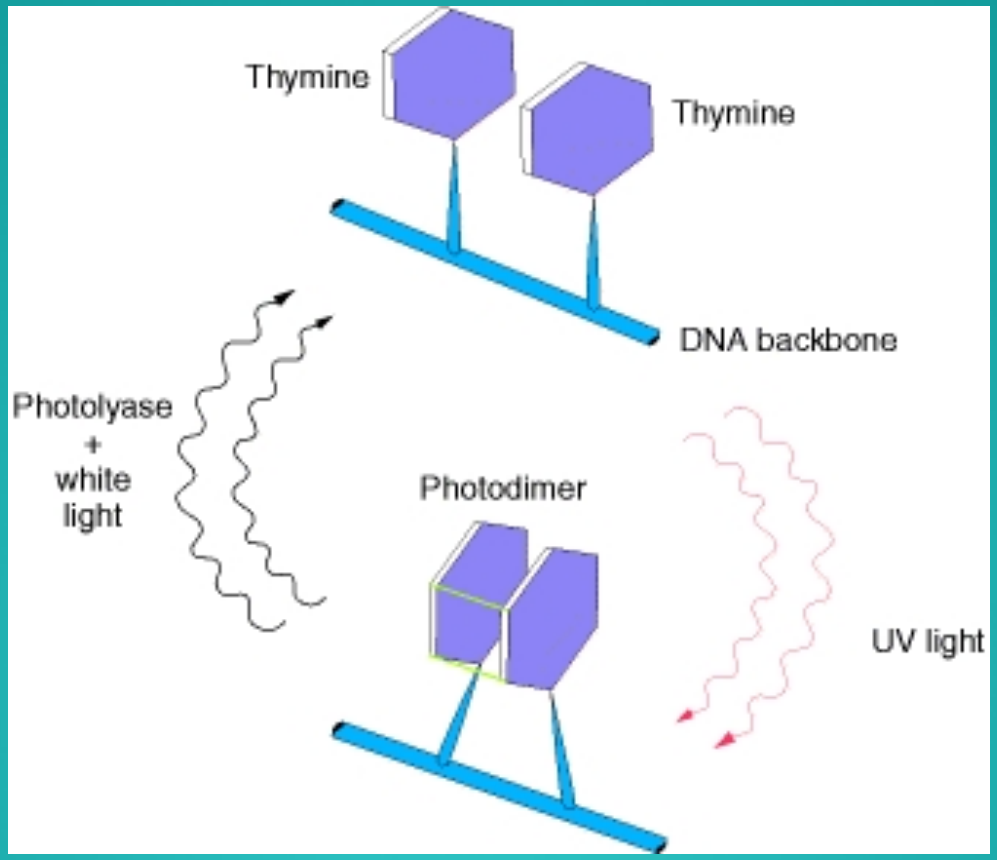
Ligation of simple strand breaks:
Simple breaks in one strand are repaired by DNA ligase

Photoactivation Repair in *E. coli*

- Exposing UV treated cells to blue light results in a reversal of the thymine dimer formation

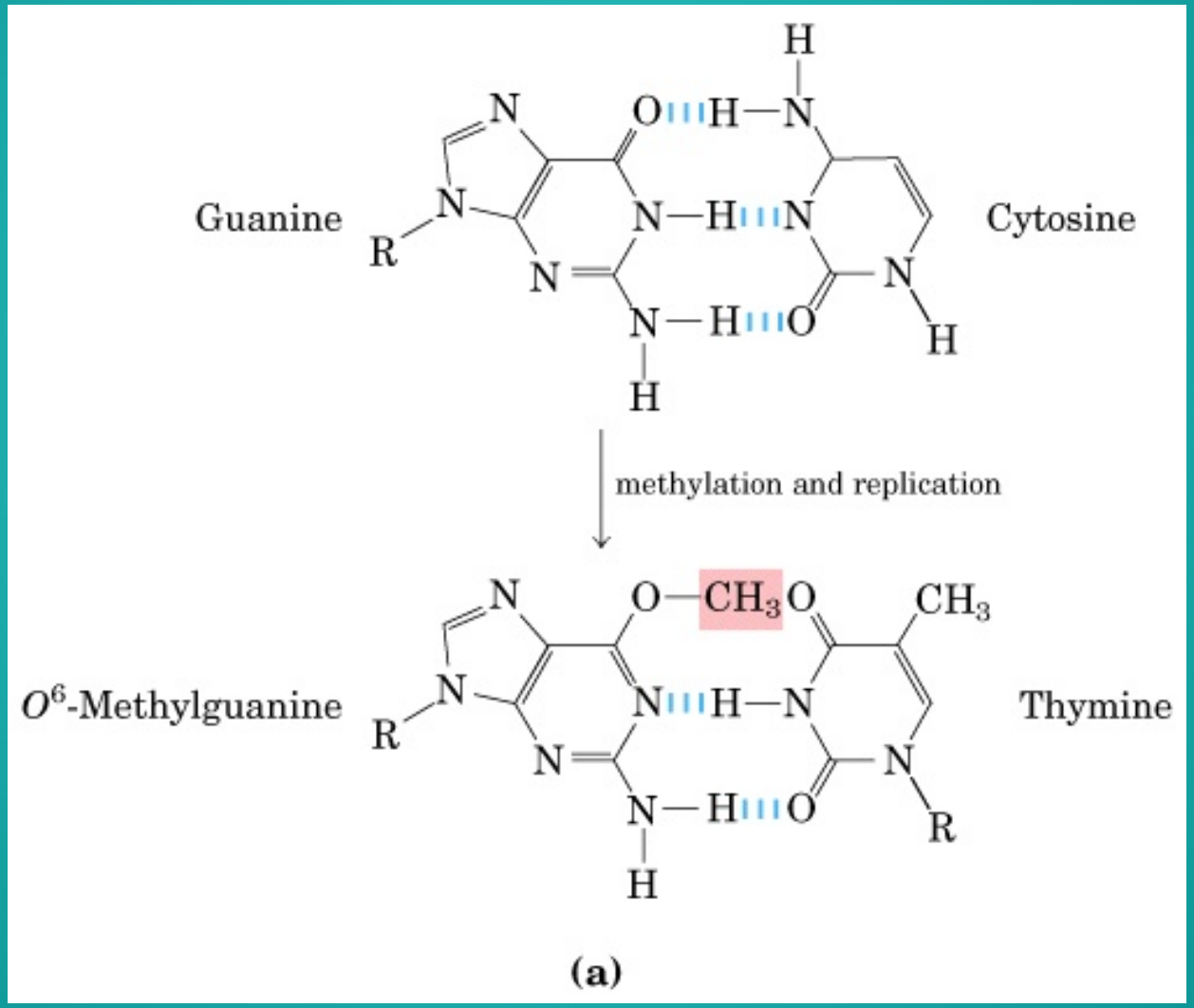
- Enzyme, photoactivation repair enzyme (PRE) absorbs a photon of light (from blue light) and is able to cleave the bond forming the thymine dimer.

