DNA damage and Repair ;

and Diseases caused due to impairment in repair mechanism

I have never known a dull enzyme.

—*Arthur Kornberg, in the essay "For Love of Enzymes," 1975*

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- DNA is a relatively stable molecule, but Earth's natural environment is quite toxic, and damage to DNA is inevitable. Because each cell contains only one or two copies of its DNA, the DNA sequence is highly protected from harm.
- But DNA can be altered by mistakes made during its own replication or recombination.
- Damage and sequence alterations to DNA are often quickly repaired, but when they are not, the DNA becomes permanently altered and harbors a mutation. Mutations are changes in DNA sequence, and when mutations occur in germ-line cells, these changes are inheritable.

- **DNA damage** is distinctly different from mutation, although both are types of error in DNA.
- DNA damage is an abnormal chemical structure in DNA, while a mutation is a change in the sequence of standard base pairs.

- DNA damages cause changes in the structure of the genetic material and prevents the replication mechanism from functioning and performing properly.
- DNA damage and mutation have different biological consequences.
- While most DNA damages can undergo DNA repair, such repair is not 100% efficient.

Why a DNA damage needs restoration ?

- A cancer cell has mutations that prevent cell death, resulting in loss of cell cycle control and unregulated cell division, which leads to malignant tumors that can end the life of the entire organism.

- The cell has a limited amount of time to fix the initial alteration and restore the DNA to its normal sequence, before replication converts the alteration into a mutation that will be passed on to the next generation.

DNA damage may lead to Mutation

- Somatic mutations : occur in somatic cells and only affect the individual in which the mutation arises.
- Germ-line mutations: alter gametes and passed to the next generation.
- **The most harmful mutations are those occurring in the genes involved in DNA repair, because these often result in cancer.**
- Many mutations in one cell can result in cancer because a mutation will occur in a gene (or genes) that encodes a protein needed to control cell division.
- In normal cells, **oncogenes** encode proteins that drive the cell division cycle forward, and **tumor suppressor genes** encode proteins that suppress cell division.
- Many tumor suppressors are transcription factors that regulate the expression of genes that drive the cell cycle.
- The transcription factor p53 and the retinoblastoma protein are examples of tumor suppressors that are mutated in many types of cancer.

DNA damage and repair

- Sources of DNA damage
  - Spontaneous
  - Environmental
    - Radiation
    - Carcinogens
  - Mistakes in replication

Estimated rates of DNA damage per human cell per day:

Single strand breaks	50,000
Depurination	10,000
Deamination	600
Oxidative base damage	2000
Alkylated bases	5000
Intrastrand cross links	10
DNA double-strand break	10

Total DNA damaging events per cell per day: 60,000

Total DNA damaging events per cell per hour: 2,500

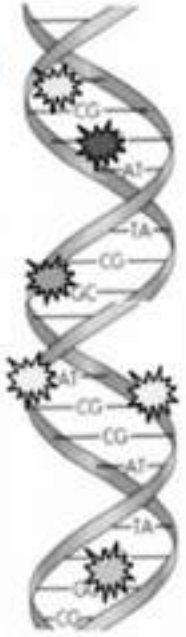
Estimate 10^{13} - 10^{14} cells in human body
– 3 x 10^{17} DNA damaging events per hour!

Commonly occurring types of DNA damage:

Spontaneous loss of bases

Alkylation of bases

Oxidation of bases



DNA strand breaks:
Natural cellular processes, exposure to radiation (cosmic, medical e.g. X-rays, radiation therapy) and some forms of chemotherapy

UV-light induced damage:
Cyclobutane dimers
6,4,-photoproducts

DNA damage, repair and its significance

DNA damage, repair mechanisms and consequences

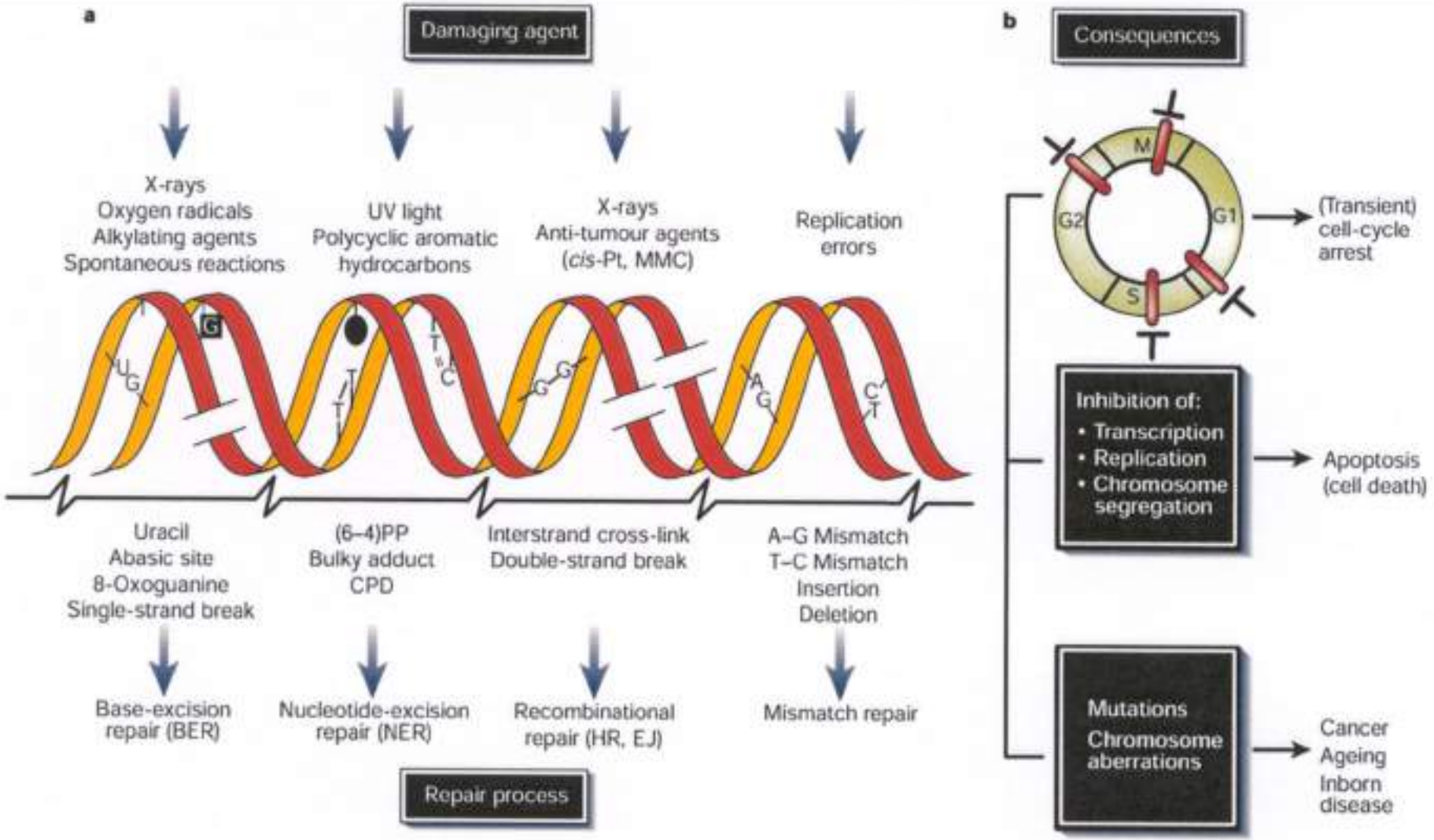


Figure source: Karp , Cell and Molecular Biology

DNA Damage

Spontaneous DNA damage:

