DNA REPAIR

OUTLINE

- Introduction
- Sources of DNA damage
- Causes of DNA Damage
- Mechanism of DNA repair
- Global Response to DNA Damage
- Medicine and DNA damage Repair
- DNA repair and Cancer
- DNA repair and ageing

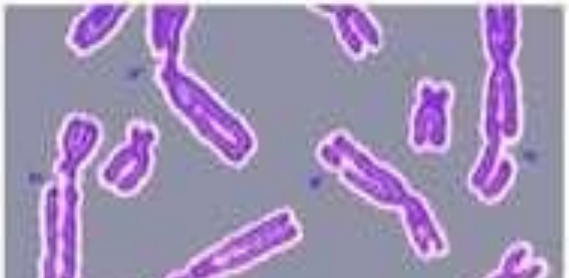
DNA REPAIR

- **DNA repair is a collection of processes by which a cell identifies and corrects damage to the DNA molecules that encode its genome.**
- In human cells, both normal metabolic activities and environmental factors such as **UV light** and radiation can cause **DNA damage**, resulting in as many as 1 million individual molecular lesions per cell per day.
- Many of these lesions cause structural damage to the DNA molecule and can alter or eliminate the cell's ability to transcribe the gene that the affected DNA encodes. Other lesions induce potentially harmful mutations in the cell's genome, which affect the survival of its daughter cells after it undergoes mitosis.

- The DNA repair process is constantly active as it responds to damage in the DNA structure.
- When normal repair processes fail, and when cellular apoptosis does not occur, irreparable DNA damage may occur, including double-strand breaks and DNA crosslinkages.
- The rate of DNA repair is dependent on many factors
- **the cell type,**
- **the age of the cell**
- **the extracellular environment**

DNA DAMAGE

- DNA damage is an alteration in the chemical structure of DNA, such as a break in a strand of DNA, a base missing from the backbone of DNA, or a chemically changed base such as 8-OHdG. Damage to DNA that occurs naturally can result from metabolic DNA damage, due to environmental factors and normal metabolic processes inside the cell.
- The vast majority of DNA damage affects the primary structure of the double helix.



SOURCE OF DAMAGE

- DNA damage can be subdivided into two main types:
- **endogenous damage** such as attack by reactive oxygen species produced from normal metabolic byproducts (spontaneous mutation), especially the process of oxidative deamination
- also includes replication errors
- **exogenous damage** caused by external agents such as
- ultraviolet [UV 200-400 nm] radiation from the sun
- other radiation frequencies, including x-rays and gamma rays
- certain plant toxins

CAUSES OF DNA DAMAGE

- The most significant consequence of oxidative stress in the body is thought to be damage to DNA.
- DNA may be modified in a variety of ways, which can ultimately lead to mutations and genomic instability.
- This could result in the development of a variety of cancers including colon, breast, and prostate.

TYPES OF DNA DAMAGE:

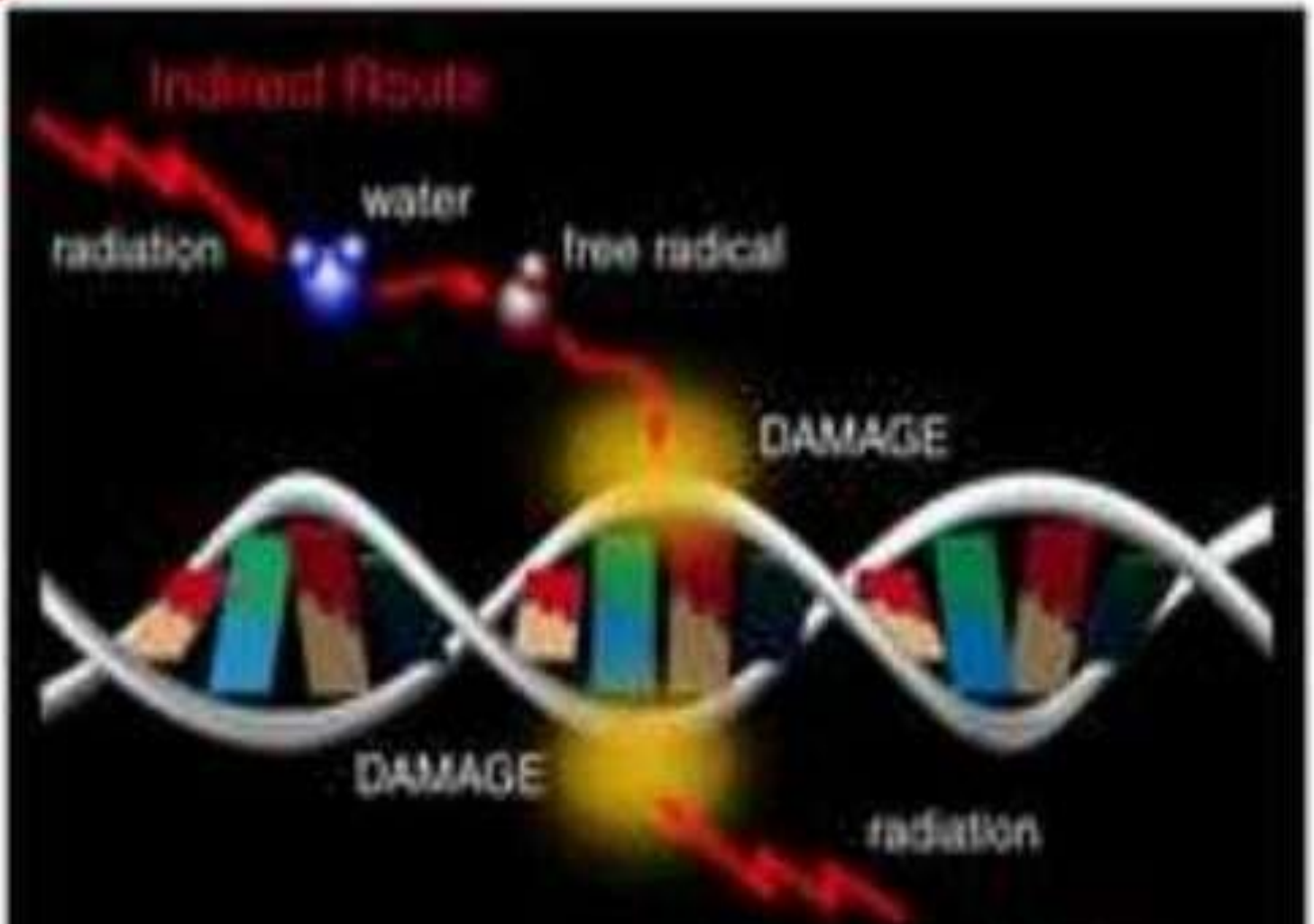
Here we discuss the various types of damage to DNA, including:

- **oxidative damage,**
- **hydrolytic damage,**
- **DNA strand breaks,**
- and others.