- Once bond is cleaved, DNA is back to normal

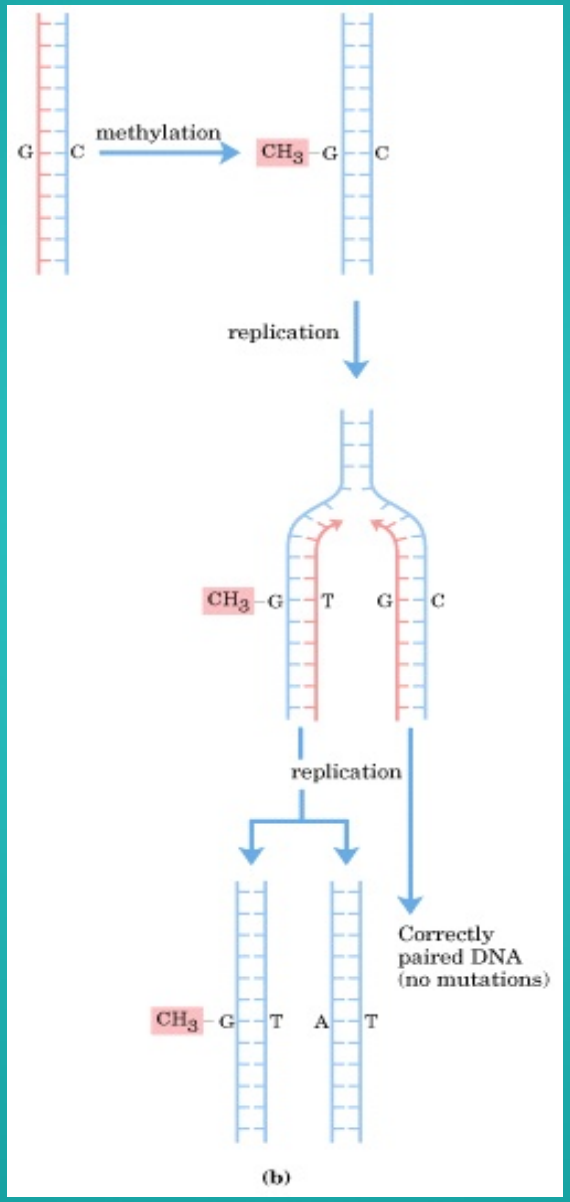


Repair of a UV-induced pyrimidine photodimer by a photoreactivating enzyme, or photolyase. The enzyme recognizes the photodimer (here, a thymine dimer) and binds to it. When light is present, the photolyase uses its energy to split the dimer into the original monomers.

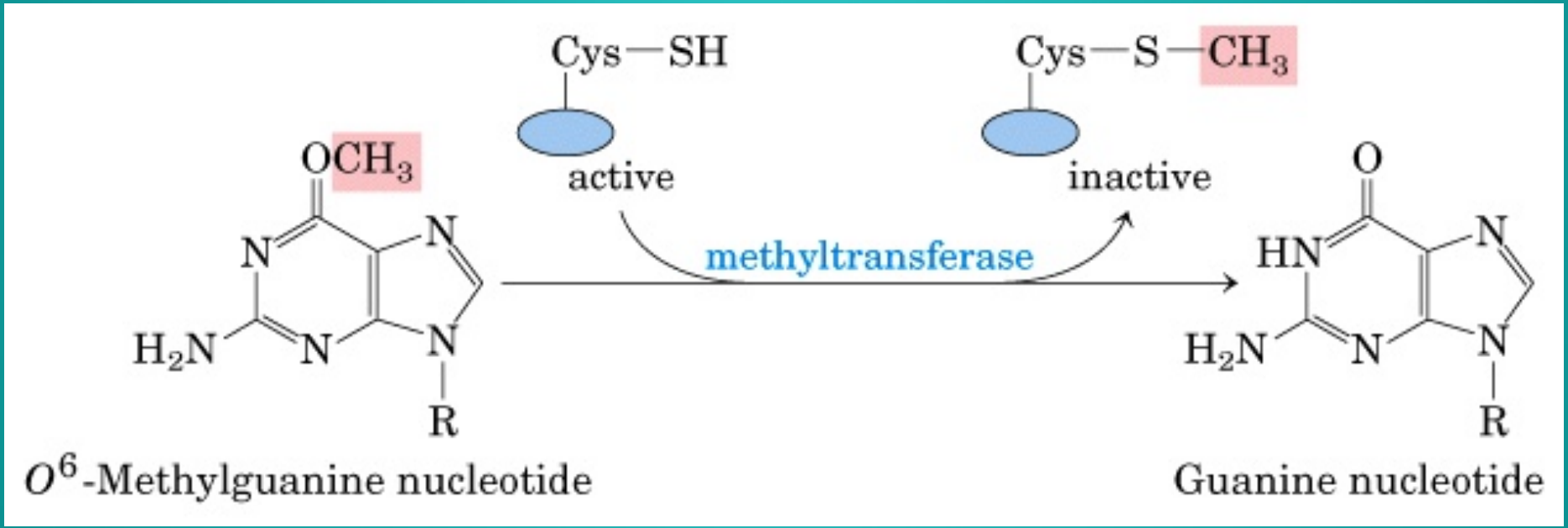
Alkylation of DNA by alkylating agents



O^6-methyl G, if not repaired, may produce a mutation



Direct Repair: Reversal of O6 methyl G to G by methyltransferase



Damage removal

Excision Repair

- Conserved throughout evolution, found in all prokaryotic and eukaryotic organisms

- Three step process:
  - 1. Error is recognized and enzymatically clipped out by a nuclease that cleaves the phosphodiester bonds (*Uvr* gene products operate at this step)

  - 2. DNA Polymerase I fills in the gap by inserting the appropriate nucleotides

  - 3. DNA Ligase seals the gap

Excision Repair

- Two known types of excision repair

– **Base excision repair (BER)**
- corrects damage to nitrogenous bases created by the spontaneous hydrolysis of DNA bases as well as the hydrolysis of DNA bases caused by agents that chemically alter them

– **Nucleotide excision repair (NER)**
- Repairs "bulky" lesions in DNA that alter or distort the regular DNA double helix
- Group of genes (uvr) involved in recognizing and clipping out the lesions in the DNA
- Repair is completed by DNA pol I and DNA ligase

Base excision Repair (BER)

It repairs small, non bulky DNA lesions: methylated, oxidized, reduced bases.