- **Spontaneous alteration of bases,**
- **Depurination and Deamination,**
- **Thymine dimer**

Spontaneous Alterations of nucleotides

Red: oxidative damage
blue: hydrolytic attack
green: uncontrolled methylation

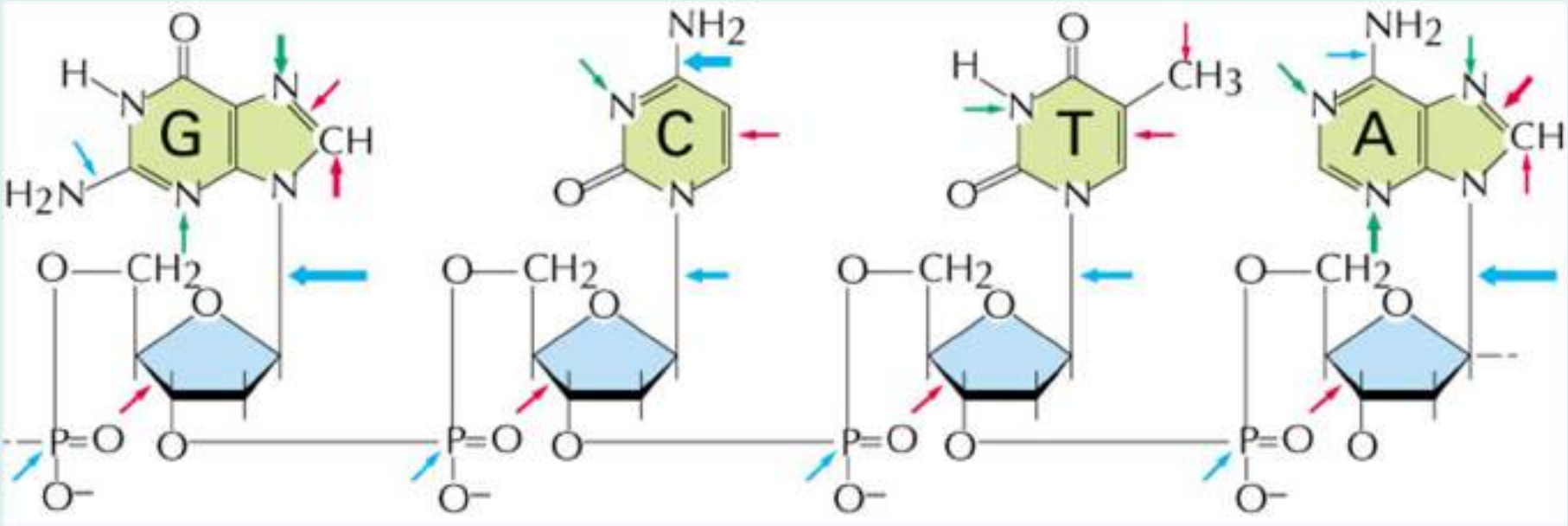


Figure source- Alberts, Molecular Biology of the Cell

Depurination and Deamination

1. Depurination: A, G
2. Deamination: C --> U, A --> Hypoxanthine

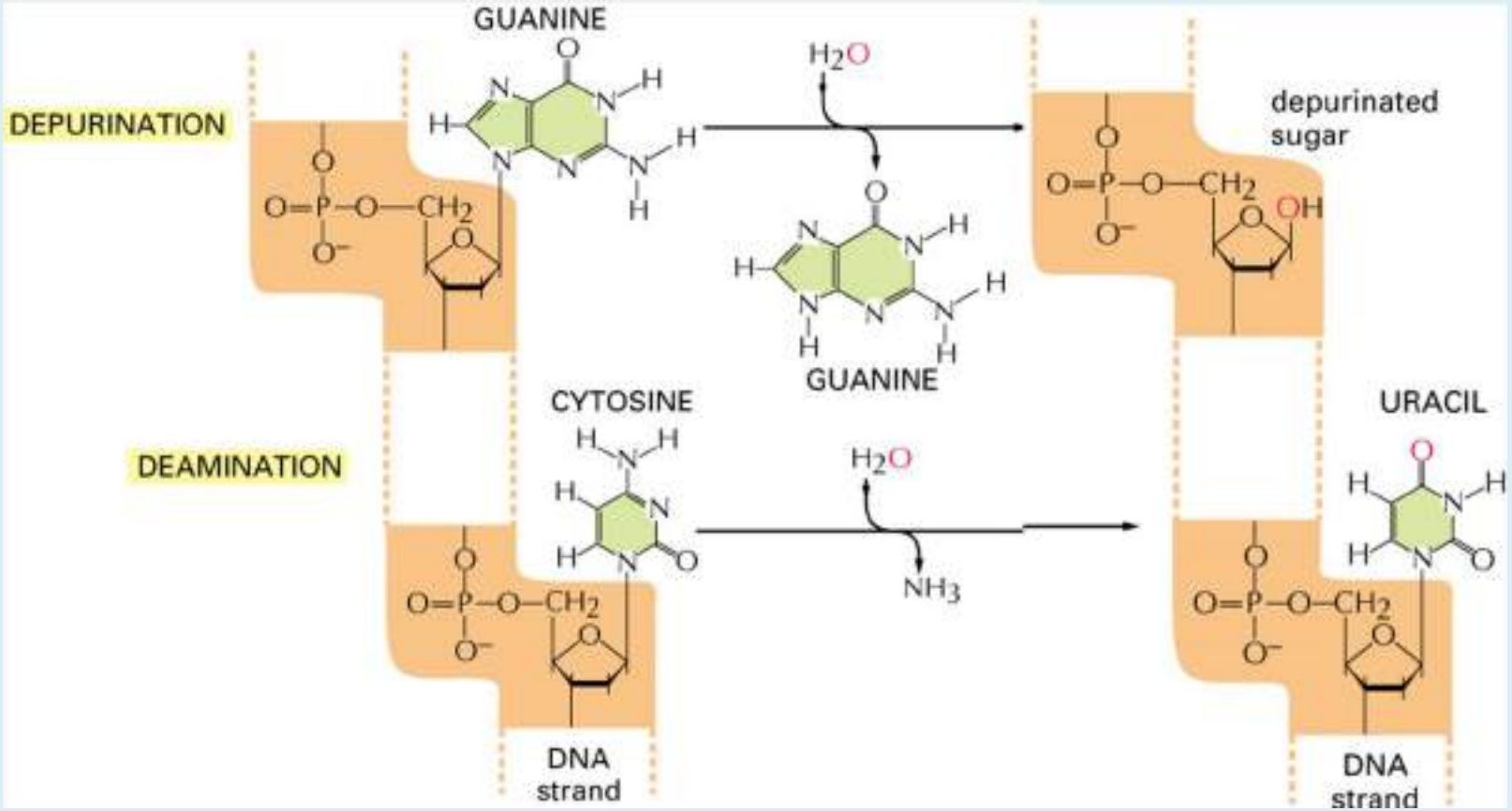


Figure source- Alberts, Molecular Biology of the Cell

Thymine dimer

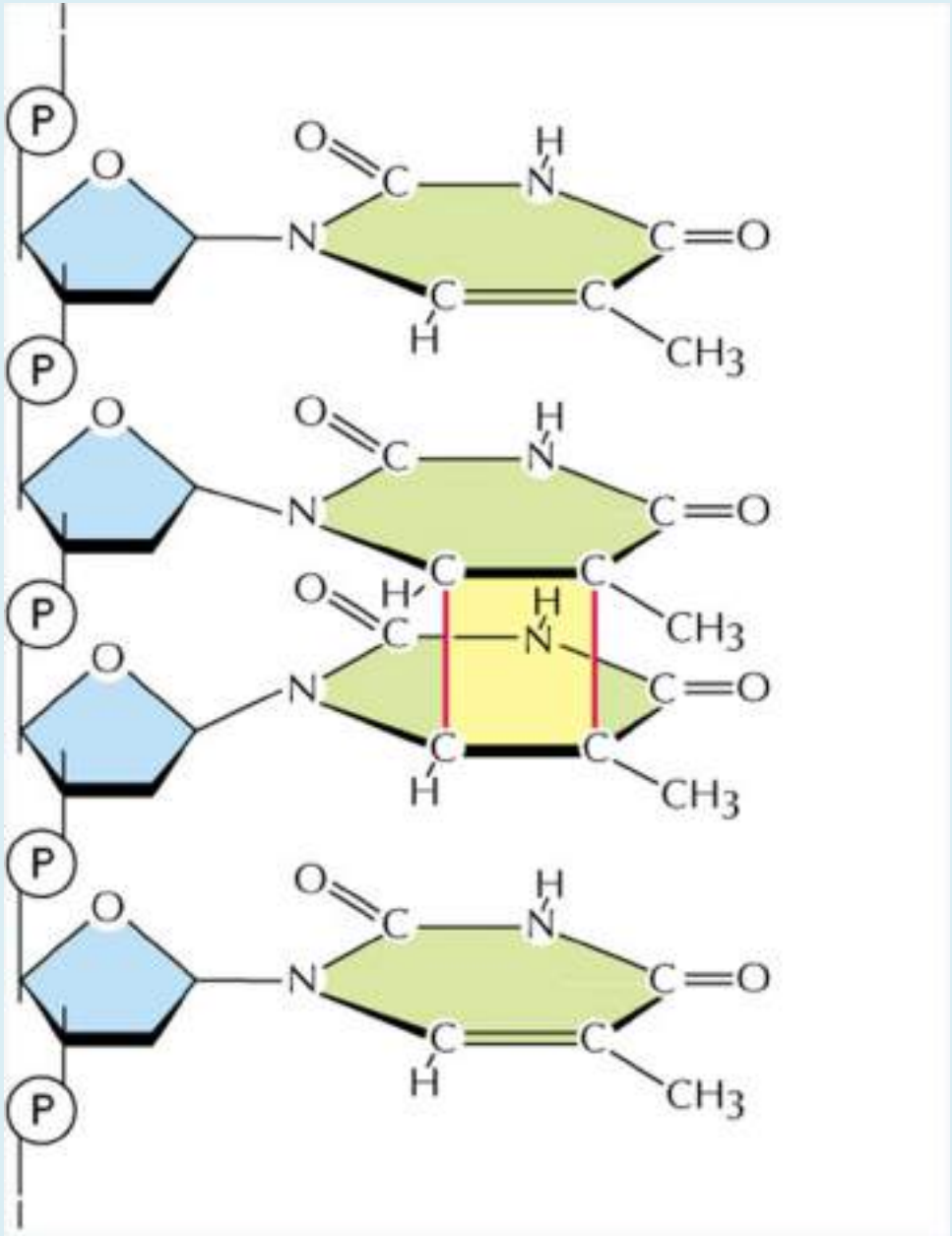


Figure source- Alberts, Molecular Biology of the Cell

DNA Repair

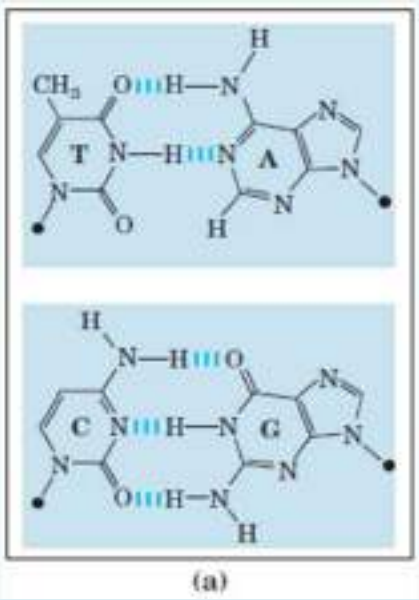
