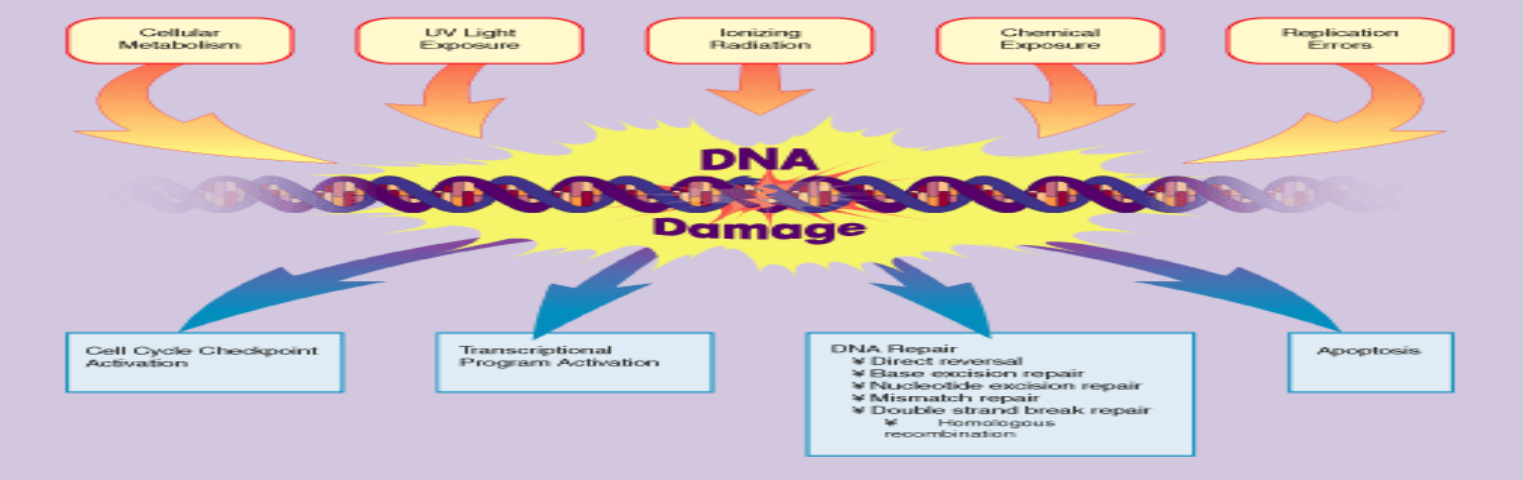

DNA Damage, Repair and Clinical significance

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Biochemistry for Medics- Lecture Notes

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Introduction

- DNA in the living cell is subjected to many chemical alterations.
- The genetic information encoded in the DNA has to remain uncorrupted
- Any chemical changes must be corrected.
- **A failure to repair DNA produces a mutation.**

Agents that Damage DNA

- **Radiations**
  - **Highly reactive oxygen radicals** produced during normal cellular respiration as well as by other biochemical pathways
  - **Ionizing radiation** such as gamma rays and x-rays
  - **Ultraviolet rays**, especially the UV-C rays (~260 nm) that are absorbed strongly by DNA but also the longer-wavelength UV-B that penetrates the ozone shield

Agents that Damage DNA

- **Chemicals in the environment**
  - Aromatic hydrocarbons, including some found in cigarette smoke
  - Plant and microbial products, e.g. the Aflatoxin produced in moldy peanuts
  - Chemicals used in chemotherapy, especially chemotherapy of cancers.

Types of DNA damage

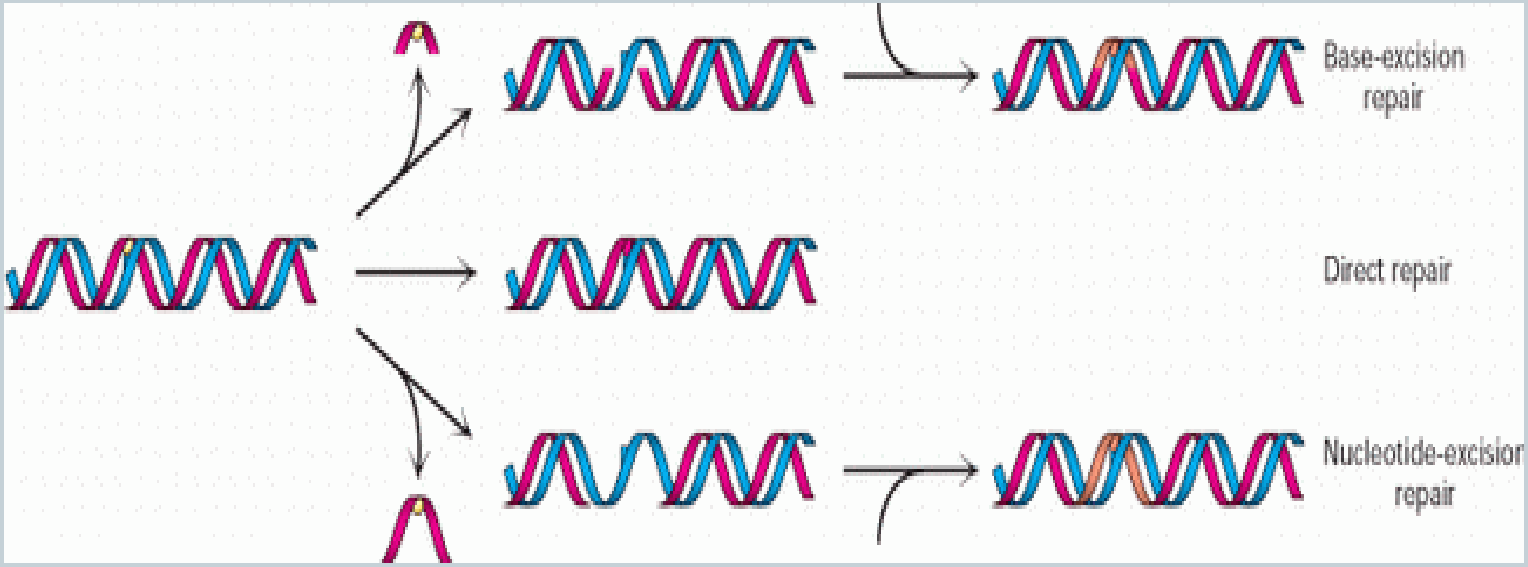
S.No.	Type of Damage	Examples
1)	Single-base alteration	A.Depurination B.Deamination of cytosine to uracil C.Deamination of adenine to hypoxanthine D.Alkylation of base E.Insertion or deletion of nucleotide F.Base-analog incorporation
2)	Two-base alterations	A. UV light–induced thymine-thymine (pyrimidine) dimer B. Bifunctional Alkylating agent cross-linkage
3)	Chain breaks	A. Ionizing radiation B. Radioactive disintegration of backbone element C. Oxidative free radical formation
4)	Cross-linkage	A. Between bases in same or opposite strands B. Between DNA and protein molecules (eg, histones)