Consist of DNA glycosylases and AP endonuclease

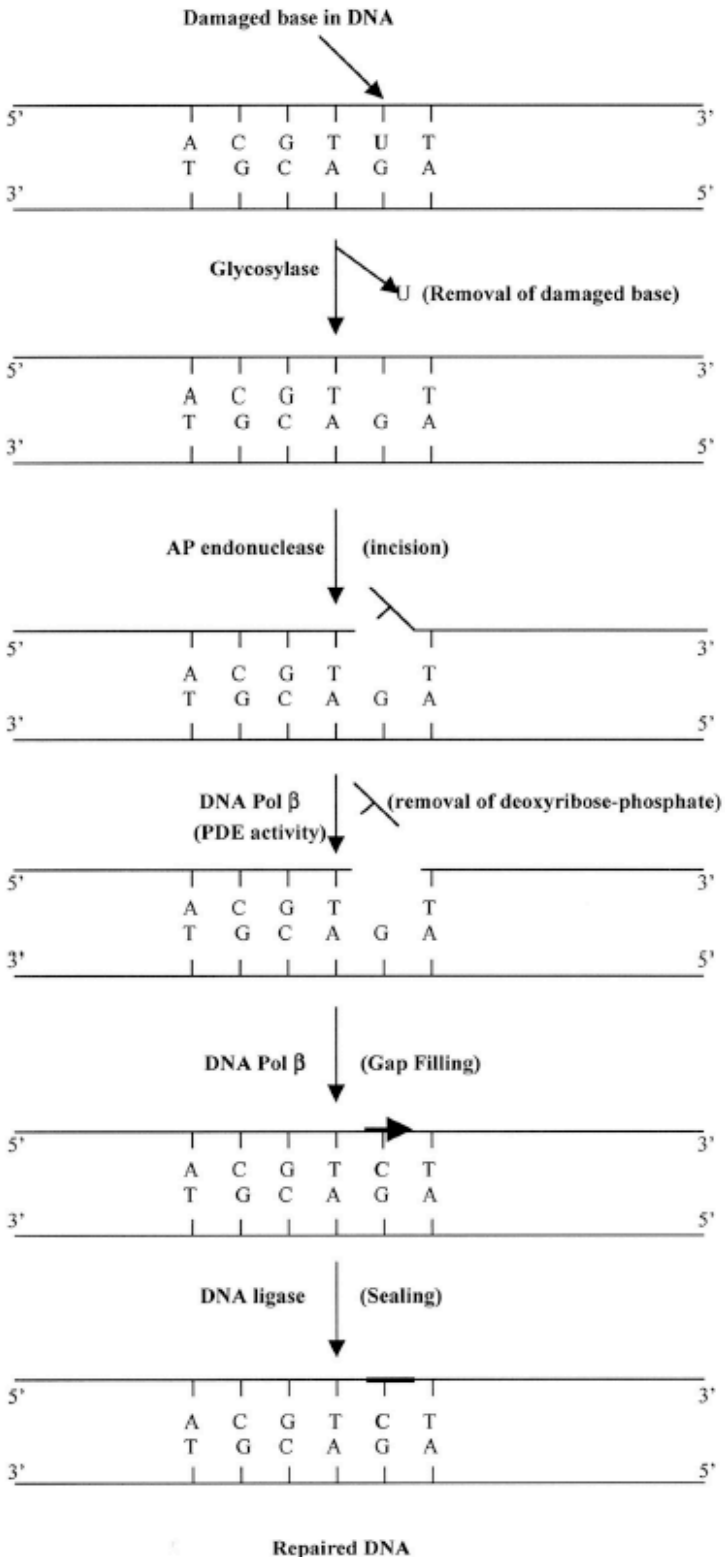
Damaged or inappropriate base is removed from its sugar linkage and replaced.

STEPS in BER

1. removal of the damaged base by a DNA glycosylase. Eight enzymes,each one responsible for identifying and removing a specific kind of base damage.
2. Removal of its deoxyribose phosphate in the backbone, producing a gap: an AP site. Two genes encoding enzymes with this function.
3. replacement with the correct nucleotide. Done by DNA polymerase beta (one of at least 11 DNA polymerases encoded by human genes), using the other strand as a template.
4. Ligation of the break in the strand.

Mechanism

1.DNA glycosylase recognizes Specific Damaged base
2. Cleaves glycosl bond to remove Base
3. AP endonuclease cleaves Backbone
4. DNA Pol removes abasic site
5. Replacement of Base



Nucleotide excision repair (N E R):

The process of NER is biochemically complicated, 30 distinct proteins that function as a large complex called the nucleotide excision repairosome.
- The most important DNA repair pathway,
- The sole repair system for bulky DNA lesions, which creates a block to DNA replication and transcription.
- Can also repair many of the same defects that are corrected by direct repair, base excision and mismatch repair

Steps in NER :

a- Recognition of damage by one or more protein factors.
b- Assembly of repair complex: nucleotide excision repairosome.
c- Double incision of the damaged strand several nucleotides away from the damaged site, on both sides, by an endonuclease.
d. Removal of the short segment (about 24 to 32 nucleotides) containing the damaged region, by an exonuclease.
e- Filling in of the resulting gap by a DNA polymerase: synthesizes DNA using the opposite strand as a template.
f- Ligation: a DNA ligase binds the synthesized piece into the backbone.

Nucleotide Excision repair

In this form of repair the gene products of the E. coli *uvrA*, *uvrB* and *uvrC* genes form an enzyme complex that physically cuts out (excises the damaged strand containing the pyrimidine dimers.

An incision is made 8 nucleotides (nt) away for the pyrimidine dimer on the 5' side and 4 or 5 nt on the 3' side.. The damaged strand is removed by *uvrD*, a helicase and then repaired by DNA pol I and DNA ligase.