BY RADIATION

- Radiation acts by damaging DNA.
- When it hurts us, it damages DNA in healthy cells which are doing their job keeping us alive and well.
- Radiation can damage DNA either by scoring a direct hit, or by breaking-up water. The broken water is very reactive and can cause damage to DNA (or anything else it comes across).

- Radiation comes in three forms, alpha, beta, and gamma.
- Radiation damages DNA - cells work hard to repair it.
- When we use it in medicine, e.g. in radiotherapy, it acts by damaging DNA in cancer cells.

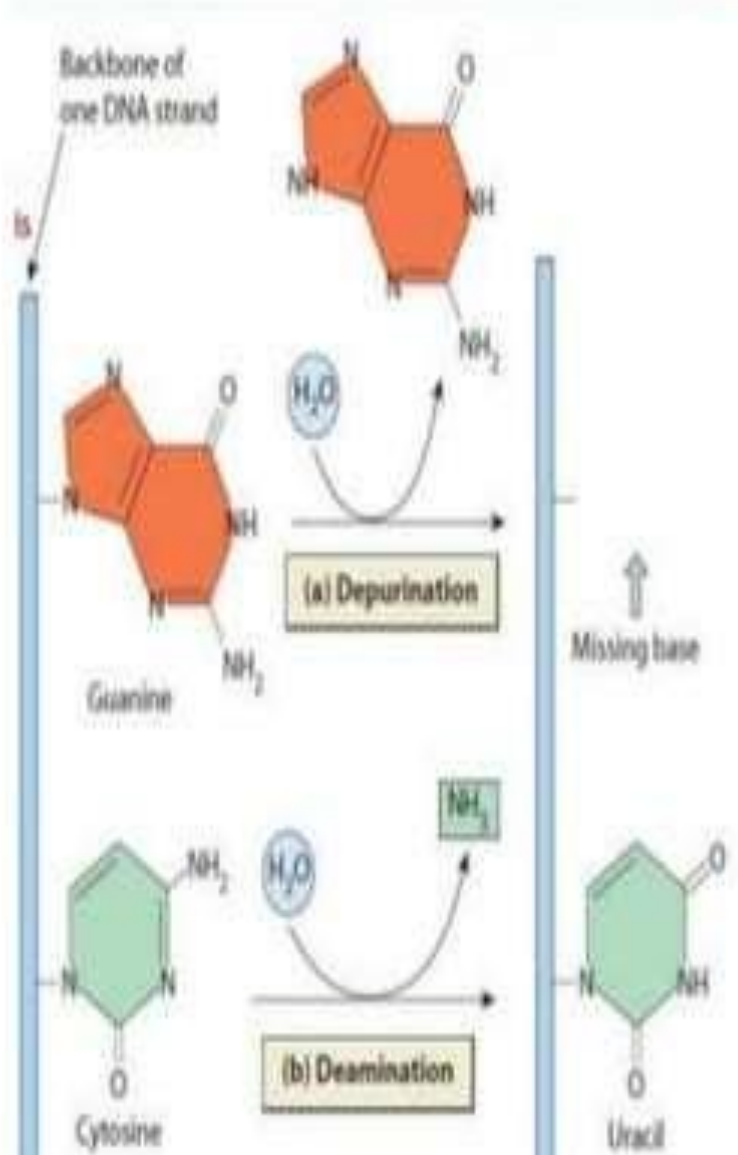

Indirect Route
water
radiation
free radical
DAMAGE
DAMAGE
radiation

BY HYDROLYSIS:

- The covalent structure of DNA is unstable in aqueous solution. It tends to hydrolyze to its monomeric components, and they themselves are subject to various hydrolytic reactions.
- A single base transformation within a DNA molecule may be sufficient to cause a mutation, or inactivate the DNA.
- Phosphodester bond and N-glycosyl bond cleavage occurs due to hydrolysis.

Hydrolysis involves two steps:

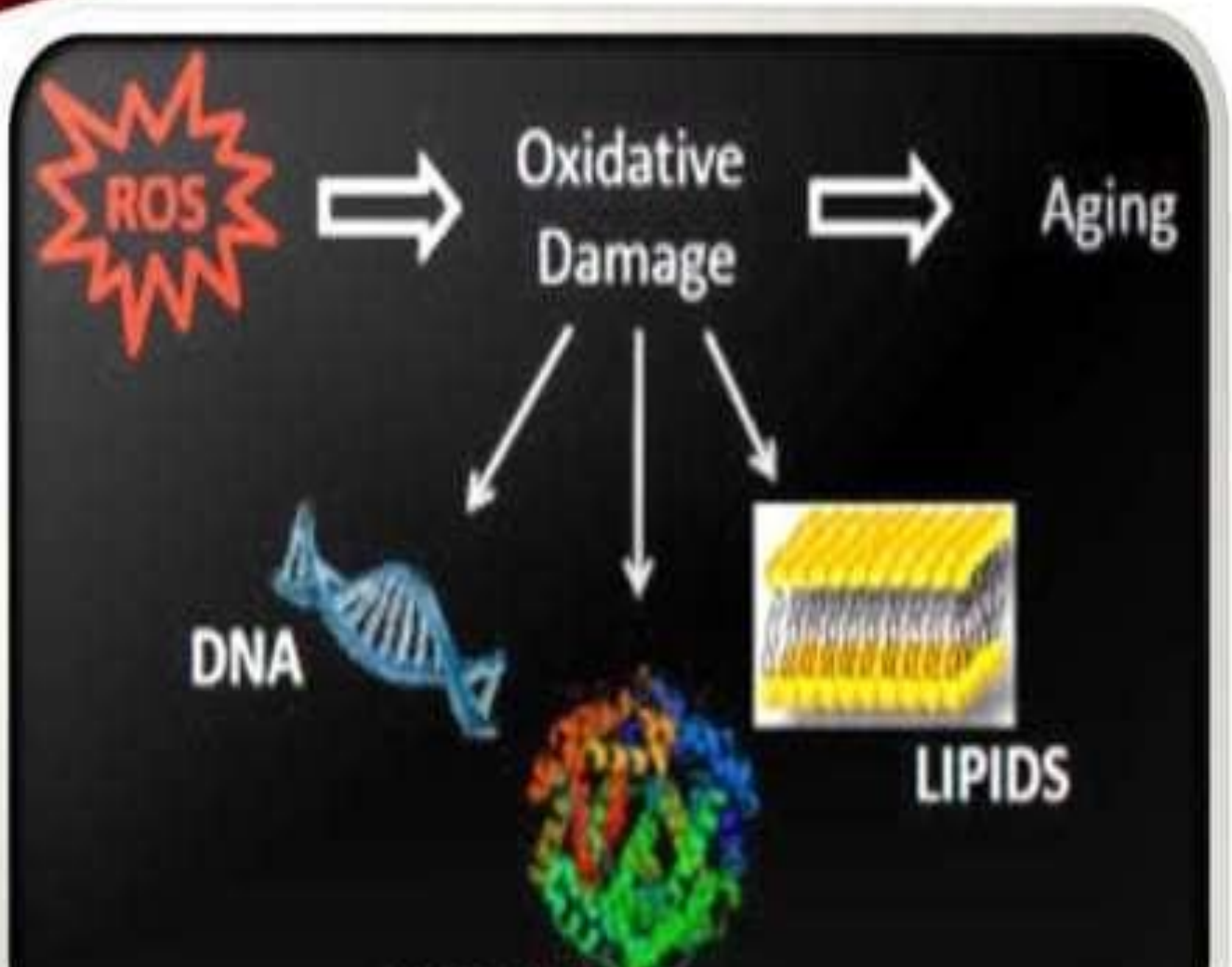
- Depurination
- deamination



BY OXIDATION:

- Mutations caused by oxidative DNA damage may contribute to human disease.
- Oxidation of **G** generates oxoG , is highly mutagenic bcz it can be base-pair with **A** as well as with **C**.
- if it pair with **A** during replication give rise **G:C** to **T:A** transvasion cause human cancer i.e. by free radicals.

- Cellular DNA is damaged by oxygen free radicals generated during cellular respiration, cell injury, phagocytosis, and exposure to environmental oxidants.
- The resultant damage to DNA bases may be a significant source of mutations that lead to cancer and other human pathology .
- Because of the multiplicity of DNA modifications produced by oxygen free radicals , it has been difficult to establish the frequency and specificity of mutations engendered by individual oxygen-induced DNA lesions.


ROS
Oxidative Damage
Aging
DNA
LIPIDS

MECHANISM OF DNA REPAIR

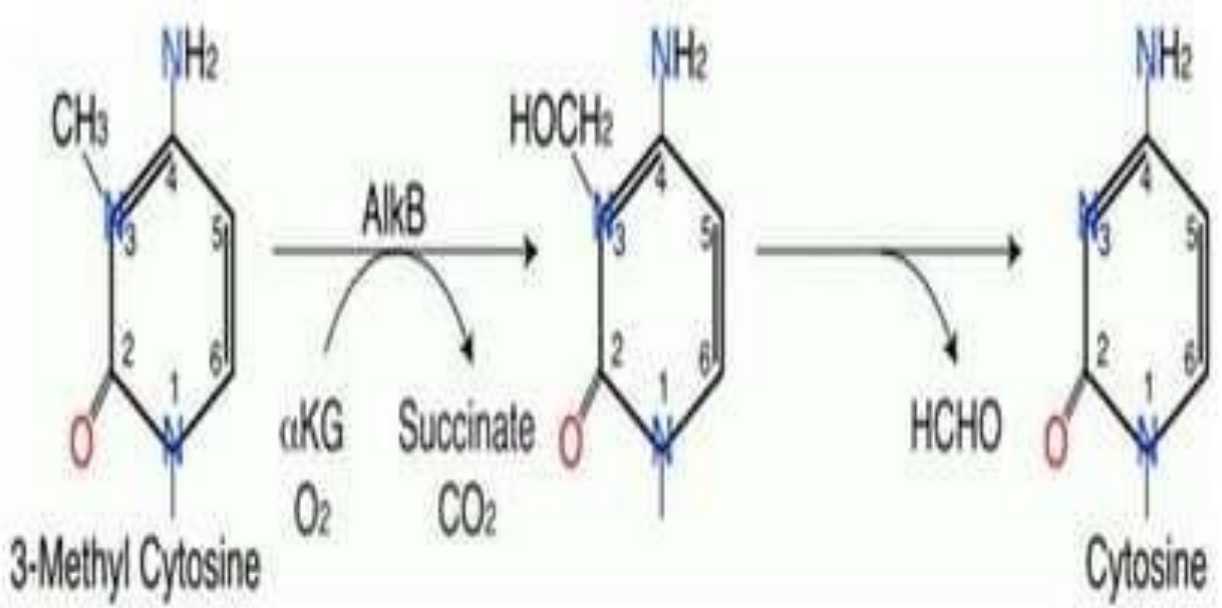
DIRECT REVERSAL REPAIR:-

Most cases of DNA damage are not reversible. For cases that are reversible, our body uses direct reversal repair mechanism to correct the damaged base.