DNA Repair

DNA repair can be grouped into two major functional categories:

A) Direct Damage reversal

B) Excision of DNA damage



A) Direct Damage Reversal

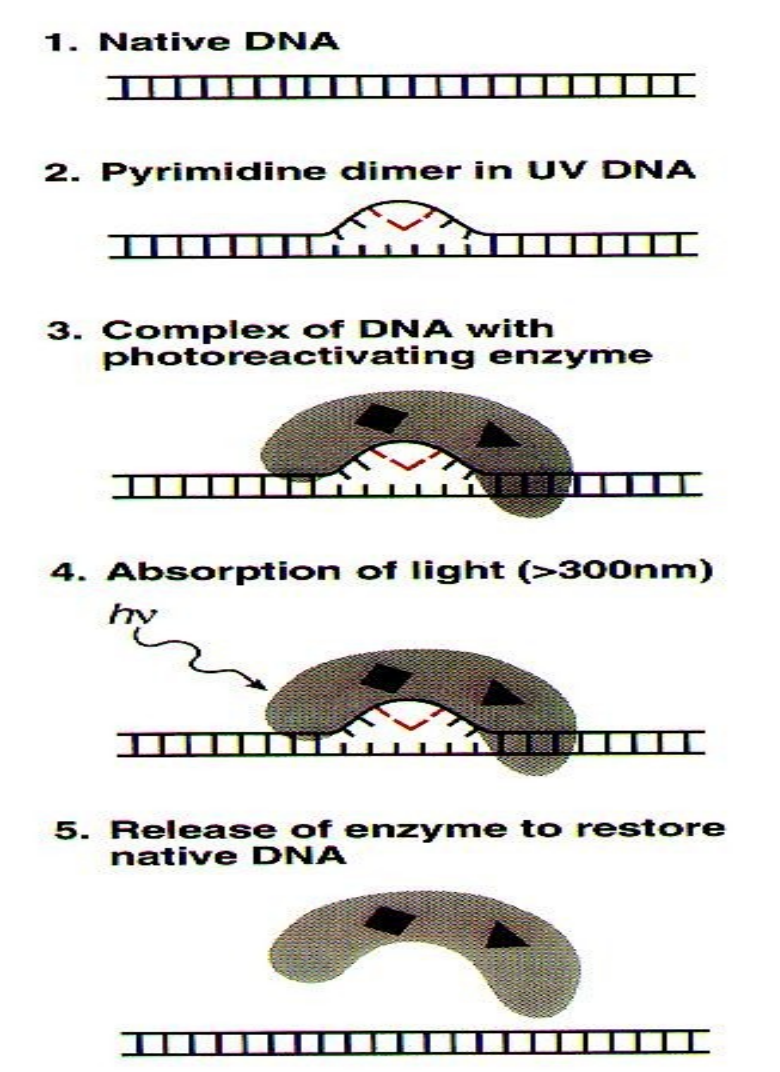
The direct reversal of DNA damage is by far the simplest repair mechanism that involves a single polypeptide chain, with enzymatic properties which binds to the damage and restores the DNA genome to its normal state in a single-reaction step. The major polypeptides involved in this pathway are:

i) DNA photolyases, the enzymes responsible for removing cyclobutane pyrimidine dimers from DNA in a light-dependent process called as photo reactivation

A) Direct Damage Reversal

ii) O6-methylguanine-DNA methyltransferase I and II (MGMT), also called DNA-alkyltransferases, remove the modified bases like O6-alkylguanine and O4-alkylthymine.

- The photolyase protein is not found in all living cells. However, the DNA-alkyltransferases are widespread in nature.



B) Excision of DNA damage

i) Base excision repair (BER)

ii) Nucleotide excision repair (NER),

iii) Mismatch repair (MMR) and

iv) Strand break repairs.

- In these reactions a nucleotide segment containing base damage, double-helix distortion or mispaired bases is replaced by the normal nucleotide sequence in a new DNA polymerase synthesis process.
- All of these pathways have been characterized in both bacterial and eukaryotic organisms.