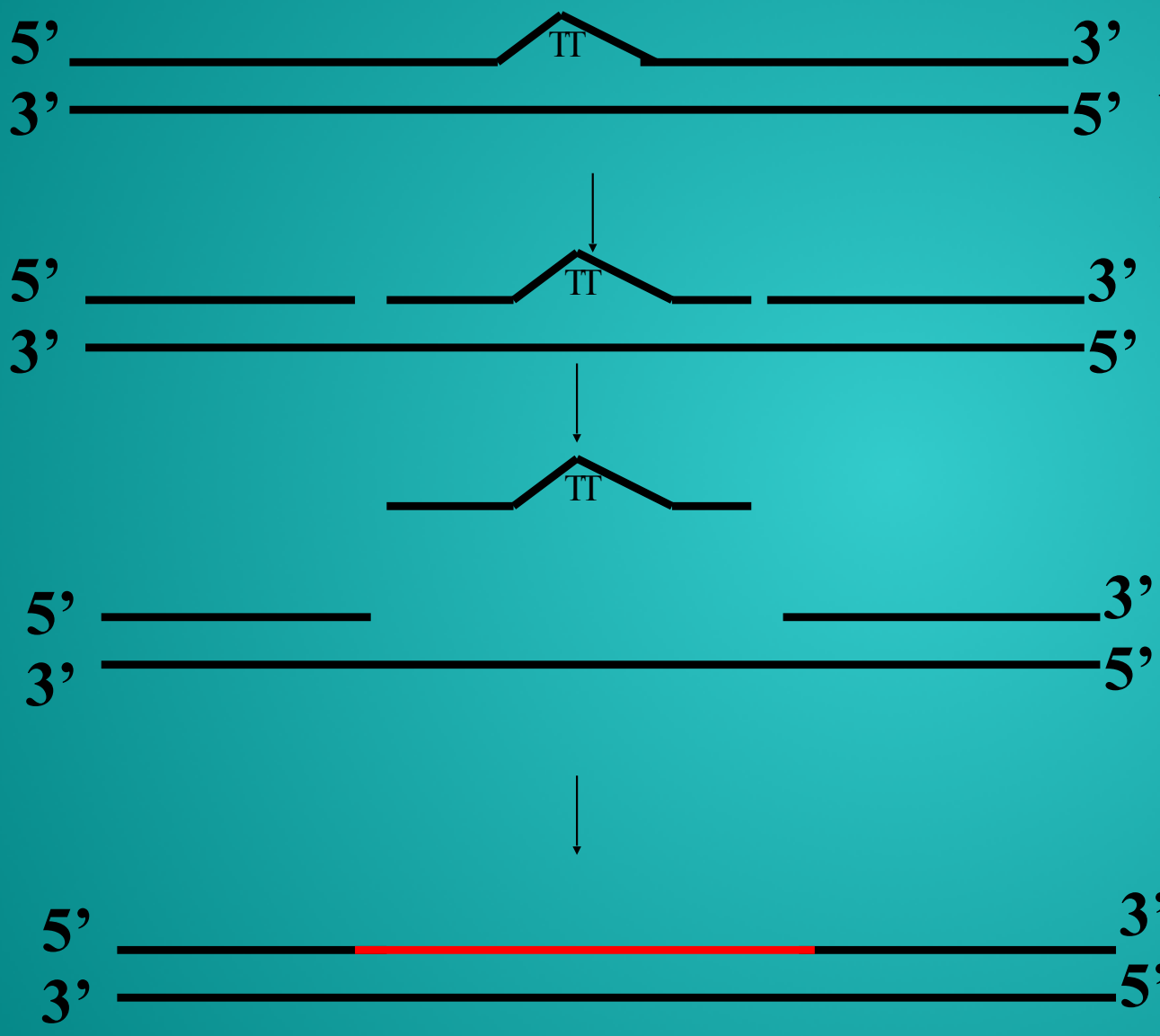
It is error-free.
The UvrABC complex is referred to as an exinuclease.

UvrAB proteins identify the bulky dimer lesion, UvrA protein then leaves, and UvrC protein then binds to UvrB protein and introduces the nicks on either side of the dimer.

In humans there is a similar process carried out by 2 related enzyme complexes: global excision repair and transcription coupled repair.

Several human syndromes deficient in excision repair, Xeroderma pigmentosum, Cockayne Syndrome, and are characterised by extreme sensitivity to UV light (& skin cancers)