MECHANISM:-

- Direct reversal repair is a mechanism of repair where the damaged area or lesion is repaired directly by specialised proteins in our body. It is the simplest form of DNA repair and also, the most energy efficient method.
- It does not require a reference template unlike the other single-strand repair mechanism.

EXAMPLE


NH2
CH3
3-Methyl Cytosine
AlkB
αKG
O2
Succinate
CO2
NH2
HOCH2
HCHO
NH2
Cytosine

EXCISION REPAIR:-

- Most such damage products involve neither pyrimidine dimers nor O6-alkylguanine, so they must be handled by a different mechanism. Most are removed by a process called **excision repair. The damaged DNA is first removed, then replaced** with fresh DNA.

MECHANISMS:-

- The damaged DNA is first removed, then replaced with fresh DNA, by one of two mechanisms:
- base excision repair or
- nucleotide excision repair.
- Mismatch repair.
- Double stranded break repair
- Recombinant repair

BASE EXCISION REPAIR:-

Base excision repair is more prevalent and usually works on common, relatively subtle changes to DNA bases, such as chemical modifications caused by cellular agents.

MECHANISM:-

- This process begins with DNA glycosylase, which extrudes a base in a damaged base pair, then clips out the damaged base,
- leaving an apurinic or apyrimidinic site that attracts the DNA repair enzymes that remove the remaining deoxyribose phosphate and replace it with a normal nucleotide.
- In bacteria, DNA polymerase I is the enzyme that fills in the missing nucleotide in BER;
- In eukaryotes, DNA polymerase b plays this role.
- However, this enzyme makes mistakes, and has no

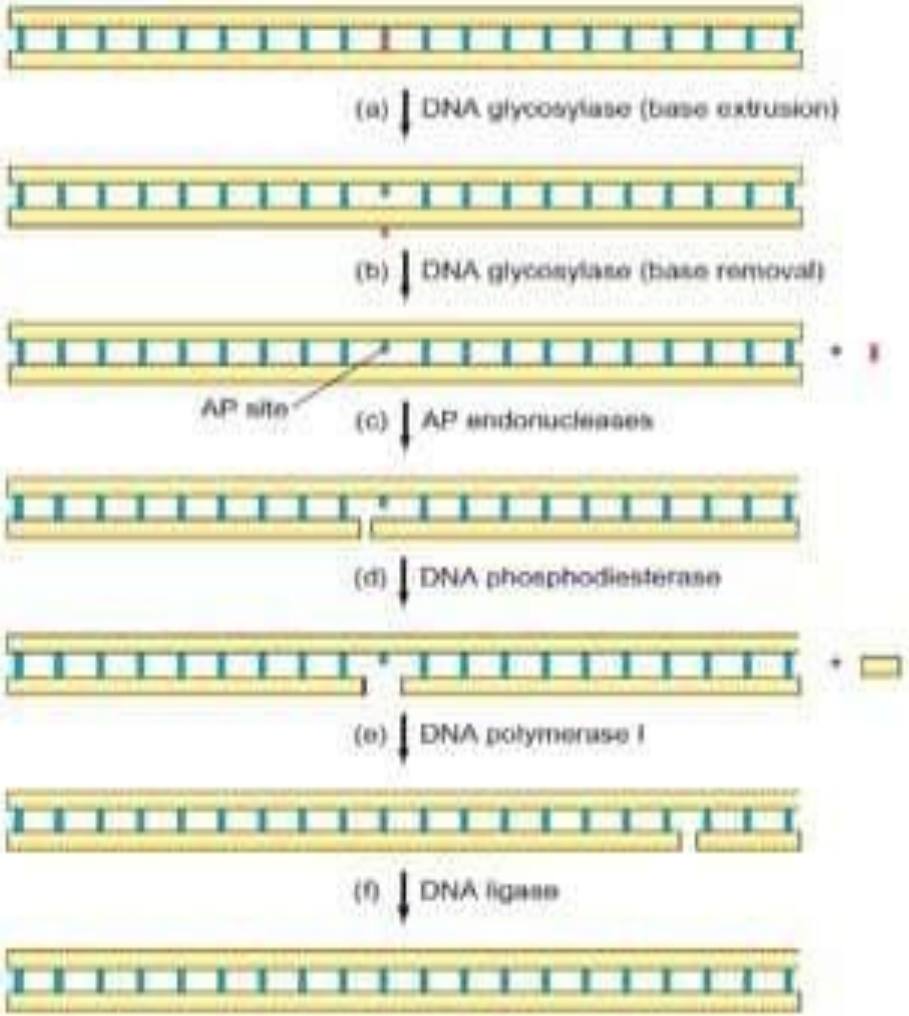


Figure 20.30 Base excision repair in *E. coli*. (a) DNA glycosylase extrudes the damaged base (red). (b) DNA glycosylase removes the extruded base, leaving an apurinic or apyrimidinic site on the bottom DNA strand. (c) An AP endonuclease cuts the DNA on the 5′-side of

NUCLEOTIDE EXCISION REPAIR:-

- Nucleotide excision repair generally deals with more drastic changes to bases, many of which distort the DNA double helix. These changes tend to be caused by mutagenic agents from outside of the cell.
- A good example of such damage is pyrimidine dimer caused by UV light.

MECHANISM:-

- Nucleotide excision repair typically handles bulky damage that distorts the DNA double helix.
- NER in E. coli begins when the damaged DNA is clipped by an endonuclease on either side of the lesion, at sites 12–13 not apart. This allows the damaged DNA to be removed as part of the resulting 12–13-base oligonucleotide. DNA polymerase I fills the gap and DNA ligase seals the final nick.