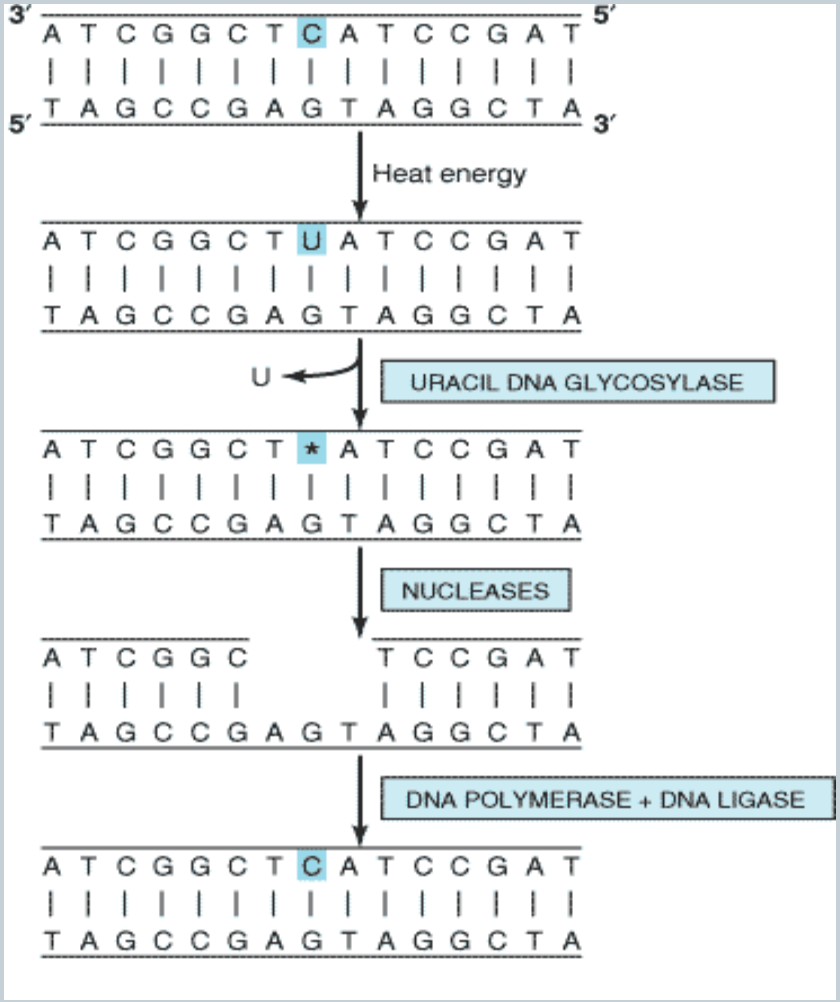
i) Base Excision Repair (BER)

- BER is initiated by DNA glycosylases, which catalyze the hydrolysis of the N-glycosidic bonds, linking particular types of chemically altered bases to the deoxyribose-phosphate backbone.
- DNA damage is excised as free bases, generating sites of base loss called apurinic or apyrimidinic (AP) sites.

i) Base Excision Repair (BER)

- The AP sites are substrates for AP endonucleases.
- These enzymes produce incisions in duplex DNA as a result of the hydrolysis of a phosphodiester bond immediately 5' or 3' to each AP site.
- The ribose-phosphate backbone is then removed from the DNA through the action of a specific exonuclease called deoxy ribophosphodiesterase or dRpase.
- Finally, the DNA polymerase and a ligase catalyze the incorporation of a specific deoxyribonucleotide into the repaired site, enabling correct base pairing

i) Base Excision Repair (BER)



Base excision-repair of DNA

- The enzyme uracil DNA glycosylase removes the uracil created by spontaneous deamination of cytosine in the DNA.
- An endonuclease cuts the backbone near the defect
- An endonuclease removes a few bases
- The defect is filled in by the action of a DNA polymerase and
- The strand is rejoined by a ligase.

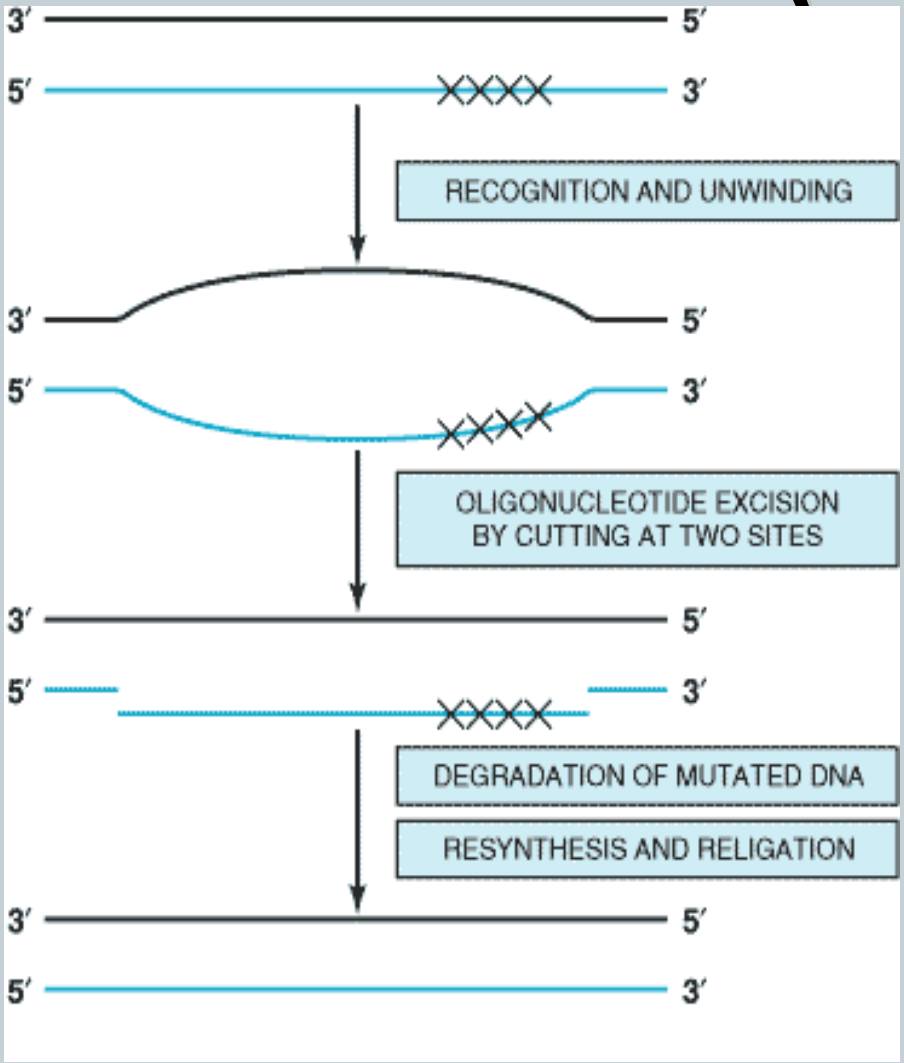
ii) Nucleotide excision repair (NER)