Excision Repair in *E.coli*

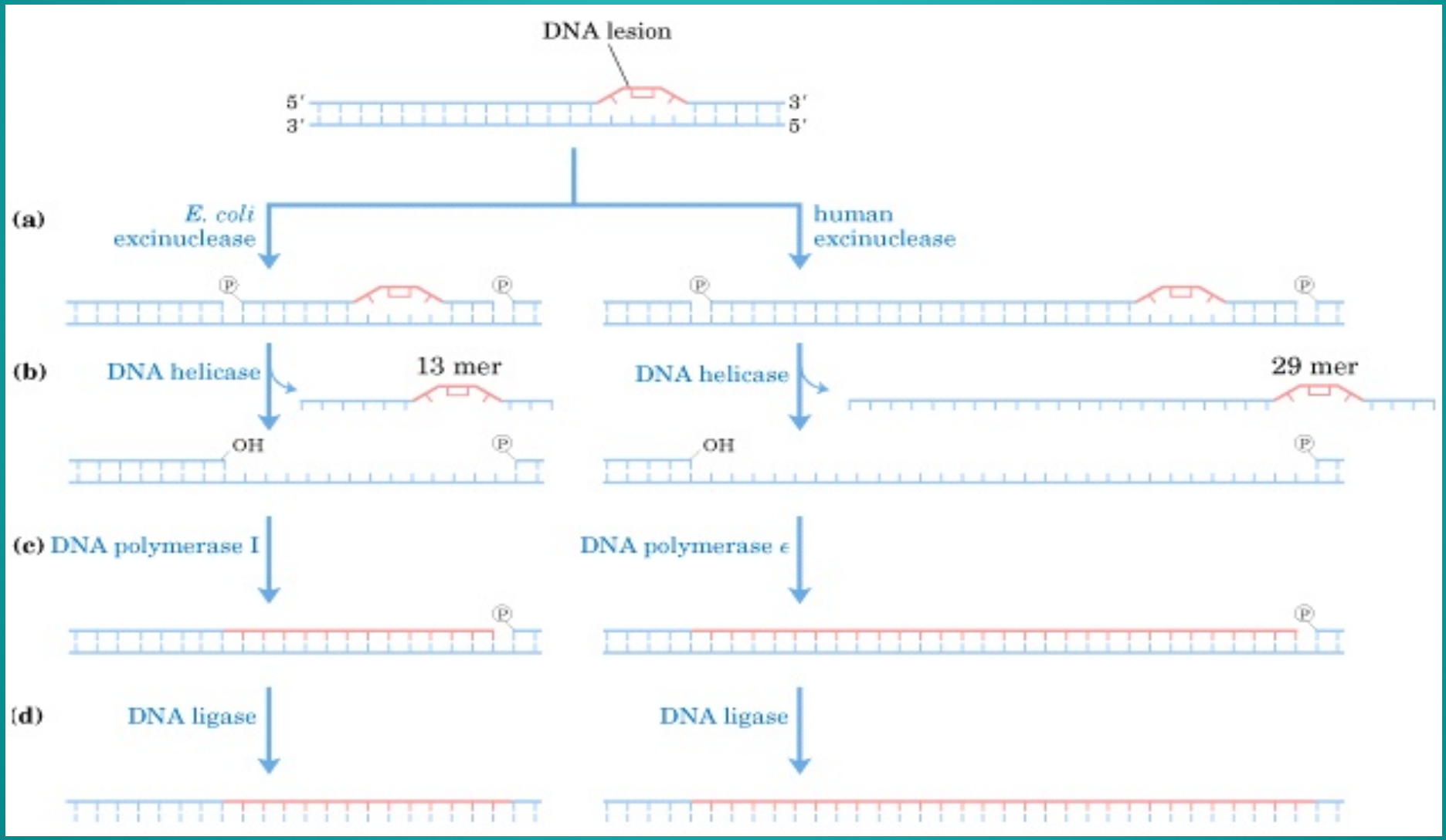


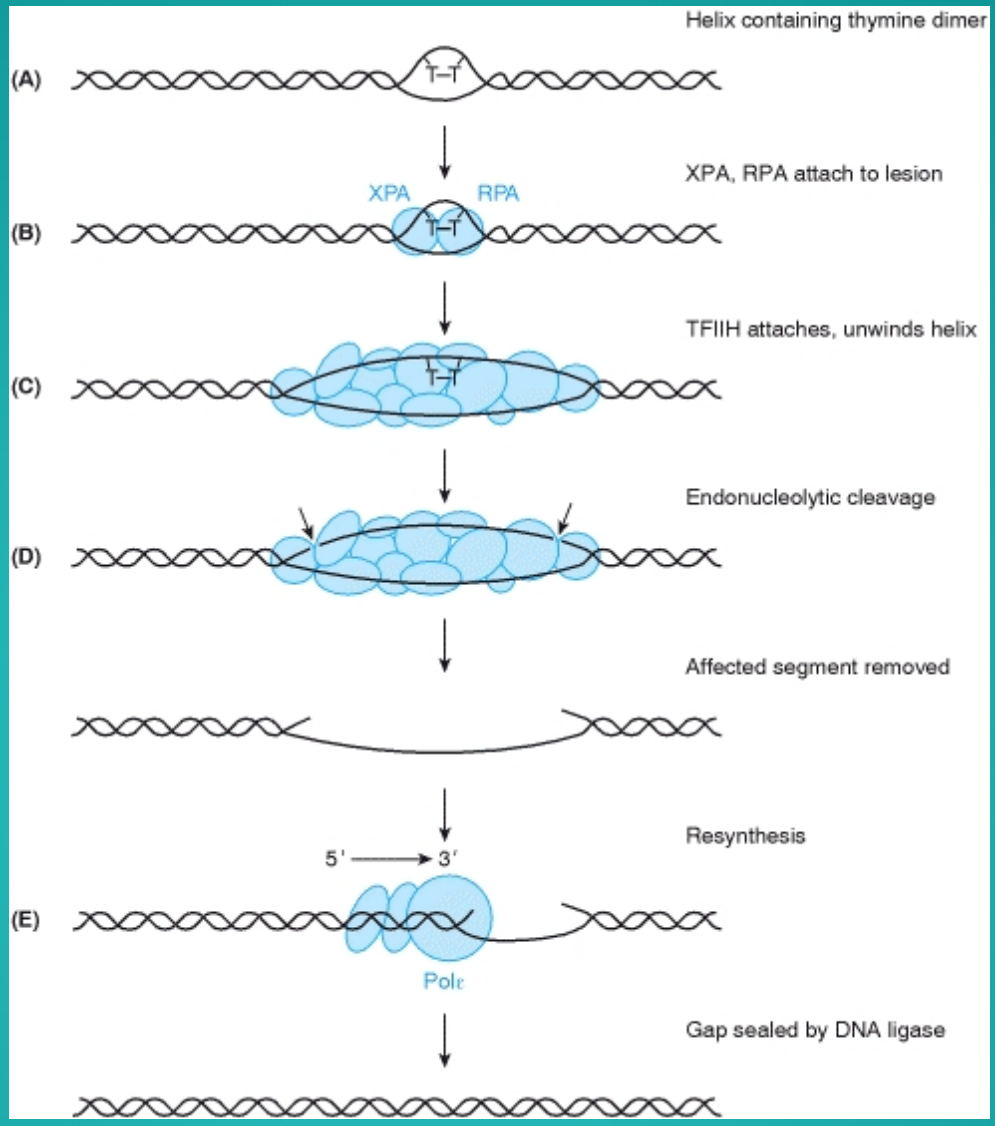
Damage recognised by UvrABC, nicks made on both sides of dimer

Dimer removed by UvrD, a helicase

Gap filled by DNA pol I and the nick sealed by DNA ligase

Nucleotide-Excision Repair in *E. coli* and Humans





A possible scheme for nucleotide excision repair in humans
(**A**) XPA protein recognizes damaged DNA and binds to it, directly or by binding to RPA, a single-strand binding protein. (**B**) The DNA-XPA-RPA complex recruits the TFIIH transcription factor. TFIIH is a multiprotein complex that includes the XPB and XPD proteins. These are helicases of opposite polarity, and they open up a single-stranded bubble in the DNA, about 30 nucleotides long. (**C**) Two cuts are made in the sugar-phosphate backbone of the damaged strand. XPF + ERCC1 cut at the 5′ end, and XPG cuts the 3′ end. (**D**) DNA polymerase ε together with replication factor C and the DPE2 subunit synthesize DNA to fill the gap. (**E**) DNA ligase seals the gap. Over 30 proteins are involved in mammalian nucleotide excision repair, and this simplified scheme does not include the likely requirement to remodel chromatin structure as part of the process

NER Defects cause

- Xeroderma Pigmentosum
  - 1874, when Moriz Kaposi used this term for the first time to describe the symptoms observed in a patient. XP patients exhibit an extreme sensitivity to sunlight and have more than 1000-fold increased risk to develop skin cancer, especiallyin regions exposed to sunlight such as hands, face, neck
- Cockayne Syndrome
  - A second disorder with UV sensitivity was reported by Edward Alfred Cockayne in 1936. Cockayne syndrome CS) is characterized by additional symptoms such as short stature, severe neurological abnormalities caused by dysmyelination, bird-like faces, tooth decay, and cataracts. CS patients have a mean life expectancy of 12.5 years but in contrast to XP do not show a clear predisposition to skin cancer. CS cells are deficient in transcription-coupled NER but are proficient in global genome NER.
- Trichothiodystrophy

  - A third genetic disease characterized by UV sensitivity, trichothiodystrophy (TTD, literally: "sulfur-deficient brittle hair"), was reported by Price in 1980. In addition to symptoms shared with CS patients, TTD patients show characteristic sulfur-deficient, brittle hair and scaling of skin. This genetic disorder is now known to correlate with mutations in genes involved in NER (*XPB*, *XPD*, and *TTDA* genes). All of these genes are part of the 10-subunit transcription/repair factor TFIIH, and TTD is likely to reflect an impairment of transcriptional transactions rather than regular defect in DNA repair. This disorder is therefore sometimes referred as a "transcriptional syndrome".