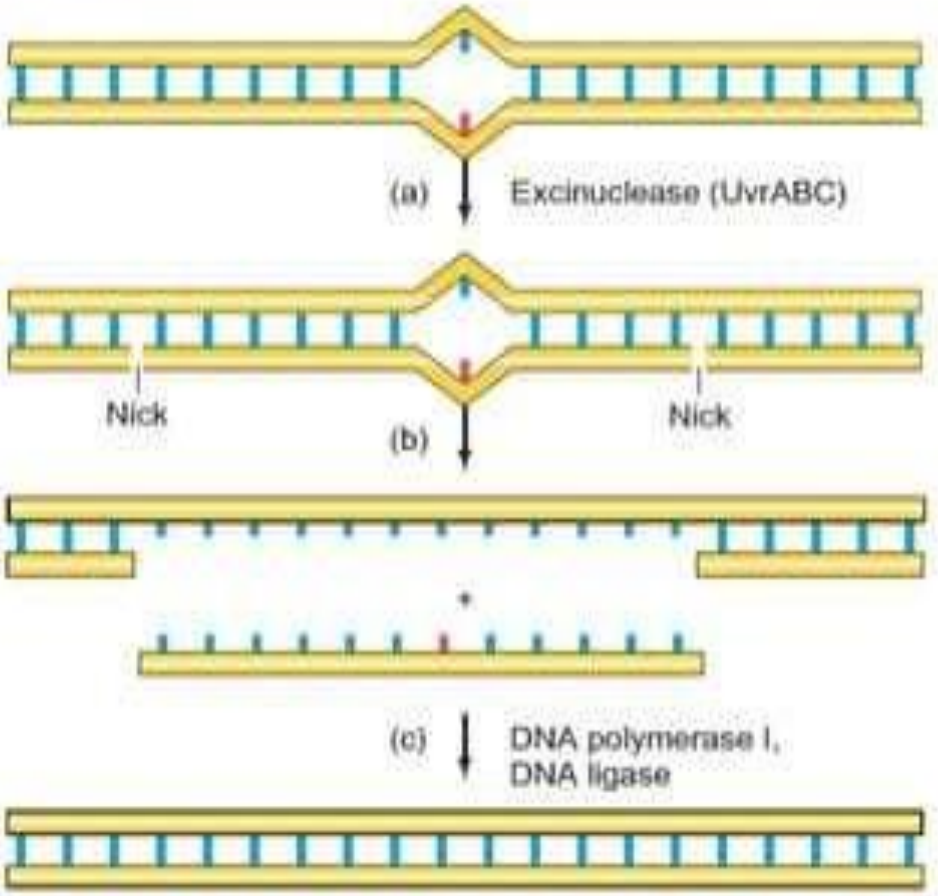


Figure 20.32 Nucleotide excision repair in *E. coli* **(a)** The UvrABC excinuclease cuts on either side of a bulky damaged base (red). This causes removal **(b)** of an oligonucleotide 12 nt long. If the damage

MISMATCH REPAIR:-

- Mismatch repair deals with correcting mismatches of the normal bases; that is, failures to maintain normal Watson-Crick base pairing (A•T, C•G)
- Recognition of a mismatch requires several different proteins.

- DOUBLE-STRAND BREAK REPAIR

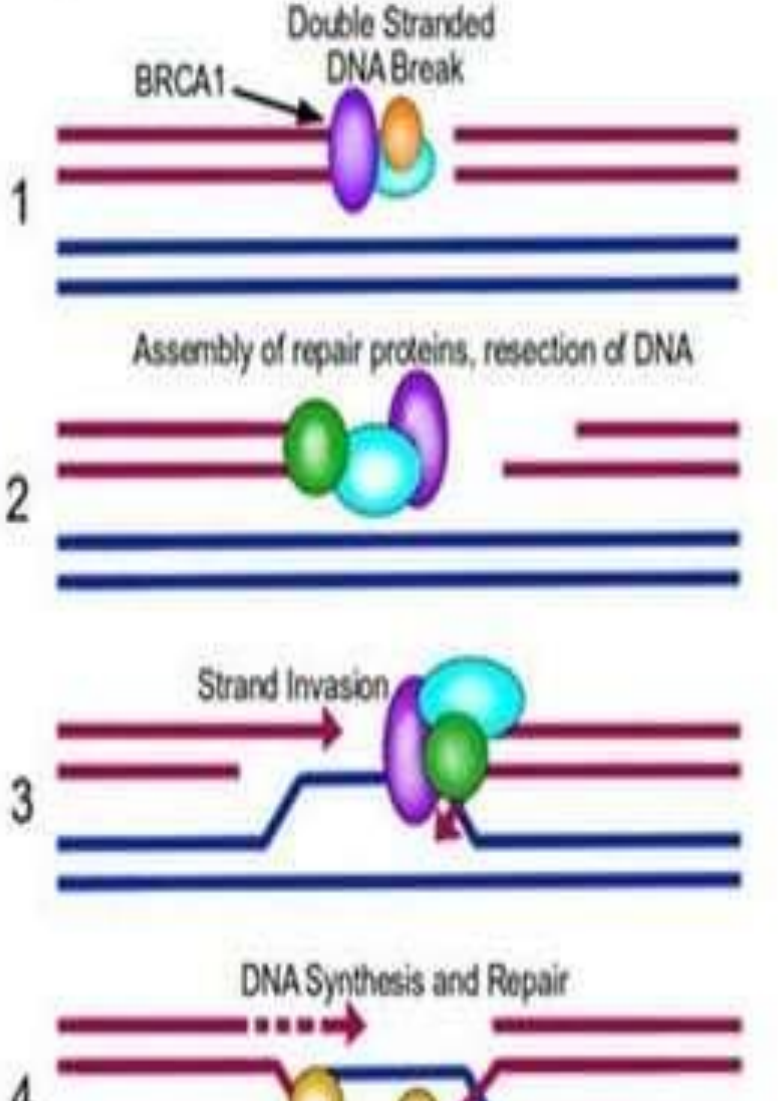
Two method for repairing Double strand Break

- By homologous recombination
- By non homologous ending joints

BY HOMOLOGOUS RECOMBINATION

- Recombinase as RecA bind to the ss-DNA
- Here the broken ends are repaired using the information on the intact sister chromatid, or on the homologous chromosome
- Two of the proteins used in homologous recombination are encoded by the genes BRCA1 and BRCA2
- Accessory factors as Rad54, Rad54B, and Rdh54 help recognize and invade the homologous region
- After the formation of D-loop, DNA polymerase involved to