- This mechanism is used to replace regions of damaged DNA up to 30 bases in length.
- Common causes of such DNA damage include ultraviolet (UV) light, which induces the formation of cyclobutane pyrimidine-pyrimidine dimers, and smoking, which causes formation of benzo[*a*]pyrene-guanine adducts.
- Ionizing radiation, cancer chemotherapeutic agents, and a variety of chemicals found in the environment cause base modification, strand breaks, cross-linkage between bases on opposite strands or between DNA and protein, and numerous other defects.
- These are repaired by a process called nucleotide excision-repair

ii) Nucleotide excision repair (NER)

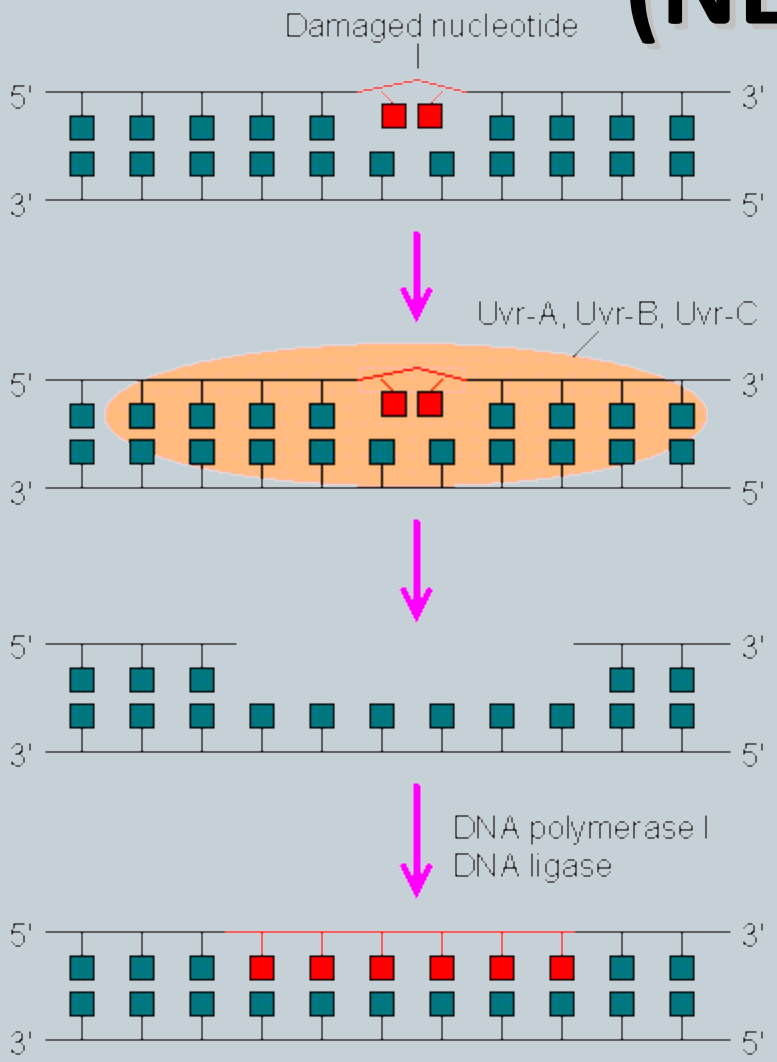
- NER is a much more complex biochemical process than BER, especially in eukaryotic cells.
- Several gene products are required in a multiple step process, during which the ordered assembly of DNA proteins provides an enzymatic complex that discriminates damaged from undamaged DNA.

ii) Nucleotide excision repair (NER)



- In eukaryotic cells the enzymes cut between the third to fifth phosphodiester bond 3' from the lesion, and on the 5' side the cut is somewhere between the twenty-first and twenty-fifth bonds.
- Thus, a fragment of DNA 27–29 nucleotides long is excised.
- After the strand is removed it is replaced, again by exact base pairing, through the action of yet another polymeras e,and the ends are joined to the existing strands by DNA ligase.

ii) Nucleotide excision repair (NER)



- In *Escherichia coli* there are three specific proteins, called UvrA, B and C, involved in lesion recognition and endonuclease incision.
- This fragment is released by UvrD helicase action, generating a gap that is finally submitted to repair synthesis

Transcription-Coupled NER

- Nucleotide-excision repair proceeds most rapidly in cells whose genes are being actively transcribed on the DNA strand that is serving as the template for transcription.
- If RNA polymerase II, tracking along the template (antisense) strand), encounters a damaged base, it can recruit other proteins, to make a quick fix before it moves on to complete transcription of the gene.