Mismatch repair (MMR)

Incorrect bases incorporated as a result of mistakes during DNA replication (base mispairs, short insertions and deletions) are excised as single nucleotides by a group of repair proteins which can scan DNA and look for incorrectly paired bases (or unpaired bases) which will have aberrant dimensions in the double helix.
Synthesis of the repair patch is done by a DNA polymerase **.**

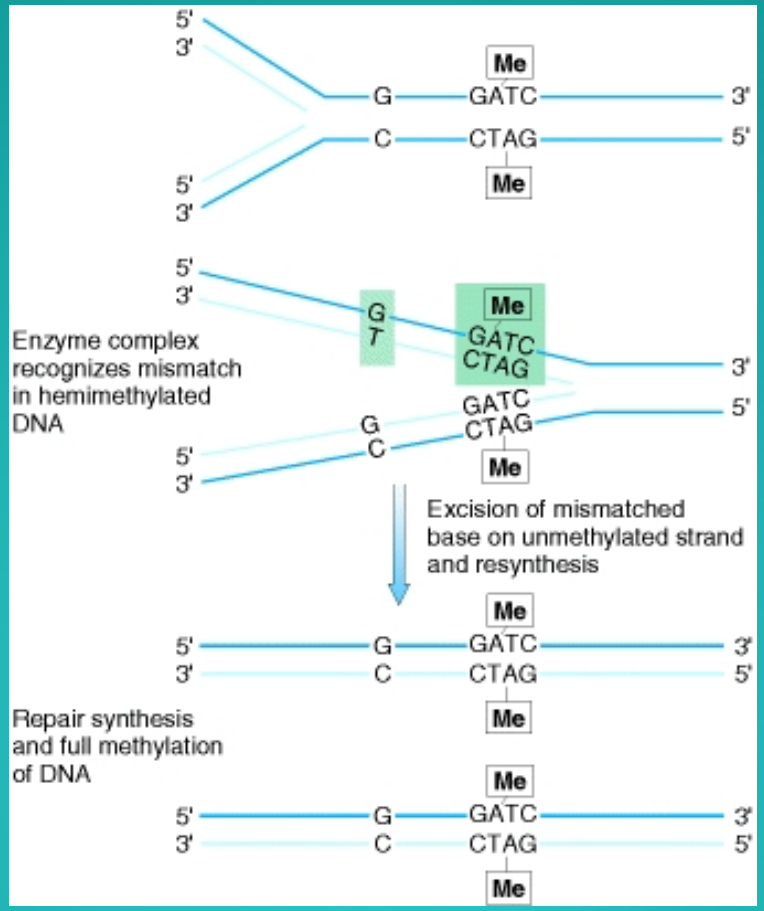
- MMR system is an excision/resynthesis system that can be divided into 4 phases:
- (i) recognition of a mismatch by MutS proteins,
- (ii) recruitment of repair enzymes
- (iii) excision of the incorrect sequence,
- (iv) resynthesis by DNA polymerase using the parental strand as a template.

Mismatch Repair in *E.coli*

- MutS is responsible for initiation of *E. coli* mismatch repair.
  - 95 kDa polypeptide, which exists as an equilibrium mixture of dimers and tetramers
  - recognizes mismatched base pairs.
- MutL, a 68 kDa polypeptide that is dimeric in solution, is recruited to the heteroduplex in a MutS- and ATP-dependent fashion.
- The MutL, MutS,heteroduplex complex is believed to be a key intermediate in the initiation of mismatch repair

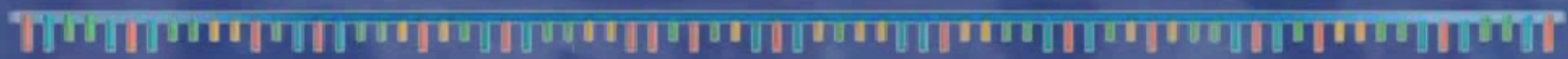
Methyl-directed mismatch repair

- If any mismatch escapes the proof reading mechanisms it will cause distortion of the helix.
- This can be detected and repaired but it is important that the repair enzyme can distinguish the new strand from the old.
- This is possible in *E. coli* because there is an enzyme which methylates the A in a sequence GATC. This methylation does not occur immediately after synthesis and until it does the two strands are distinguishable.



Model for mismatch repair in *E. coli.* Because DNA is methylated by enzymatic reactions that recognize the A in a GATC sequence, directly after DNA replication the newly synthesized strand will not be methylated. The “hemimethylated” DNA duplex serves as a recognition point for the mismatch-repair system in discerning the old from the new strand. Here a G·T mismatch is shown. The mismatch- repair system can recognize and bind to this mismatch, determine the correct (old) strand because it is the methylated strand of a hemimethylated duplex, and then excise the mismatched base from the new strand. Repair synthesis restores the normal base pair.

Methyl-directed mismatch repair



Genes Encoding Enzymes of Mismatch Repair

E. coli	*S. cerevisiae*	Human	Functions of Eukaryotic Proteins
MutS	MSH2	MSH2	MutSα (with MSH6; 80-90%); MutSβ (with MSH3)
"	MSH3	MSH3	MutSβ (with MSH2); repair of larger loops
"	MSH6	MSH6	MutSα (with MSH2); repair of mismatches and small loops
MutL	MLH1	MLH1	Forms heterodimers with the other three MutL homologs
"	PMS1	PMS2	MutLα (90%); Mismatch repair; endonuclease motif
"	MLH2	PMS1	MutLβ; Role unknown
"	MLH3	MLH3	MutLγ; Mismatch repair; endonuclease motif
MutH	?	?	?
uvrD	?	?	?
?	Exonuclease I	Exonuclease I	Excision (5' to 3' polarity)
?	RFC, PCNA, Polδ	RFC, PCNA, Polδ	Nick identification; gap filling

Damage tolerance:

1- Double strand break (D S B):

Naturally occurring reactive oxygen molecules and ionizing radiation are prevalent sources of such damage.
DSB's are a major cytotoxic lesion : even a single unrepaired DSB can be a lethal event.
There are two different mechanisms of repair:

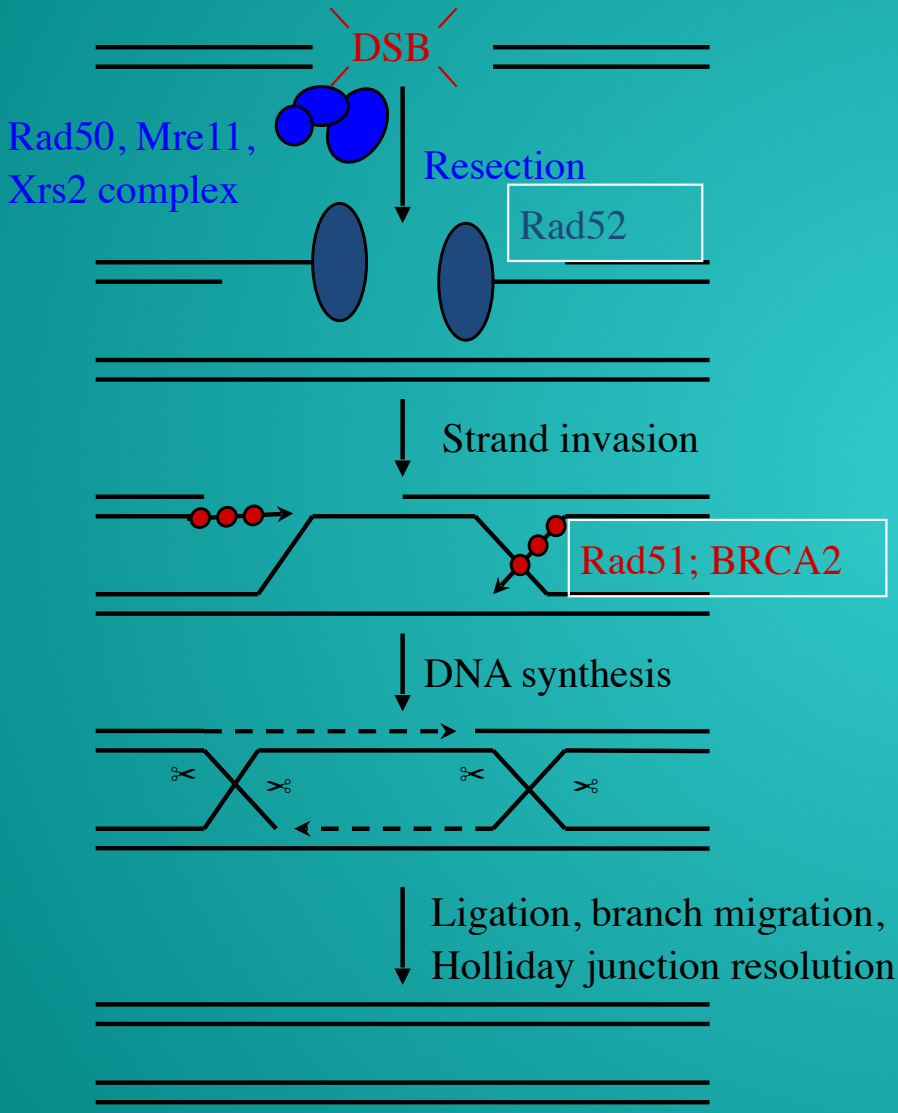
1. **End joining repair of DSB's:**

Joins broken chromosome ends in a manner that does not depend on sequence homology and may not be error free. incorrect ends may be joined, and repair mechanism causes sequence errors.
Three steps in end joining repair of DSB's:
1- Recognition of broken ends.
2- Unwinding of short stretch of DNA to uncover short regions of homology "microhomologies"
3- Removal of unpaired regions and ligation of products.
This mechanism: called non homologous end-joining.

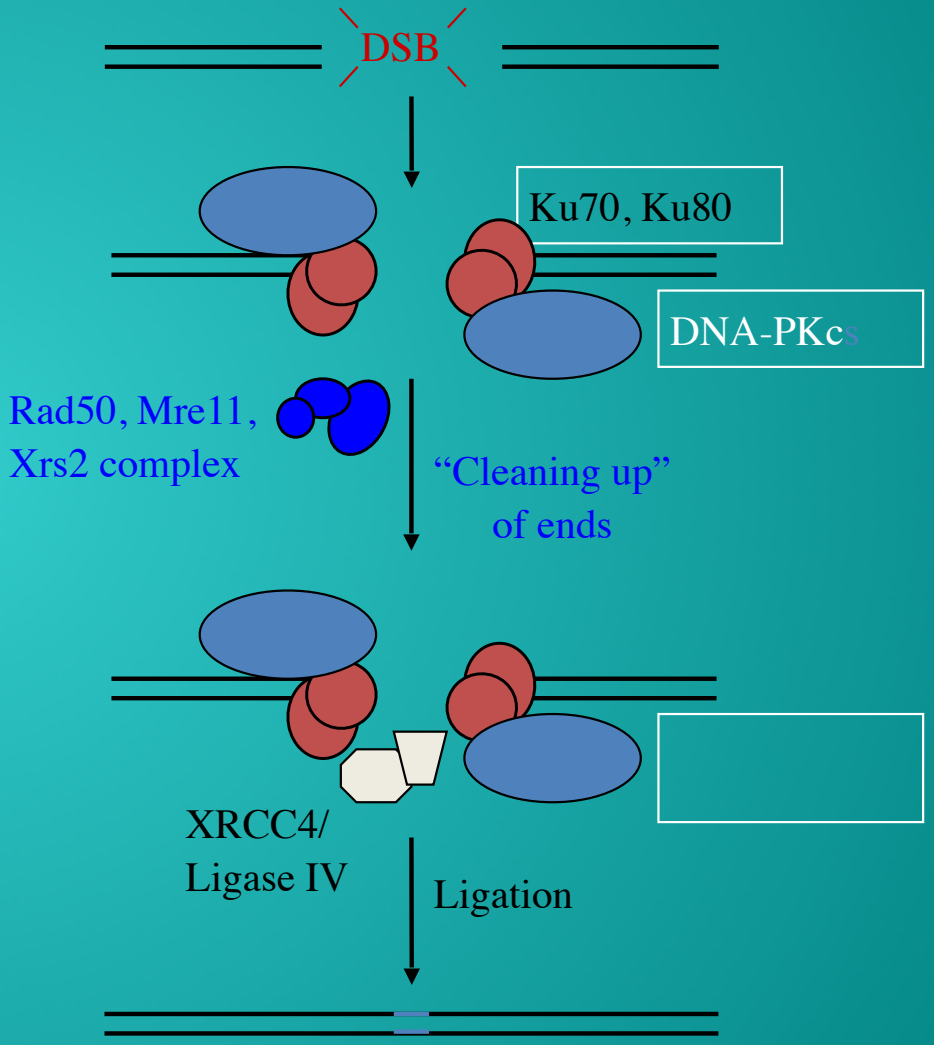
DNA non-homologous end-joining (NHEJ)

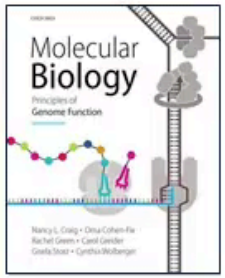
- Predominant mechanism for DSB repair in mammals.
- Also exists in single-celled eukaryotes, e.g. *Saccharomyces cerevisiae*
- Particularly important in G0/G1

Homologous recombination



Non-homologous end-joining





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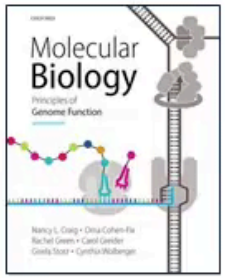
Animation 13: **Homology-dependent double strand break repair**

Animation produced by Connor Hendrich



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Animation 12: **Non-homologous end joining**

Animation produced by Connor Hendrich

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.

How is this SOS repair activated?

table 25–6

Genes Induced as Part of the SOS Response in *E. coli*

Gene name	Protein encoded and/or role in DNA repair
Genes of known function	
*pol*B (*din*A)	Encodes polymerization subunit of DNA polymerase II, required for replication restart in recombinational DNA repair
*uvr*A, *uvr*B	Encode ABC excinuclease subunits UvrA and UvrB
*umu*C, *umu*D	Encode DNA polymerase V
*sul*A	Encodes protein that inhibits cell division, possibly to allow time for DNA repair
*rec*A	Encodes RecA protein required for error-prone repair and recombinational repair
*din*B	Encodes DNA polymerase IV
Genes involved in DNA metabolism, but role in DNA repair unknown	
ssb	Encodes single-stranded DNA-binding protein (SSB)
*uvr*D	Encodes DNA helicase II (DNA-unwinding protein)
*him*A	Encodes subunit of integration host factor, involved in site-specific recombination, replication, transposition, regulation of gene expression
*rec*N	Required for recombinational repair
Genes of unknown function	
*din*D	
*din*F	

Note: Some of these genes and their functions are further discussed in Chapter 28.