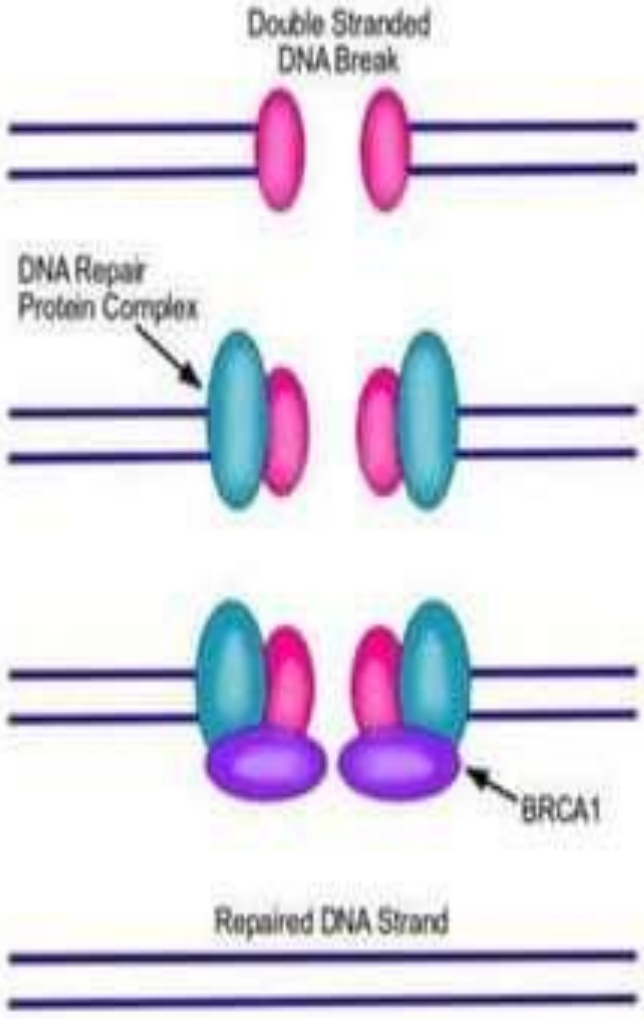
Homologous Recombination DNA Repair



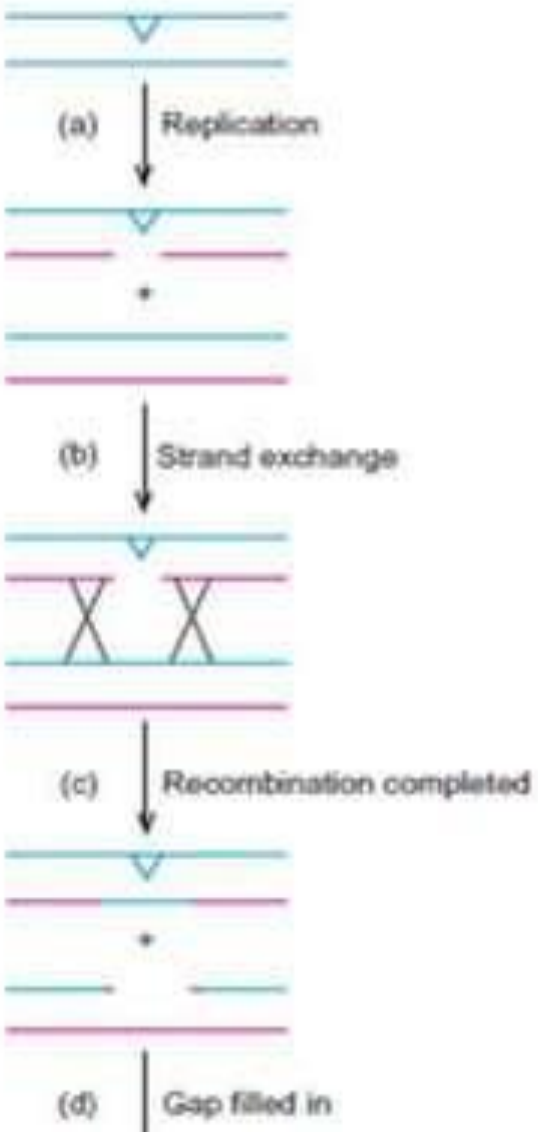
BY NON HOMOLOGOUS ENDING JOINTS

- Non-homologous end-joining (NHEJ) is used at other points of the cell cycle when sister chromatids are not available for use as HR templates. When these breaks occur, the cell has not yet replicated the region of DNA that contains the break, so unlike the HR pathway, there is no corresponding template strand available.
- 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 Non homologous End-Joining (NHEJ). A protein called Ku is essential for NHEJ