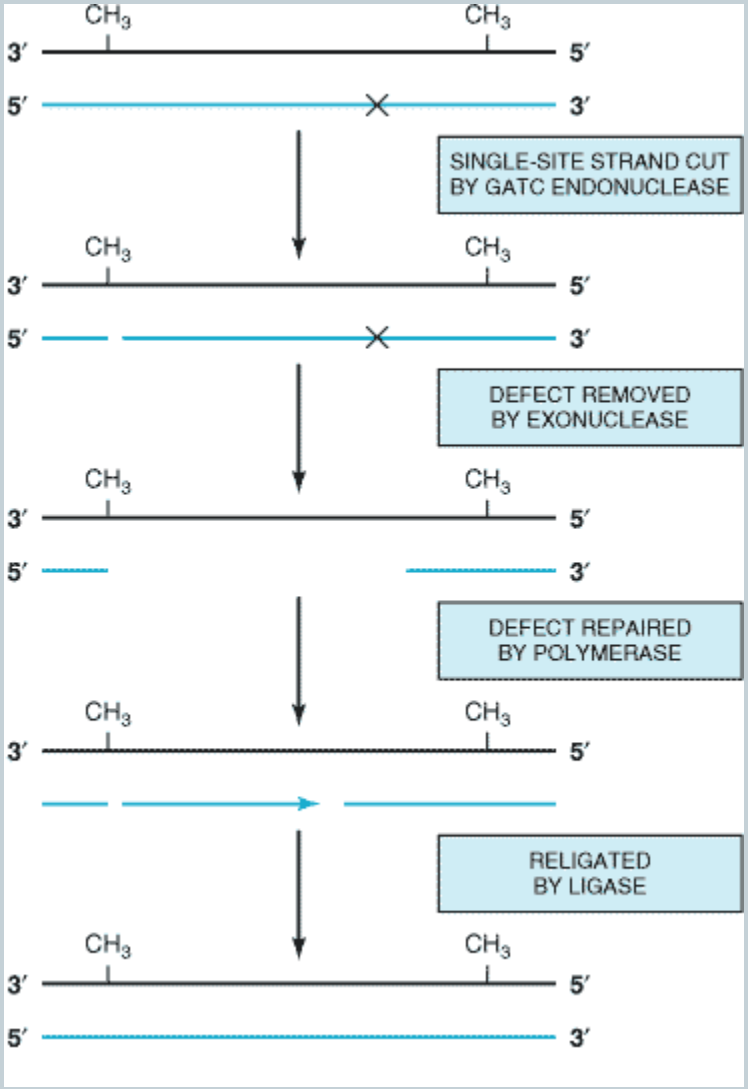
iii) Mismatch repair (MMR)

- Mismatch repair corrects errors made when DNA is copied.
- For example, a C could be inserted opposite an A, or the polymerase could slip or stutter and insert two to five extra unpaired bases.
- Specific proteins scan the newly synthesized DNA, using adenine methylation within a GATC sequence as the point of reference
- The template strand is methylated, and the newly synthesized strand is not.

iii) Mismatch repair (MMR)

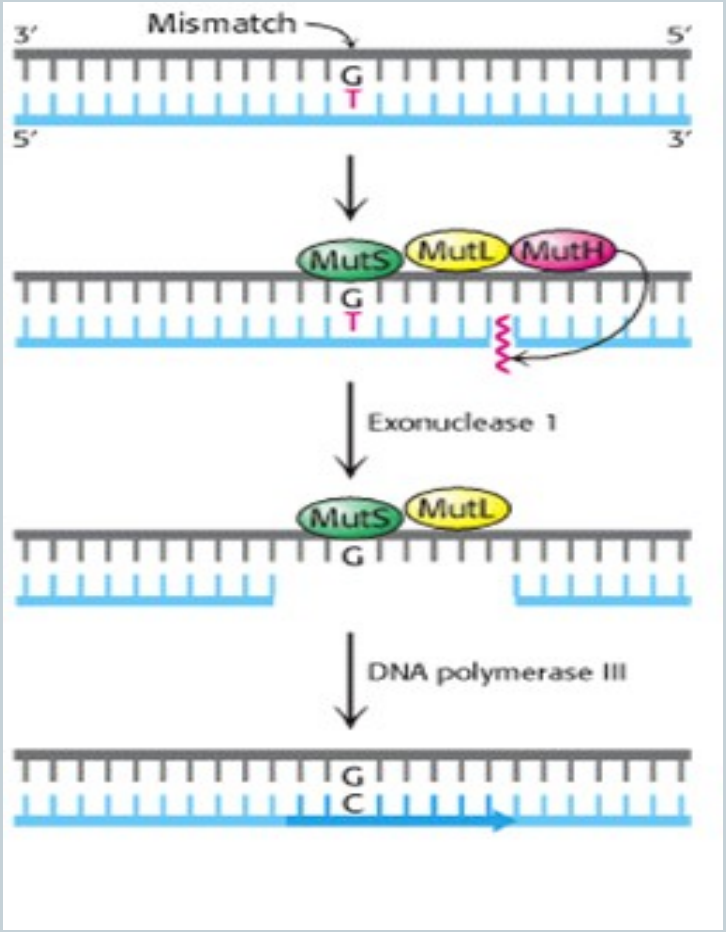
- This difference allows the repair enzymes to identify the strand that contains the errant nucleotide which requires replacement.
- If a mismatch or small loop is found, a GATC endonuclease cuts the strand bearing the mutation at a site corresponding to the GATC.
- An exonuclease then digests this strand from the GATC through the mutation, thus removing the faulty DNA. This can occur from either end if the defect is bracketed by two GATC sites.
- This defect is then filled in by normal cellular enzymes according to base pairing rules

iii) Mismatch repair (MMR)



- This mechanism corrects a single mismatch base pair (eg, C to A rather than T to A) or a short region of unpaired DNA.
- The defective region is recognized by an endonuclease that makes a single-strand cut at an adjacent methylated GATC sequence.
- The DNA strand is removed through the mutation, replaced, and religated.

iii) Mismatch repair (MMR) IN E.coli



- In *E coli,* three proteins (Mutt S, Mutt L, and Mutt H) are required for recognition of the mutation and nicking of the strand.
- Other cellular enzymes, including ligase, polymerase, and SSBs, remove and replace the strand.
- The process is more complicated in mammalian cells, as about six proteins are involved in the first steps.
- Faulty mismatch repair has been linked to hereditary nonpolyposis colon cancer (HNPCC), one of the most common inherited cancers.

