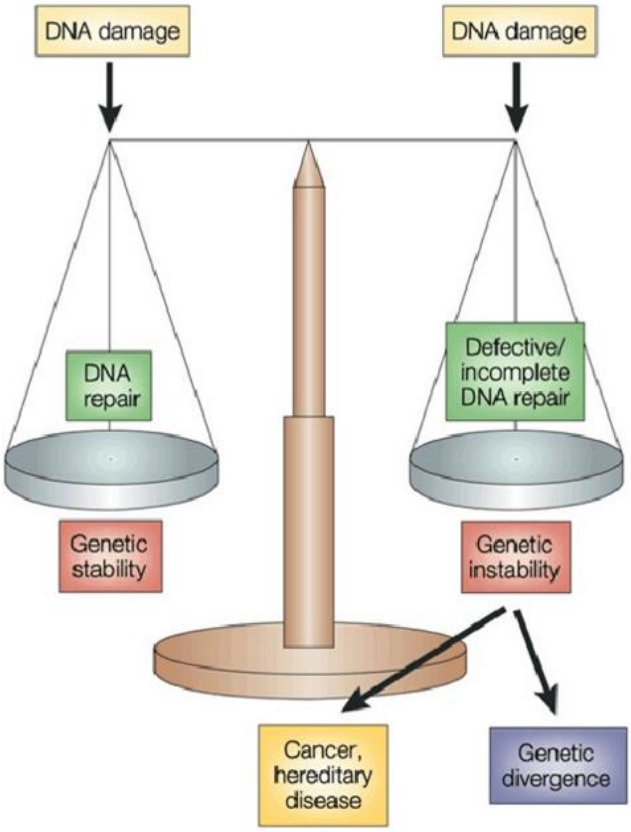
DNA Repair Systems

Replicating and non-replicating DNA is vulnerable to various forms of errors and lesions that constitute or lead to gene mutations. Living systems have evolved a variety of very elaborate repair systems that are able to counteract many of the forms of DNA damage that lead to mutation. The repair systems are essential to the survival of organisms on Earth. In humans, the loss of just one type of repair system leads to a devastating, life-shortening genetic disorders.

- Mutations are rare events,
- 1 per 10^9-10^{10} base pairs per cell division.
- misincorporation during DNA synthesis yields noncomplementary base pairs or mismatches.
- Mutations can also arise via incorporation of chemically damaged nucleotides or by incorporation of an undamaged nucleotide opposite a damaged base within the template strand.
- Strand slippage or formation of unusual secondary structures within DNA, especially within repetitive sequences, can also result in mutations when processed aberrantly during replication, recombination, or repair.



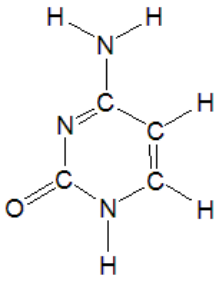
Information must be transmitted intact to daughter cells.

Accuracy is maintained by:

1- High fidelity in replication

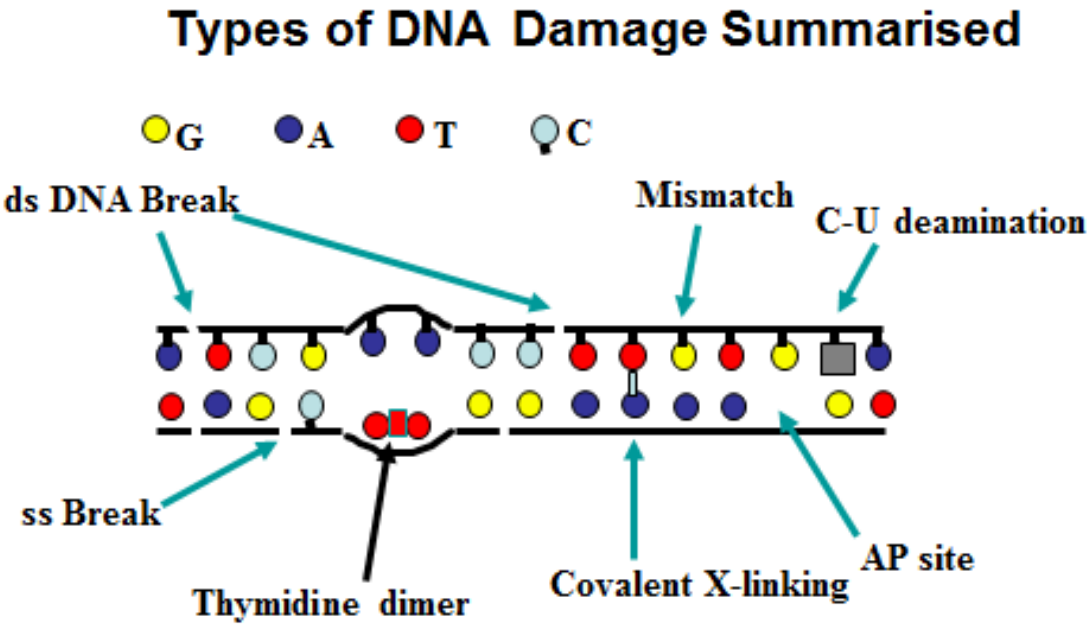
- 3'- exonuclease activity of DNA pol I
- Uracil-DNA N-glycosylase pathway (corrects mutations from deamination of cytosine)