Comparison of Three DNA Polymerases of *E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	*polA*	*polB*	*polC (dnaE)*
Subunits (number of different types)	1	7	≥10
M_r	103,000	88,000†	791,500
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/s)	16–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

Source- Lehninger, Principles of Biochemistry , By Nelson and Cox

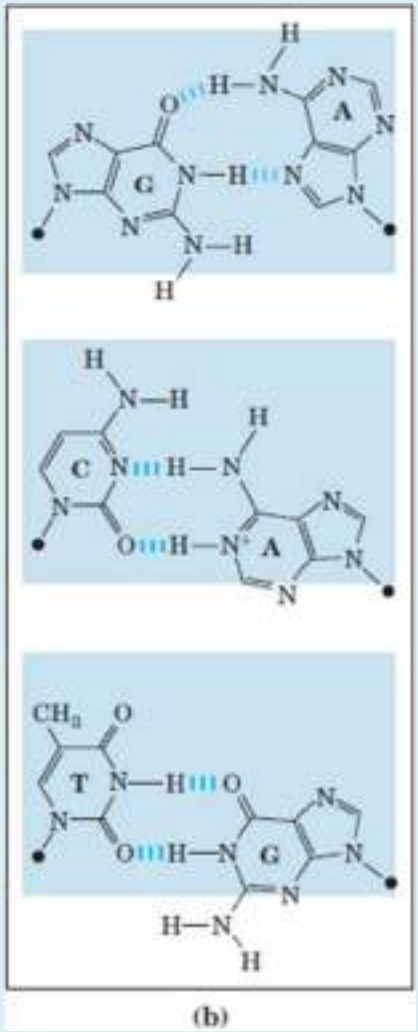
1. **During Replication**
   - i. DNA Proofreading
   - ii. **Mismatch Repair**

2. **Post Replication**
   - i. **Excision Repair**
     - a. **Base Excision Repair**
     - b. **Nucleotide Excision Repair**
   - ii. Double-strand break Repair (NEJ and HEJ)
   - iii. Direct Repair