B) Repairing Strand Breaks

- Ionizing radiation and certain chemicals can produce both single-strand breaks (**SSB**s) and double-strand breaks (**DSB**s) in the DNA backbone.

i) Single-Strand Breaks (SSBs)

- Breaks in a single strand of the DNA molecule are repaired using the same enzyme systems that are used in Base-Excision Repair (BER).

B) Repairing Strand Breaks

ii) Double-Strand Break Repair

- There are two mechanisms by which the cell attempts to repair a complete break in a DNA molecule:
- **1) Direct joining** of the broken ends. This requires proteins that recognize and bind to the exposed ends and bring them together for ligating. This type of joining is also called **Nonhomologous End-Joining** (**NHEJ**). A protein called Ku is essential for NHEJ.

B) Repairing Strand Breaks

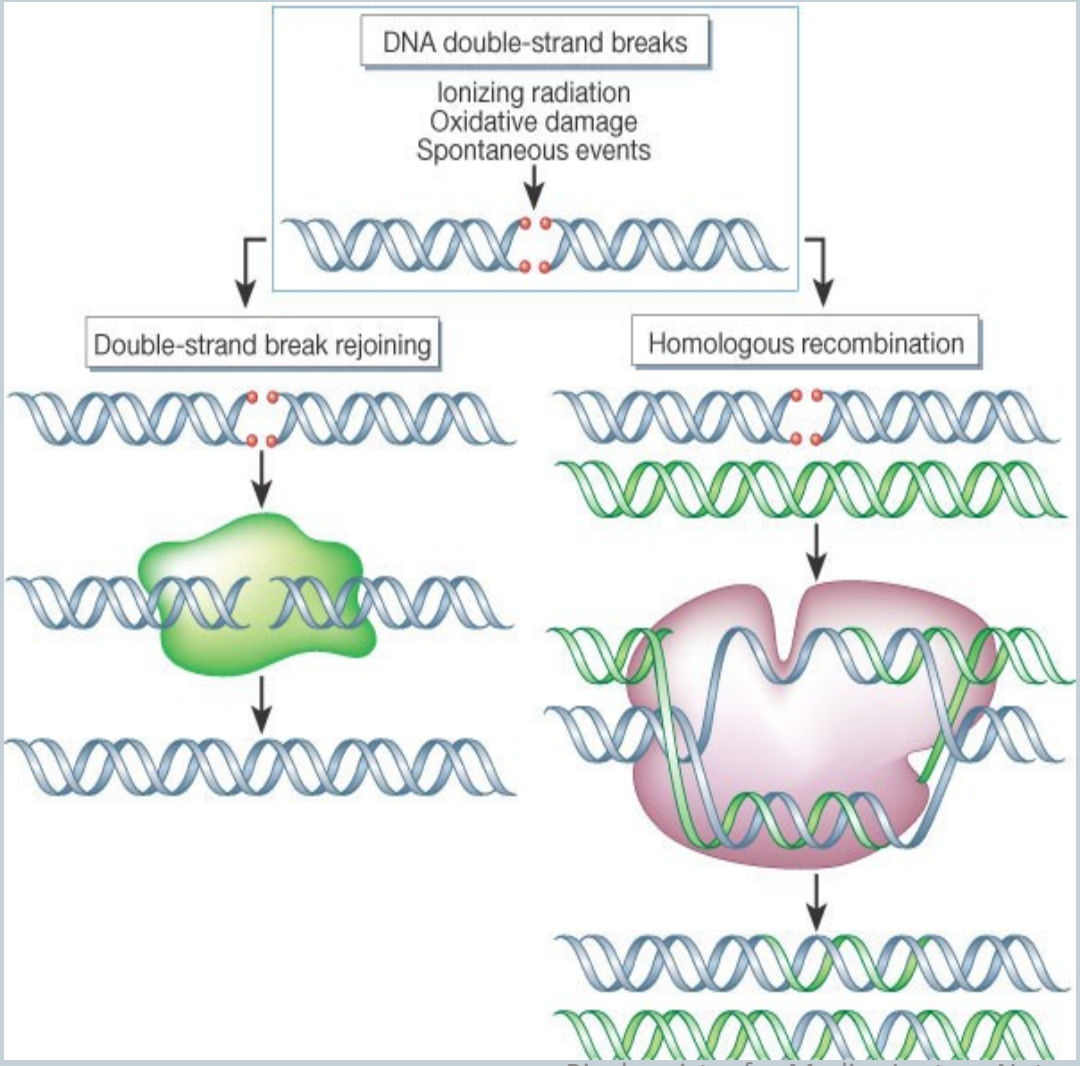
- Errors in direct joining may be a cause of the various **translocations** that are associated with cancers. Examples:
- Burkitt's lymphoma
- Philadelphia chromosome in chronic myelogenous leukemia (CML)
- B-cell leukemia

B) Repairing Strand Breaks

2) **Homologous Recombination.** Here the broken ends are repaired using the information on the intact

- **sister chromatid**, **or** on the
- **homologous chromosome**
- **same chromosome** if there are duplicate copies of the gene on the chromosome oriented in opposite directions (head-to-head or back-to-back).
- Two of the proteins used in homologous recombination are encoded by the genes ***BRCA1*** and ***BRCA2***.
- Inherited mutations in these genes predispose women to breast and ovarian cancers.