Non Homologous End Joining



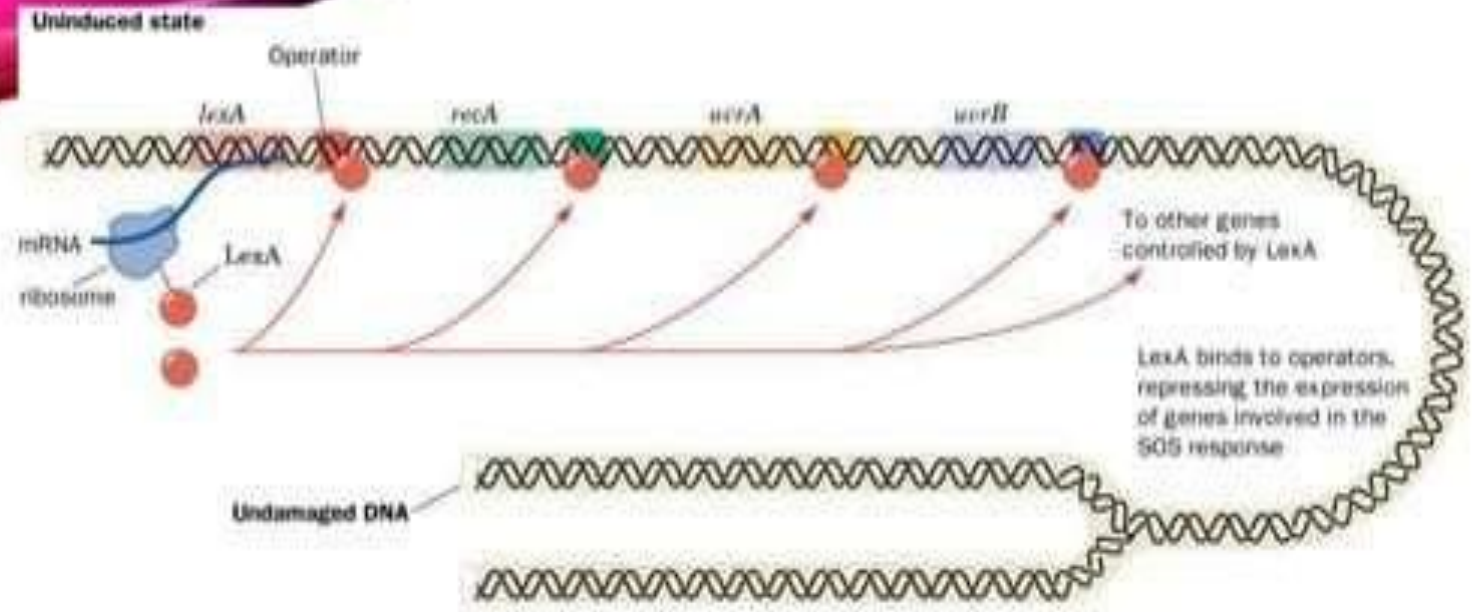
4-RECOMBINANT REPAIR

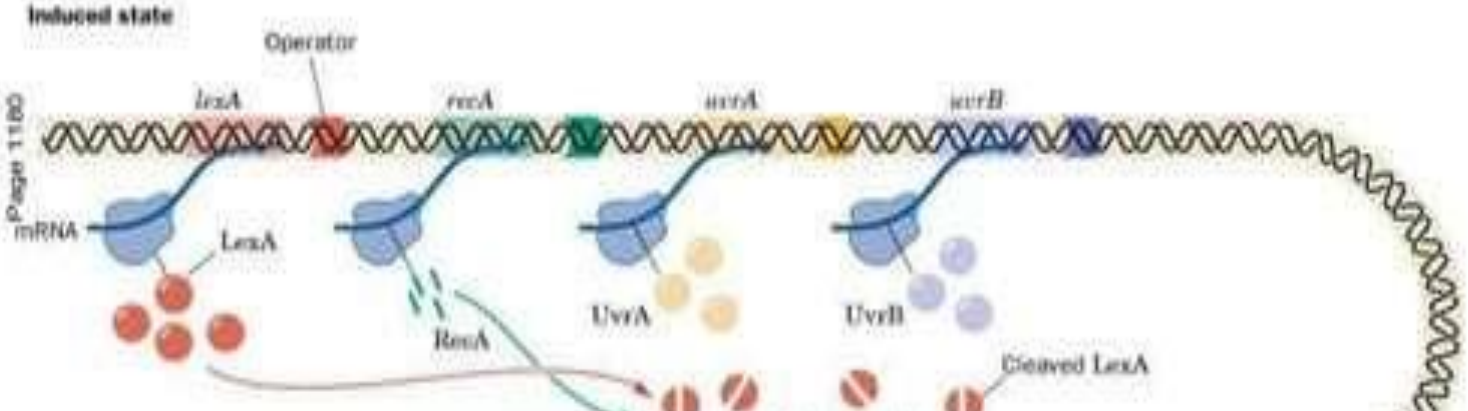

(a)
Replication
(b)
Strand exchange
(c)
Recombination completed
(d)
Gap filled in

GLOBAL RESPONSE TO DNA DAMAGE

SOS response

- SOS response causes cells to stop dividing and repair damaged DNA.
- LexA and RecA mutants always have the SOS response on.
- When *E. coli* is exposed to agents that damage DNA, RecA mediates proteolytic cleavage of LexA. This is induced by RecA binding to ssDNA.
- LexA is a repressor of 43 genes involved in DNA repair (all


Uninduced state
Operator
lexA
recA
uvrA
uvrB
mRNA
ribosome
LexA
To other genes controlled by LexA
LexA binds to operators, repressing the expression of genes involved in the SOS response
Undamaged DNA


Induced state
Operator
lexA
recA
uvrA
uvrB
mRNA
LexA
RecA
UvrA
UvrB
Cleaved LexA

SOS Repair

- *E. coli* Pol III is unable to replicate through lesions (AP sites, thymine dimers), causing a replication fork "collapse"
- To restore the replication fork, can either induce **recombination repair** which uses a homologous chromosome as the template or **SOS repair.**
- Uses 2 **bypass DNA polymreases (Pol IV and PolV).**
- These are **error-prone** DNA polymerases (lack the 3' → 5' exonuclease)
- SOS is a mutagenic process. *This is a last resort if DNA has not*

CONTROL OF THE CELL CYCLE

Three checkpoints:

- The **G1/S cell cycle checkpoint**
- **G2/M DNA damage checkpoint**
- **Mitosis checkpoint**

G1/S CELL CYCLE CHECKPOINT

controls the passage of eukaryotic cells from the first 'gap' phase (G1) into the DNA synthesis phase (S).

Checks:

- That the size is CORRECT
- That the environment is CORRECT

G1/S CELL CYCLE CHECKPOINT
HOW DO THEY DO THAT?