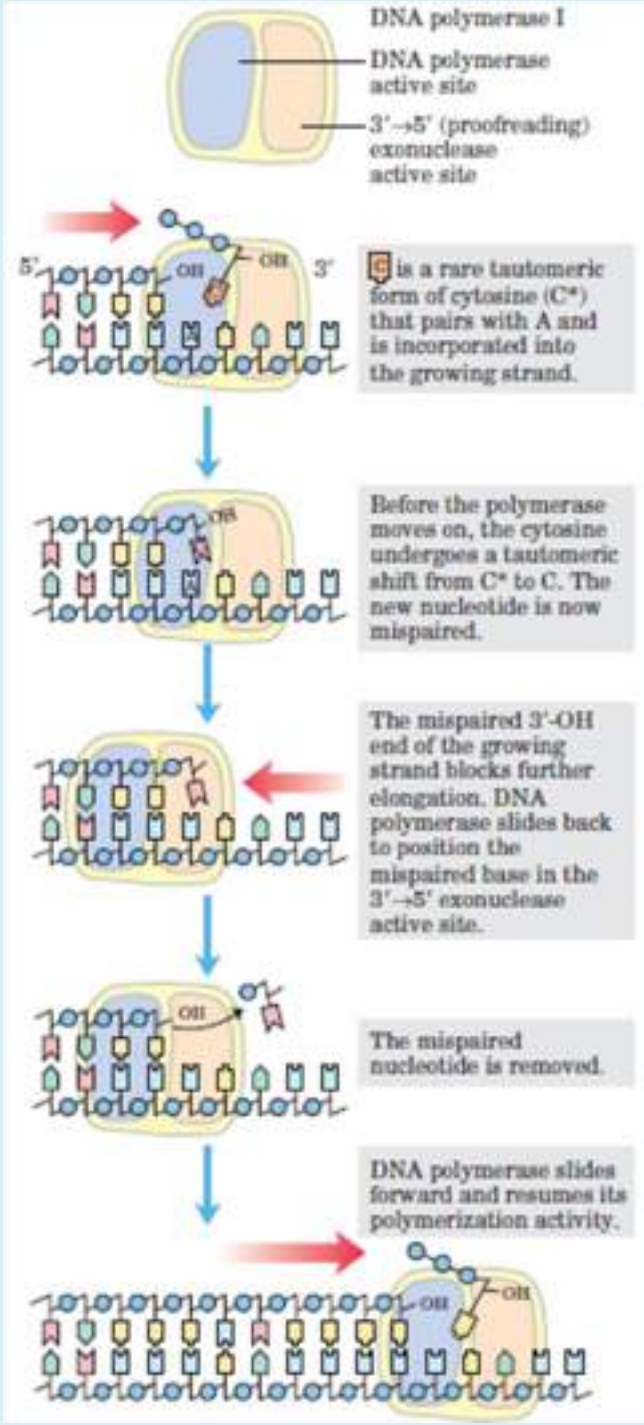


Standard Watson Crick pairing

A = T, G ≡ C



Incorrect pairing, detected by DNA polymerase proofreading activity



An example of error correction by the 3' → 5' exonuclease activity of DNA polymerase I.

Source- Lehninger, Principles of Biochemistry , By Nelson and Cox

Enzymes/proteins	Type of damage
Mismatch repair	
Dam methylase MutH, MutL, MutS proteins DNA helicase II SSB DNA polymerase III Exonuclease I Exonuclease VII RecJ nuclease Exonuclease X DNA ligase	Mismatches
Base-excision repair	
DNA glycosylases AP endonucleases DNA polymerase I DNA ligase	Abnormal bases (uracil, hypoxanthine, xanthine); alkylated bases; in some other organisms, pyrimidine dimers
Nucleotide-excision repair	
ABC excinuclease DNA polymerase I DNA ligase	DNA lesions that cause large structural changes (e.g., pyrimidine dimers)
Direct repair	
DNA photolyases	Pyrimidine dimers
O^6-Methylguanine-DNA methyltransferase	O^6-Methylguanine
AlkB protein	1-Methylguanine, 3-methylcytosine

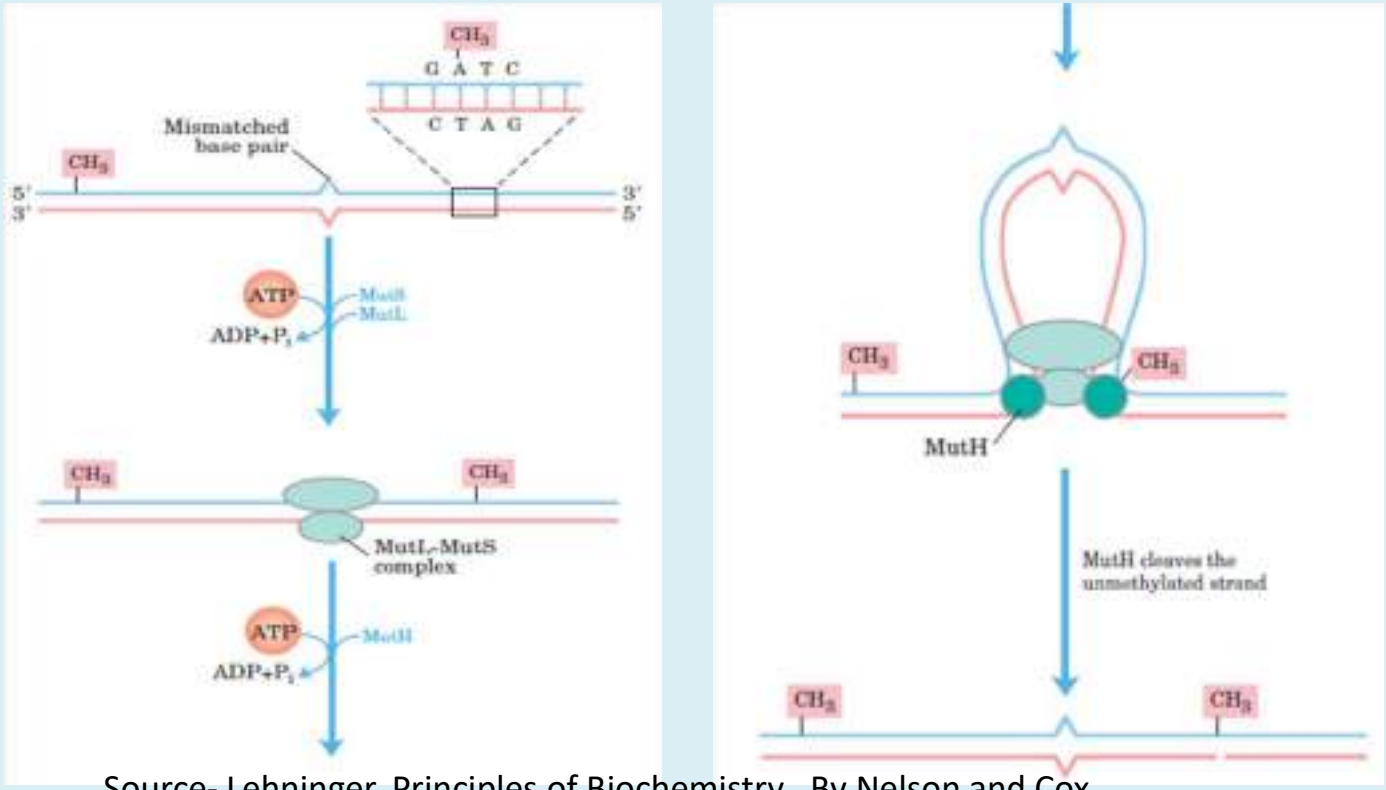
Types of DNA repair in E.coli

The number and diversity of repair systems reflect both the importance of DNA repair to cell survival and the diverse sources of DNA damage. DNA repair is possible largely because the DNA molecule consists of two complementary strands. DNA damage in one strand can be removed and accurately replaced by using the undamaged complementary strand as a template. We consider here the principal types of repair
Systems.