The SOS response

In response to extensive genetic damage there is a regulatory system that co-ordinates the bacterial cell response. This results in the increased expression of >30 genes, involved in DNA repair, these include:

recA - activator of SOS response, recombination

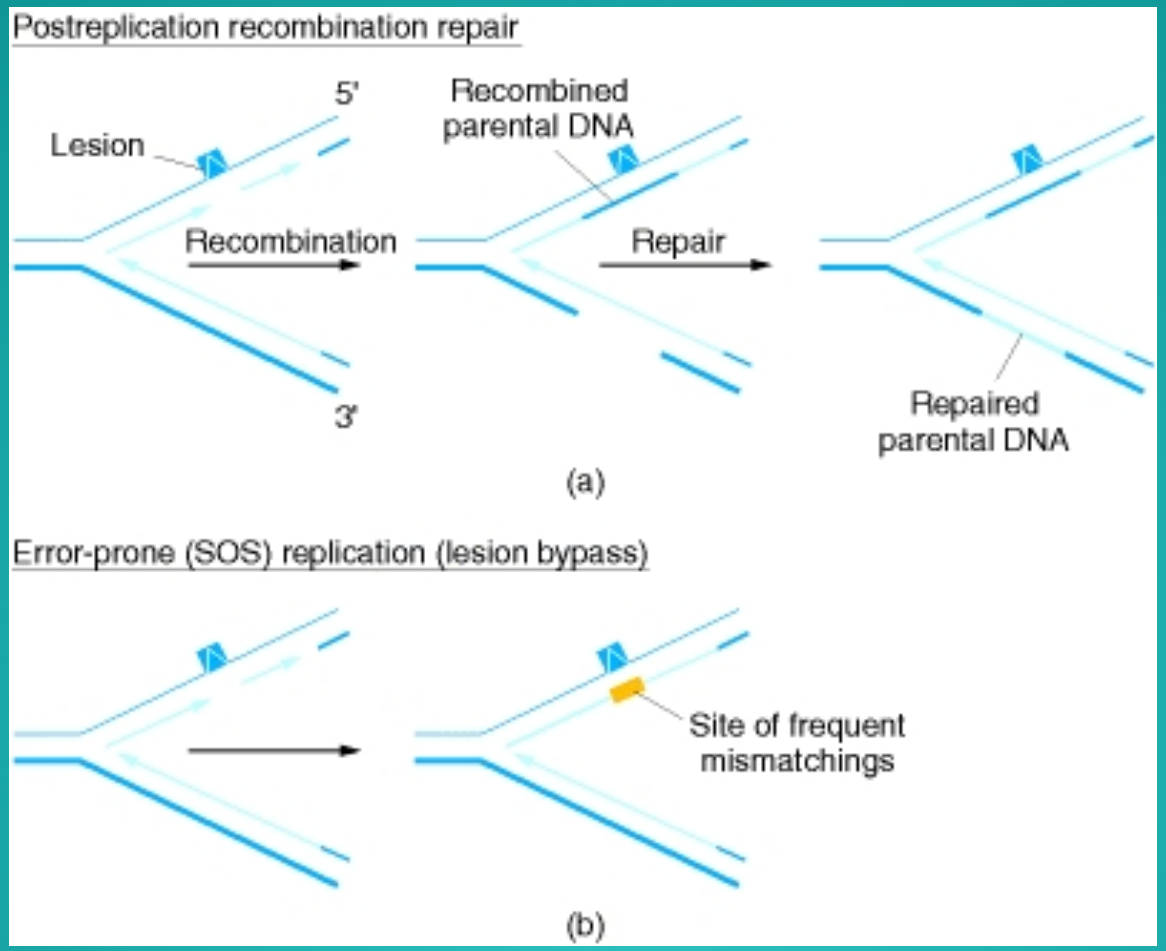
sfiA (sulA) - a cell division inhibitor (repair before replication)

umuC, D - an error prone bypass of thymine dimers (loss of fidelity in DNA replication)

uvrA,B,C,D - excision repair

The SOS response is regulated by two key genes:

recA & lexA



Schemes for postreplication repair. (a) In recombinational repair, replication jumps across a blocking lesion, leaving a gap in the new strand. A *recA*-directed protein then fills in the gap, using a piece from the opposite parental strand (because of DNA complementarity, this filler will supply the correct bases for the gap). Finally, the RecA protein repairs the gap in the parental strand. (b) In SOS bypass, when replication reaches a blocking lesion, the SOS system inserts the necessary number of bases (often incorrect ones) directly across from the lesion and replication continues without a gap. Note that, with either pathway, the original blocking lesion is still there and must be repaired by some other repair pathway.

Genetic diseases associated with defects in DNA repair systems

Disease	Symptoms	Genetic defect
Xeroderma pigmentosum	Frecklelike spots on skin, sensitivity to sunlight, predisposition to skin cancer	Defects in nucleotide-excision repair
Cockayne syndrome	Dwarfism, sensitivity to sunlight, premature aging, deafness, mental retardation	Defects in nucleotide-excision repair
Trichothiodystrophy	Brittle hair, skin abnormalities, short stature, immature sexual development, characteristic facial features	Defects in nucleotide-excision repair
Hereditary nonpolyposis colon cancer	Predisposition to colon cancer	Defects in mismatch repair
Fanconi anemia	Increased skin pigmentation, abnormalities of skeleton, heart, and kidneys, predisposition to leukemia	Possibly defects in the repair of interstrand cross-links
Ataxia telangiectasia	Defective muscle coordination, dilation of blood vessels in skin and eyes, immune deficiencies, sensitivity to ionizing radiation, predisposition to cancer	Defects in DNA damage detection and response
Li-Fraumeni syndrome	Predisposition to cancer in many different tissues	Defects in DNA damage response

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.

Hereditary DNA repair disorders

Defects in the NER mechanism are responsible for several genetic disorders, including:

Xeroderma pigmentosum: hypersensitivity to sunlight/UV, resulting in increased skin cancer incidence and premature aging

Cockayne syndrome: hypersensitivity to UV and chemical agents

Trichothiodystrophy: sensitive skin, brittle hair and nails

Mental retardation often accompanies the latter two disorders, suggesting increased vulnerability of developmental neurons.

Other DNA repair disorders include:

Werner's syndrome: premature aging and retarded growth

Bloom's syndrome: sunlight hypersensitivity, high incidence of malignancies (especially leukemias).

Ataxia telangiectasia: sensitivity to ionizing radiation and some chemical agents

All of the above diseases are often called "segmental progerias" ("accelerated aging diseases") because their victims appear elderly and suffer from aging-related diseases at an abnormally young age, while not manifesting all the symptoms of old age.

Other diseases associated with reduced DNA repair function include Fanconi's anemia, hereditary breast cancer and hereditary colon cancer.