B) Repairing Strand Breaks



Meiosis also involves DSBs

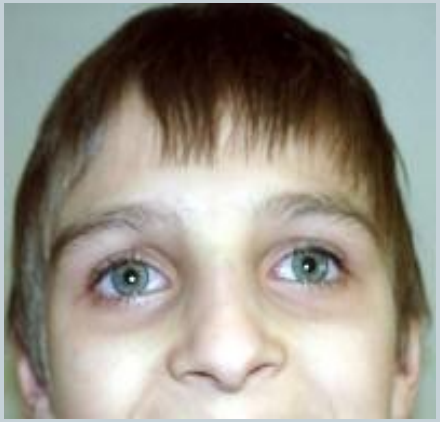
Recombination between homologous chromosomes in meiosis I also involves the formation of DSBs and their repair. Meiosis I with the alignment of homologous sequences provides a mechanism for repairing damaged DNA.

Diseases associated with defective DNA repair system

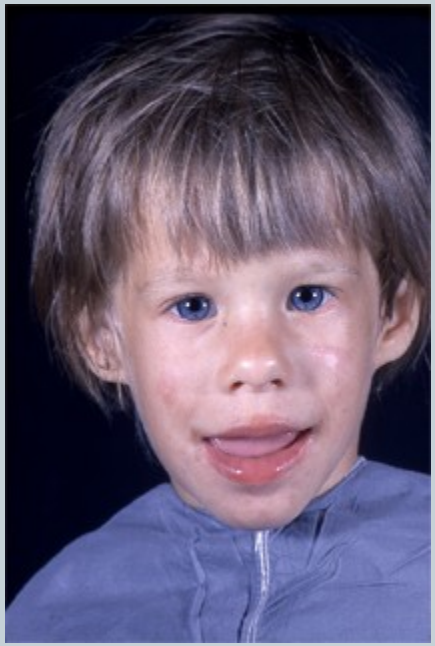
- Ataxia telangiectasia
- Bloom syndrome
- Cockayne's syndrome
- Progeria (Hutchinson-Gilford Progeria syndrome)
- Rothmund-Thomson syndrome
- Trichothiodystrophy
- Werner syndrome
- Xeroderma pigmentosum
- Hereditary non polyposis colon cancer.

Ataxia-telangiectasia (A-T)

- Ataxia-telangiectasia (A-T) is an autosomal recessive, complex, multisystem disorder characterized by progressive neurologic impairment, cerebellar ataxia, variable immunodeficiency with susceptibility to sinopulmonary infections, impaired organ maturation, ocular and cutaneous telangiectasia and a predisposition to malignancy.



Bloom syndrome



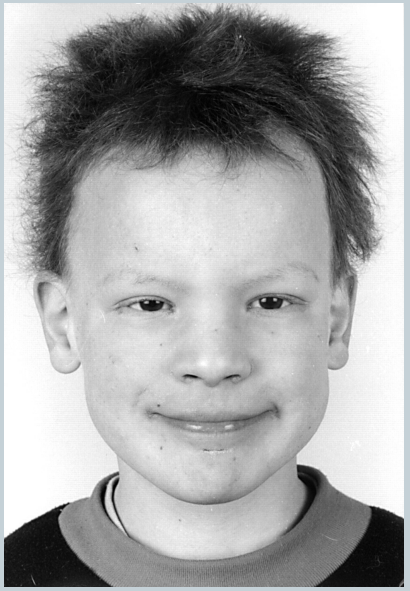
- Head is disproportionately small
- Striking paucity of subcutaneous fat tissue throughout infancy and childhood, and
- A redness of the cheeks and nose that characteristically makes its appearance in infancy after sun exposure.
- Chronic obstructive lung disease, Diabetes mellitus and malignancies of varied types are some of the common complications of Bloom syndrome

Cockayne's syndrome

- “Cockayne syndrome (also called Weber-Cockayne syndrome, or Neill-Dingwall Syndrome) is a rare autosomal recessive congenital disorder characterized by growth failure, impaired development of the nervous system, abnormal sensitivity to sunlight (photosensitivity), and premature aging.
- Hearing loss and eye abnormalities (pigmentary retinopathy) are other common features, but problems with any or all of the internal organs are possible.
- It is associated with a group of disorders called leukodystrophies.

Trichothiodystrophy

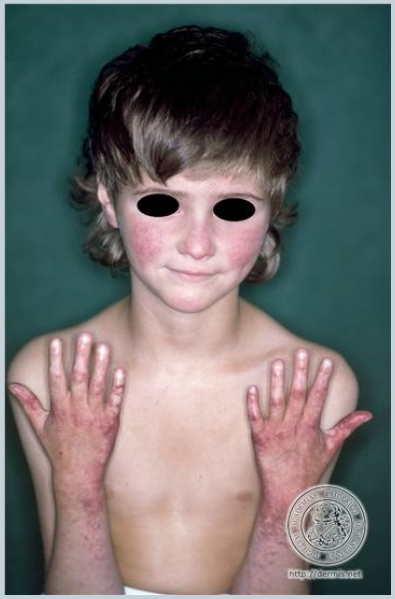
- Brittle hair, rough skin and extreme photosensitivity are the characteristic features. The trichothiodystrophies (TTD) are named primarily for the hair sulphur deficiency which is their most specific feature and which leads to brittleness of the hair.
- There is defect in DNA excision repair system along with other defects.