Source- Lehninger, Principles of Biochemistry , By Nelson and Cox

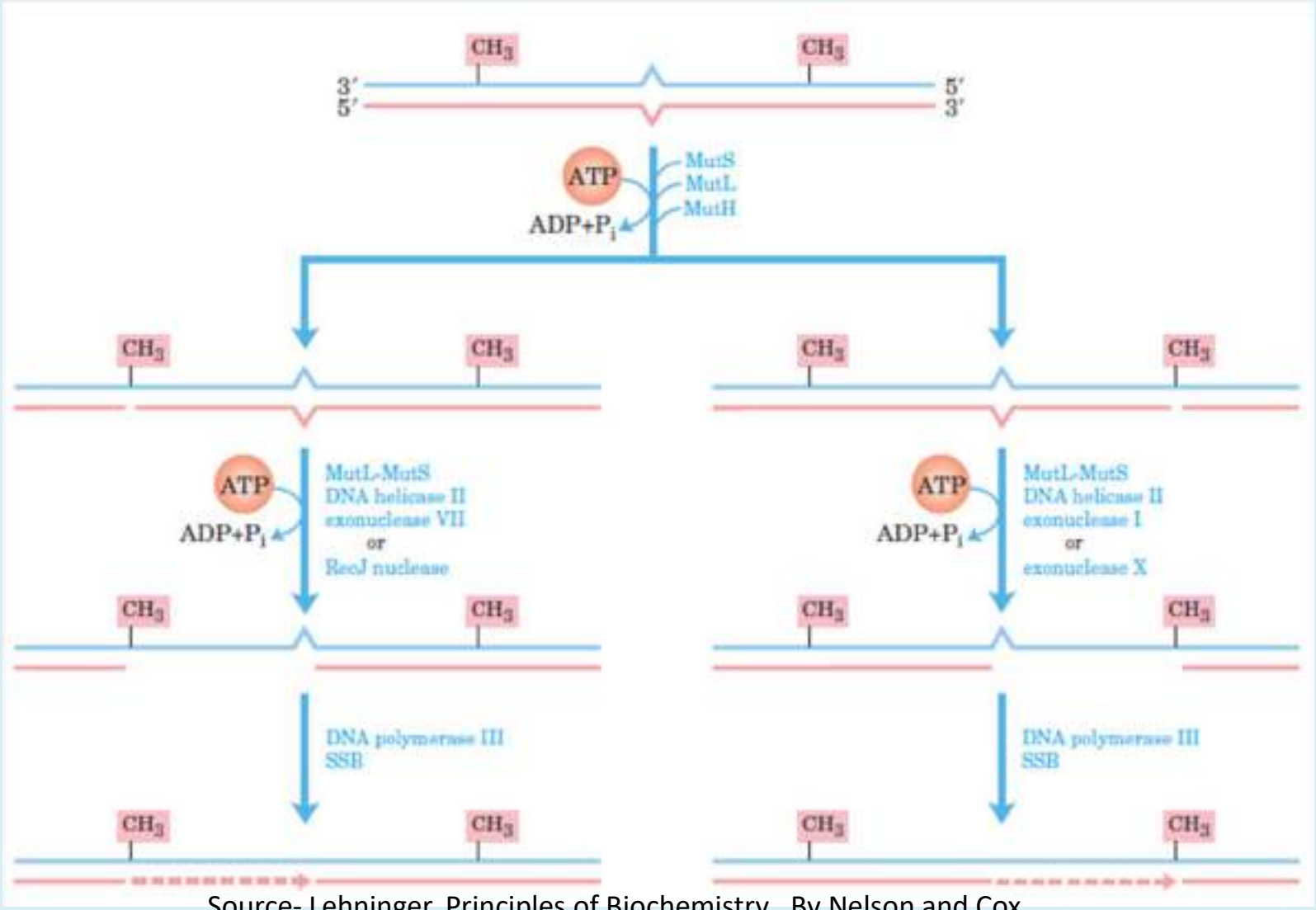
Mismatch Repair

- Mismatched nucleotides incorporated by the replication apparatus are corrected by the **mismatch repair (MMR)** system, which is conserved in all cell types from bacteria to humans. The mismatches are nearly always corrected **to reflect the information in the parent strand**.
- The *E. coli* MMR system can also recognize small loops of up to 4 bp of unpaired nucleotides. If left unrepaired, these small loops of extra DNA result in deletions or insertions. Loops of more than 4 bp are not recognized by the MMR system. Thus, larger 'indels' are simply not corrected by MMR.



Source- Lehninger, Principles of Biochemistry , By Nelson and Cox

- Early steps in MMR
- Recognition of the sequence (5)’ GATC and of the mismatch are specialized functions of the MutH and MutS proteins, respectively. The MutL protein forms a complex with MutS at the mismatch.



Source- Lehninger, Principles of Biochemistry , By Nelson and Cox

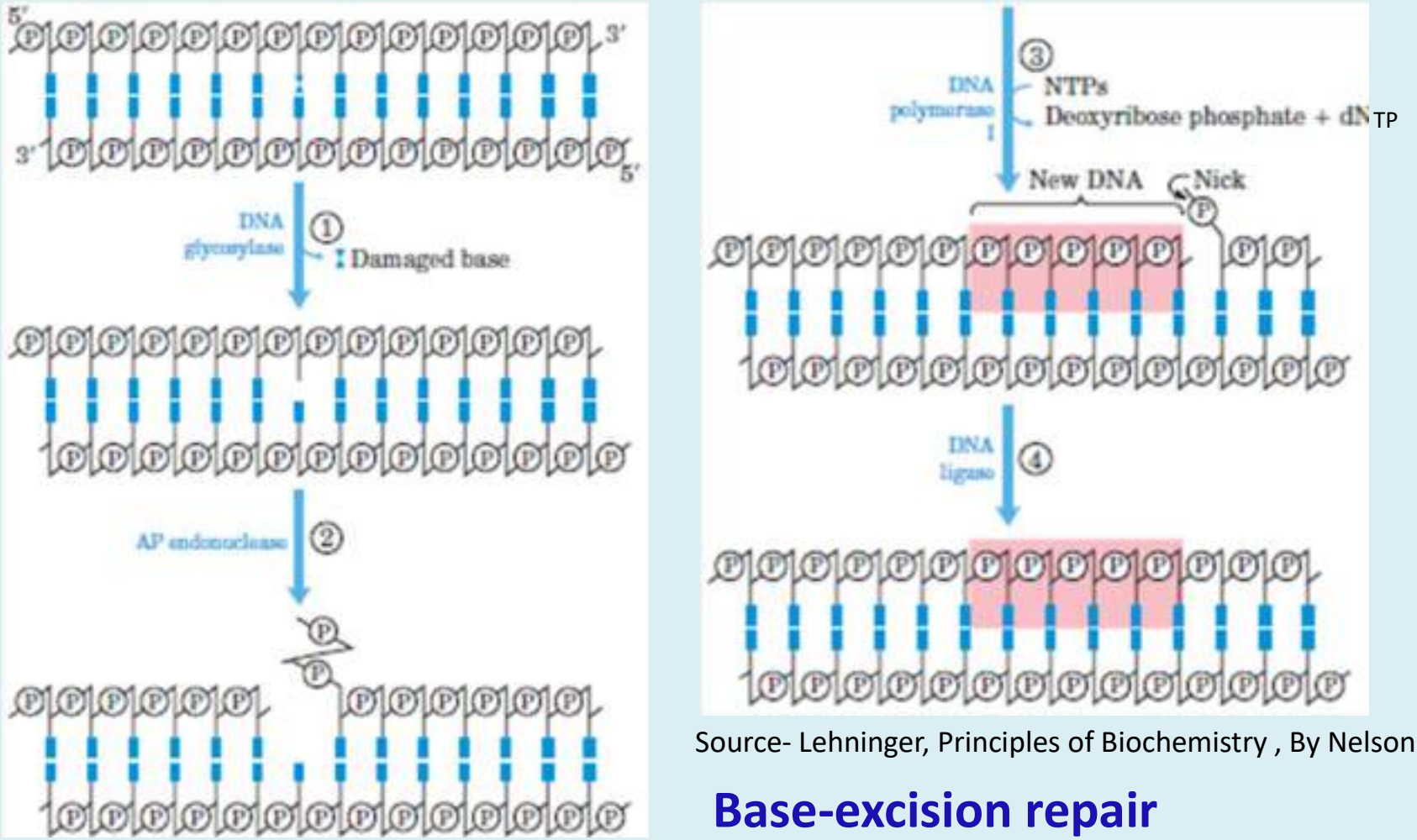
- DNA is threaded through this complex such that the complex moves simultaneously in both directions along the DNA until it encounters a MutH protein bound at a hemimethylated GATC sequence.
- MutH cleaves the unmethylated strand on the 5’ side of the G in this sequence. A complex consisting of DNA helicase II and one of several exonucleases then degrades the unmethylated DNA strand from that point toward the mismatch.
- The combined action of DNA helicase II, SSB, and one of four different exonucleases removes a segment of the new strand between the MutH cleavage site and a point just beyond the mismatch. The exonuclease that is used depends on the location of the cleavage site relative to the mismatch, as shown by the alternative pathways here.
- The resulting gap is filled in by DNA polymerase III, and the nick is sealed by DNA ligase.