Major proteins involved:

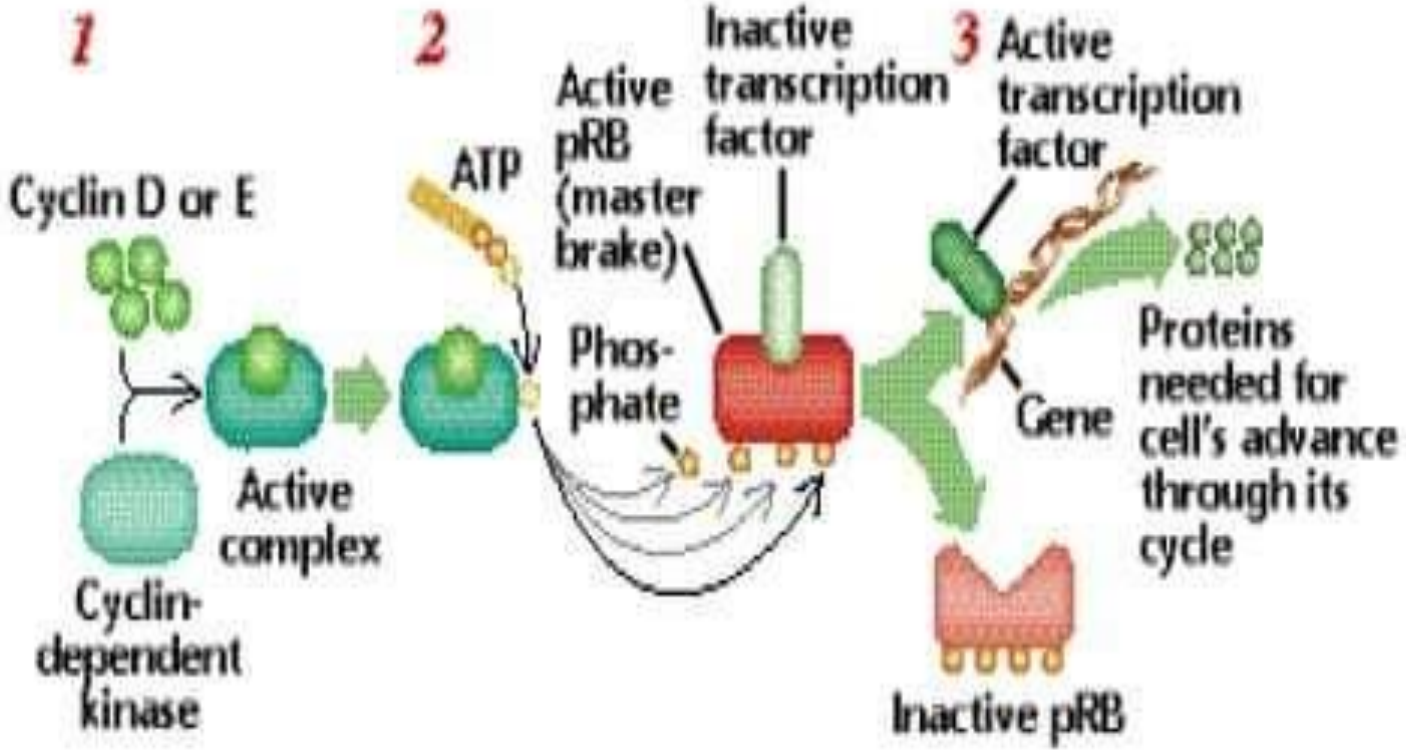
Cyclins (proteins) - level fluctuate in the cell cycle.

Cyclin dependent KINASES* **(Cdks)**

They add phosphate groups to proteins that control processes in the cell cycle.

They only do this when the cyclins are present.

FLICKING THE SWITCH ON



G2/M DNA DAMAGE CHECKPOINT

- The **G2/M DNA damage checkpoint** prevents the cell from entering mitosis (M phase) if the genome is damaged.
- It also checks if the cell is big enough (i.e. has the resources to undergo mitosis)
- Almost exclusively, internally controlled

M CHECKPOINT

- The M checkpoint is where the attachment of the spindle fibres to the centromeres is assessed.
- Only if this is correct can mitosis proceed.
- Failure to attach spindle fibres correctly would lead to failure to separate chromosomes

MEDICINE AND DNA DAMAGE REPAIR

- 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

.

Other DNA repair disorders include:

Werner's syndrome: premature aging and retarded growth

Bloom's syndrome: sunlight hypersensitivity, high incidence of 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 anemia, hereditary breast cancer and hereditary colon cancer.

DNA REPAIR AND CANCER

- There are at least 34 Inherited human DNA repair gene mutations that increase cancer risk. Many of these mutations cause DNA repair to be less effective than normal. In particular, Hereditary nonpolyposis colorectal cancer (HNPCC) is strongly associated with specific mutations in the DNA mismatch repair pathway. *BRCA1* and *BRCA2*, two famous genes whose mutations confer a hugely increased risk of breast cancer on carriers.

•Cancer therapy procedures such as chemotherapy and radiotherapy work by overwhelming the capacity of the cell to repair DNA damage, resulting in cell death. Cells that are most rapidly dividing — most typically cancer cells — are preferentially affected

Colon cancer is the second leading cause of cancer death in the United States. More than 15% of cancer deaths worldwide are linked to underlying infections or inflammatory conditions.

- An irreversible state of dormancy, known as senescence
- Cell suicide, also known as apoptosis or programmed cell death
- Unregulated cell division, which can lead to the formation of a tumor that is cancerous

DNA repair and anti aging

TA 65

The winners of the **2009 NOBEL PRIZE in MEDICINE** were scientists who discovered that a certain part of our DNA was responsible for the aging and death of our cells. This DNA segment is called the telomere. Telomeres are like the little plastic caps on the ends of your shoelaces. When the plastic cap wears away, the shoelaces fray, unravel, and don't work anymore. The same goes for telomeres. Over time, these "DNA caps" breakdown and begin to shorten. When they shorten far enough, they are unable to protect the rest of the DNA, and the DNA becomes non-functional. The cell dies, or more precisely it

telomere shortening in humans include: Inflammation, Oxidation, and Glycation (think sugar). In layman's terms we are talking: bad diet, stress, lack of exercise, stress, smoking, pollution, stress, plastics, sugar, stress, heavy metal exposure…

Back to DNA. Telomerase lengthens short telomeres. It rebuilds the plastic cap on your shoelaces. Telomerase has the potential to make our DNA stay active and healthy. It has the potential to make people live longer and stay healthier. Research on animals (and some humans) has shown unequivocally that re-lengthening short telomeres with telomerase can reverse heart disease, vascular disease, diabetes, cancer, Parkinson's, etc.


THANK YOU