Rothmund-Thomson syndrome

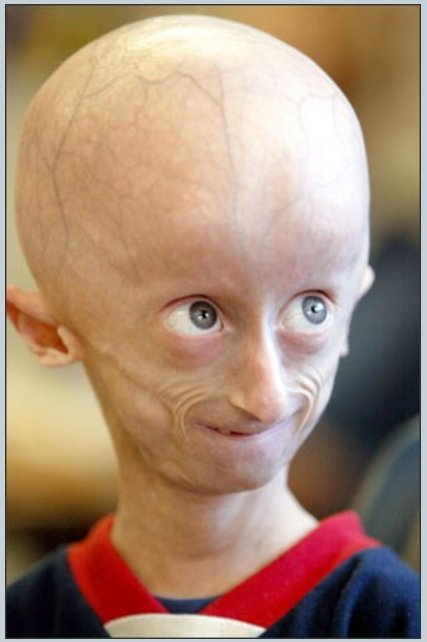
The common clinical findings are-

- Sun-sensitive rash with telangiectasias
- Juvenile cataracts
- Saddle nose
- Congenital bone defects, including short stature and anomalies such as absent thumbs
- Hair growth problems (absent eyelashes, eyebrows and/or hair)
- Osteosarcoma

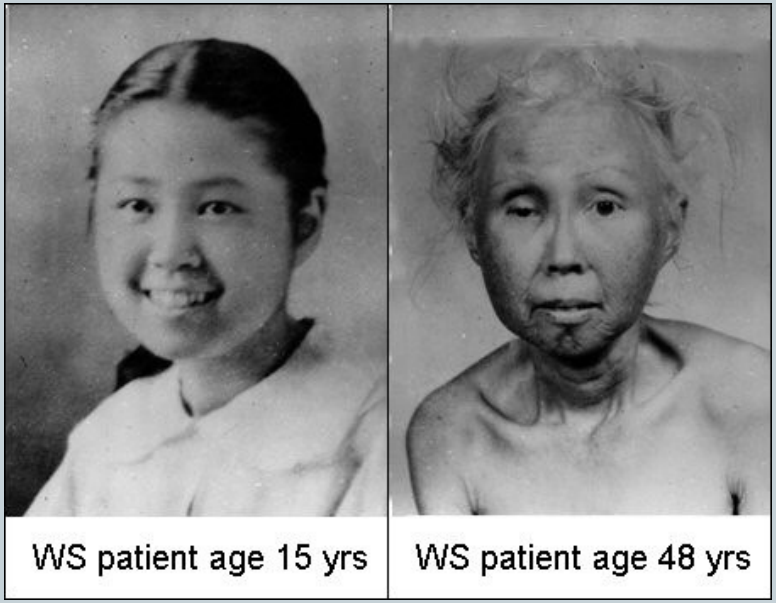


Progeria

- **Progeria** (Hutchinson-Gilford Progeria Syndrome) is an extremely rare genetic disorder that causes the affected individual to undergo advanced aging at an early age.
- The symptoms closely resemble aging and include wrinkles, hair loss, and delayed growth.
- Affected individuals have normal development up to 18 months and suddenly stop gaining weight and display stunted height.
- As the individual ages, Progeria becomes more severe with an average life expectancy of 12 years.



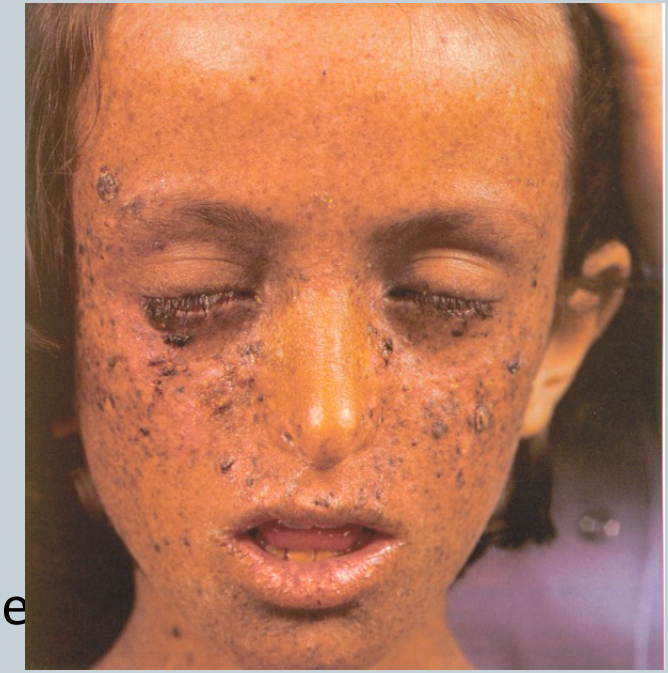
Werner syndrome(Adult Progeria)



- Werner syndrome is a hereditary condition associated with premature aging and an increased risk of cancer and other diseases. The signs of Werner syndrome usually develop in the teenage years.
- A person with Werner syndrome does not have the usual growth spurt typical of a teenager and is shorter on average. Signs of aging, including gray hair and hair loss, may appear in the 20's.

Xeroderma pigmentosum (XP)

- **Xeroderma pigmentosum (XP)** is an autosomal recessive genetic disease.
- The clinical syndrome includes marked sensitivity to sunlight (ultraviolet) with subsequent formation of multiple skin cancers and premature death.
- The risk of developing skin cancer is increased 1000- to 2000-fold.
- The inherited defect seems to involve the repair of damaged DNA, particularly thymine dimers.
- Cells cultured from patients with xeroderma pigmentosum exhibit low activity for the nucleotide excision-repair process.



Summary

- To revise the concepts follow the links
- http://highered.mcgraw-hill.com/sites/0072556781/student_view0/chapter11/animation_quiz_5.html
- http://highered.mcgraw-hill.com/sites/dl/free/0072835125/126997/animation34.html
- http://highered.mcgraw-hill.com/sites/dl/free/0072835125/126997/animation33.html