Excision Repair

The most prevalent means that cells use to repair damaged DNA is excision repair, of which there are two types: base excision and nucleotide excision repair.

1. **Base excision repair (BER)** functions at the level of a **single damaged nucleotide** that distorts DNA very little. It is also the main pathway for the repair of single-strand DNA breaks that lack a ligatable junction and therefore require **"cleaning"** of the 3′ or 5′ terminus for ligation.
2. **Nucleotide excision repair (NER)** targets large, **bulky lesions** and removes DNA on either side of them. In contrast to base excision repair, **NER does not require specific recognition of a damaged nucleotide and** thus it can remove **DNA lesions**.

Base excision repair (BER) - Every cell has a class of enzymes called **DNA glycosylases** that recognize particularly common DNA lesions (such as the products of cytosine and adenine deamination) and remove the affected base by cleaving the *N*-glycosyl bond. This cleavage creates an apurinic or apyrimidinic site in the DNA, commonly referred to as an **AP site** or **abasic site**. Each DNA glycosylase is generally specific for one type of lesion.



Source- Lehninger, Principles of Biochemistry , By Nelson and Cox

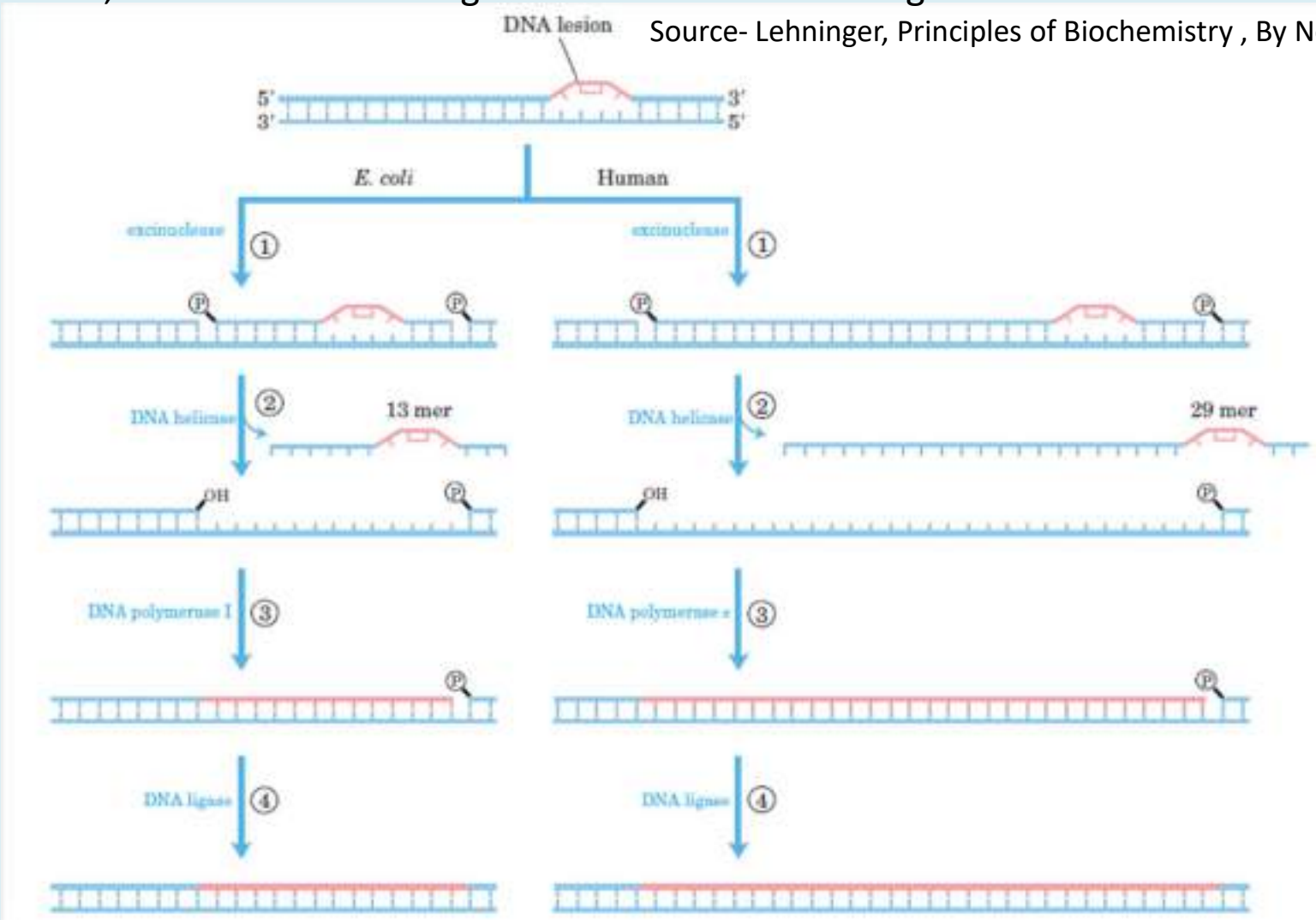
Base-excision repair

1. A DNA glycosylase recognizes a damaged base and cleaves between the base and deoxyribose in the backbone.
2. An AP endonuclease cleaves the phosphodiester backbone near the AP site.
3. DNA polymerase I initiates repair synthesis from the free 3’ hydroxyl at the nick, removing (with its 5’- 3’ exonuclease activity) and replacing a portion of the damaged strand.
4. The nick remaining after DNA polymerase I has dissociated is sealed by DNA ligase.

Nucleotide-excision repair

The general pathway of nucleotide-excision repair is similar in all organisms. 1. An excinuclease binds to DNA at the site of a bulky lesion and cleaves the damaged DNA strand on either side of the lesion. 2. The DNA segment—of 13 nucleotides (13 mer) or 29 nucleotides (29 mer)—is removed with the aid of a helicase. 3 .The gap is filled in by DNA polymerase, and 4. the remaining nick is sealed with DNA ligase.

Source- Lehninger, Principles of Biochemistry , By Nelson and Cox



- In the NER pathway of *E. coli*, the key enzymatic complex is the ABC excinuclease, which has three subunits, UvrA (*M*r 104,000), UvrB (*M*r 78,000), and UvrC (*M*r 68,000). The term "**excinuclease**" is used to describe the unique capacity of this enzyme complex to catalyze two specific endonucleolytic cleavages, distinguishing this activity from that of standard endonucleases.
- A complex of the UvrA and UvrB proteins (A2B) scans the DNA and binds to the site of a lesion. The UvrA dimer then dissociates, leaving a tight UvrB-DNA complex. UvrC protein then binds to UvrB, and UvrB makes an incision at the fifth phosphodiester bond on the 3 side of the lesion. This is followed by a UvrC-mediated incision at the eighth phosphodiester bond on the 5 side. The resulting 12 to 13 nucleotide fragment is removed by UvrD helicase.
- The short gap thus created is filled in by DNA polymerase I and DNA ligase. This pathway is a primary repair route for many types of lesions, including cyclobutane pyrimidine dimers, 6-4 photoproducts.

Double-Strand breaks repair

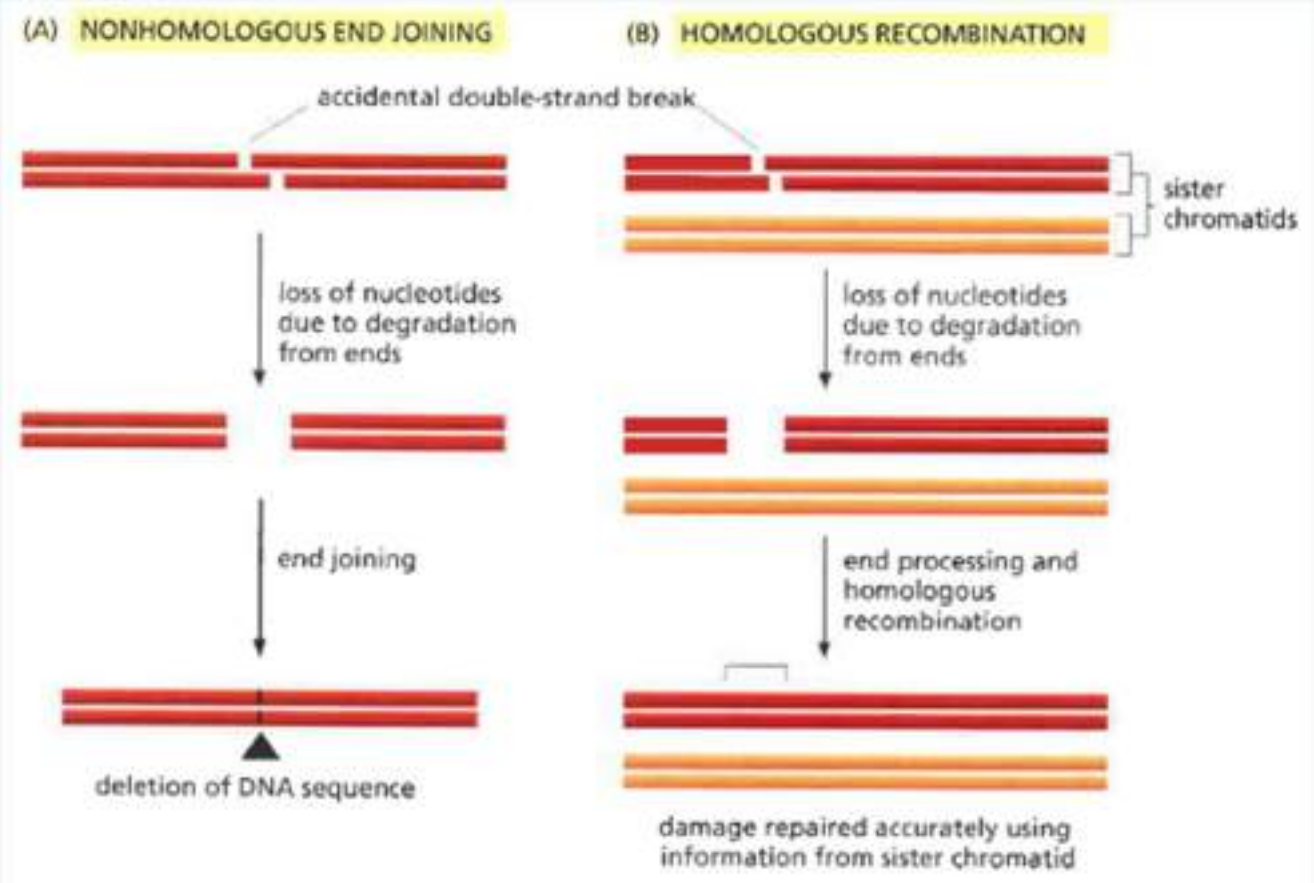


Figure source- Alberts, Molecular Biology of the Cell

Two different ways to repair double-strand breaks

(A) **Non-homologous end-joining (NHEJ)** alters the original DNA Sequence when repairing a broken Chromosome. These alteration can be either deletions or short insertions

(B) Repairing double-strand breaks by homologous re combinations mediated **homologous end joining (HEJ**) is more difficult to accomplish but this type of repair restores the original DNA sequence typically takes place after the DNA has been duplicated but before the Cell has divided.

Direct repair

- Several types of damage are repaired without removing a base or nucleotide. The best characterized example is direct photoreactivation of cyclobutane pyrimidine dimers, a reaction promoted by **DNA photolyases**.
- Pyrimidine dimers result from a UV-induced reaction, and photolyases use energy derived from absorbed light to reverse the damage. Photolyases generally contain two cofactors that serve as light-absorbing agents, or chromophores.
- One of the chromophores is always FADH. In *E. coli* and yeast, the other chromophore is a folate. The reaction mechanism entails the generation of free radicals.
- DNA photolyases are not present in the cells of placental mammals (which include humans). **
- Additional examples can be seen in the repair of nucleotides with alkylation damage. The modified nucleotide *O*6-methylguanine forms in the presence of alkylating agents and is a common and highly mutagenic lesions.
- A very different but equally direct mechanism is used to repair 1-methyladenine and 3-methylcytosine.

❖ Error-Prone Translesion DNA Synthesis

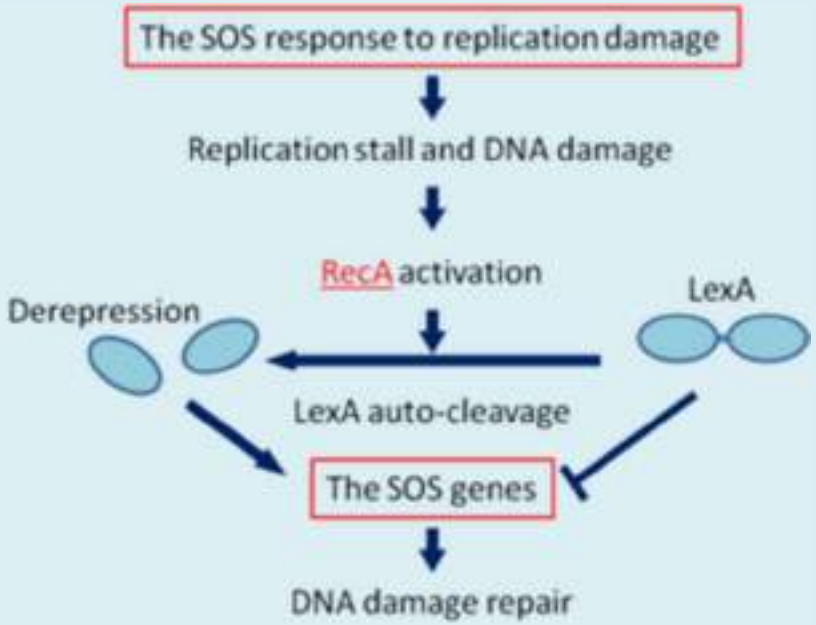
- When the Interaction of Replication Forks ocurs with DNA Damage, Under some conditions, a second repair pathway, **error-prone translesion DNA synthesis** (often abbreviated **TLS**), becomes available. When this pathway is active, DNA repair becomes significantly less accurate and a high mutation rate can result.
- In bacteria, error-prone translesion DNA synthesis is part of a cellular stress response to extensive DNA damage known, appropriately enough, as the **SOS response**.
- Some SOS proteins, such as the UvrA and UvrB proteins already described are normally present in the cell but are induced to higher levels as part of the SOS response.

The process of tolerance to DNA damage, which allows DNA replication machinery to replicate past DNA lesions, is called "translesion synthesis" (TLS).

SOS Repair

- The DNA repair systems described so far are quite accurate. However, when the DNA of *E. coli* cells is heavily damaged by mutagenic agents such as UV light, the cells take some drastic steps in their attempt to survive.
- They go through a so-called **SOS response,** during which a whole battery of DNA repair, recombination, and replication proteins are synthesized. Two of these proteins, encoded by the *umuC* and *umuD* (*U*V *mu*table) genes, are subunits of DNA polymerase V, an enzyme that catalyzes the replication of DNA in damaged regions of the chromosome—regions where replication by DNA polymerase III is blocked.
- DNA polymerase V allows replication to proceed across damaged segments of template strands, even though the nucleotide sequences in the damaged region cannot be replicated accurately. This *error-prone repair* system eliminates gaps in the newly synthesized strands opposite damaged nucleotides in the template strands but, in so doing, increases the frequency of replication errors.
- The mechanism by which the SOS system is induced by DNA damage has been worked out in considerable detail. Two key regulatory proteins—LexA and RecA— control the SOS response. Both are synthesized at low background levels in the cell in the absence of damaged DNA.

- Under this condition, LexA binds to the DNA regions that regulate the transcription of the genes that are induced during the SOS response and keeps their expression levels low.
- When cells are exposed to ultraviolet light or other agents that cause DNA damage, the RecA protein binds to single-stranded regions of DNA caused by the inability of DNA polymerase III to replicate the damaged regions.
- The interaction of RecA with DNA activates RecA, which then stimulates LexA to inactivate itself by self-cleavage. With LexA inactive, the level of expression of the SOS genes—including *recA*, *lexA, umuC, umuD,* and others— increases and the error-prone repair system is activated.
- The SOS response appears to be a somewhat desperate and risky attempt to escape the lethal effects of heavily damaged DNA. When the error-prone repair system is operative, mutation rates increase sharply.



DNA proofreading and repair; Role in human disease

Evidence for the importance of proofreading and repair mechanisms comes from human genetic disorders. In many cases, mutations in genes that encode proofreading and repair proteins are associated with heredity cancers (cancers that run in families). For example:

1. **Hereditary nonpolyposis colorectal cancer** (also called **Lynch syndrome**) is caused by mutations in genes encoding certain mismatch repair proteins. Since mismatched bases are not repaired in the cells of people with this syndrome, mutations accumulate much more rapidly than in the cells of an unaffected person. This can lead to the development of tumors in the colon.
2. People with **xeroderma pigmentosum** are extremely sensitive to UV light. This condition is caused by mutations affecting the nucleotide excision repair pathway. When this pathway doesn't work, thymine dimers and other forms of UV damage can't be repaired. People with xeroderma pigmentosum develop severe sunburns from just a few minutes in the sun, and about half will get skin cancer by the age of 10 unless they avoid the sun.

Inherited Human Diseases Caused by Defects in DNA Repair

Inherited Disorder	Gene	Chromosome	Function of Product	Major Symptoms
1. Xeroderma pigmentosum	*XPA*	9	DNA-damage-recognition protein	UV sensitivity, early onset skin cancers, neurological disorders
	XPB	2	3′ → 5′ helicase	
	XPC	3	DNA-damage-recognition protein	
	XPD	19	5′ → 3′ helicase	
	XPE	11	DNA-damage-recognition protein	
	XPF	16	Nuclease, 3′ incision	
	XPG	13	Nuclease, 5′ incision	
	XPV	6	Translesion DNA polymerase η	
2. Trichothiodystrophy	*TTDA*	6	Basal transcription factor IIH	UV sensitivity, neurological disorders, mental retardation
	XPB	2	3′ → 5′ helicase	
	XPD	19	5′ → 3′ helicase	
3. Cockayne syndrome	*CSA*	5	DNA excision repair protein	UV sensitivity, neurological and developmental disorders, premature aging
	CSB	10	DNA excision repair protein	
4. Ataxia-telangiectasia	*ATM*	11	Serine/threonine kinase	Radiation sensitivity, chromosome instability, early onset progressive neurodegeneration, cancer prone
5. Nonpolyposis colon cancer (Lynch syndrome)	*MSH2*	2	DNA mismatch recognition protein (like *E. coli* MutS)	High risk of familial colon cancer
	MLH1	3	Homolog of *E. coli* mismatch repair protein MutL	
	MSH6	2	MutS homolog 6	
	PMS2	7	Endonuclease PMS2	
	PMS1	2	Homolog of yeast mismatch repair protein	
6. Fanconi anemia	*FA* (8 genes, *A-H*, on 5 different chromosomes)			Sensitivity to DNA-cross-linking agents, chromosome instability, cancer prone
7. Bloom syndrome	*BLM*	15	BLM RecQ helicase	Chromosome instability, mental retardation, cancer prone
8. Werner syndrome	*WRN*	8	WRN RecQ helicase	Chromosome instability, progressive neurodegeneration, cancer prone
9. Rothmund-Thomson syndrome	*RECQL4*	8	RecQ helicase L4	Chromosome instability, mental retardation, cancer prone
10. Nijmegan breakage syndrome	*NBS1*	8	DNA-double-strand-break-recognition protein	Chromosome instability, microcephaly (small cranium), cancer prone

Source- Snustad and Simmons, Principles of Genetics

THANK YOU

Reference and sources

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