Cytosine **Uracil**



2-Mechanisms for correcting genetic information in damaged DNA

- e.g due to chemical modifications
- Irradiation changes



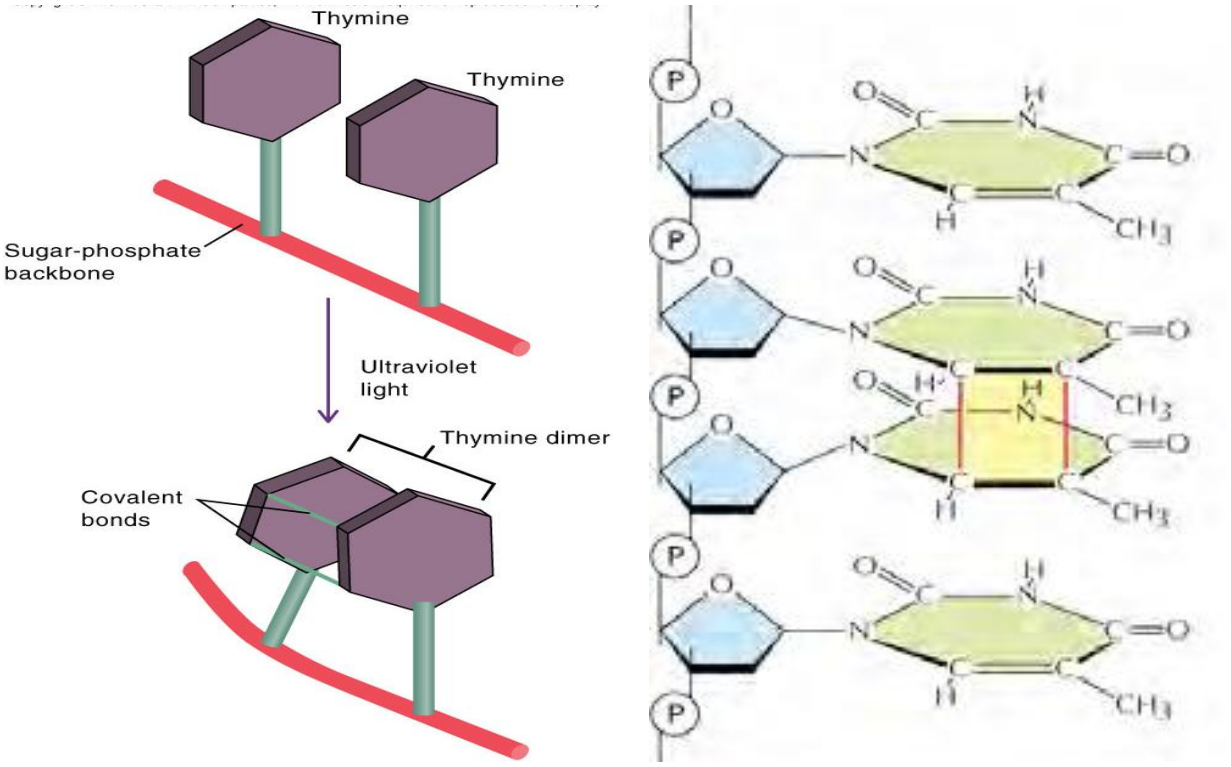
DNA REPAIR SYSTEMS

1. Photoreactivation (Light Repair)
2. Base excision Repair
3. Nucleotide Excision Repair
4. Recombination repair
5. MisMatch Repair
6. SOS response
7. Double strand break repair

(1) Photoreactivation (Light Repair)

UV light is mutagenic as a result of the creation of pyrimidine dimers. The study of mutagenicity of UV radiation paved (يسهل) the way for discovery of many forms of natural repair of DNA damage. The first relevant discovery concerning UV repair in bacteria was made in 1949 when Albert Kelner observed the phenomenon of photoreactivation repair (PR). He showed that the UV-induced damage to *E.coli* DNA could be partially reversed if, following irradiation, the cells were exposed briefly to light in the blue range of the visible spectrum.

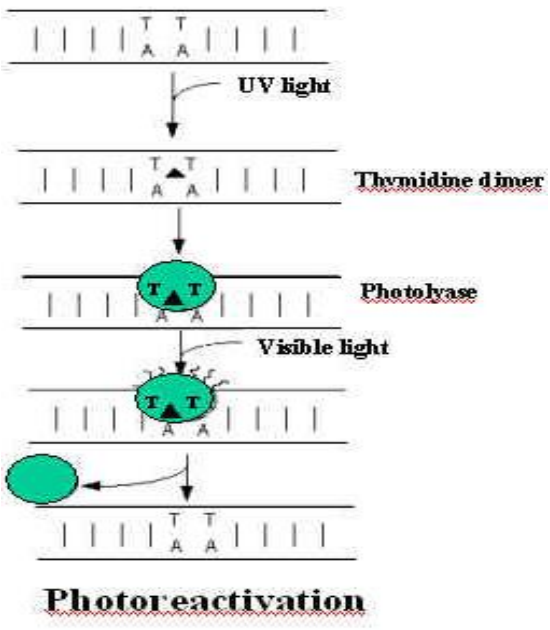
The (PR) process has subsequently been shown to be temperature dependent, suggesting that the light-induced mechanisms involve an enzymatically controlled chemical reaction. Visible light appears to induce the repair process of the DNA damage by UV irradiation.



Further studies of (PR) have revealed that the process is due to a protein called photoreactivation enzyme (PRE) or photlyase. This molecule can be isolated from extracts of *E.coli* cells. The enzyme's made of action is due to cleave the bonds (cyclobutyl bond) between thymine dimers, thus reversing the effect of UV radiation on DNA. Although the enzyme will associate with a dimer in the dark, it must absorb a photon of light to cleave the dimer.

The major components of (PR) system

- **PHR/PRE gene**
- **codes for photolyase**
- **with cofactor folic acid**
- **binds in dark to T dimer**
- **When light shines on cell**
- **folic acid absorbs the light (photon)**
- **uses the energy to break bond of T dimer**
- **photolyase then falls off DNA**



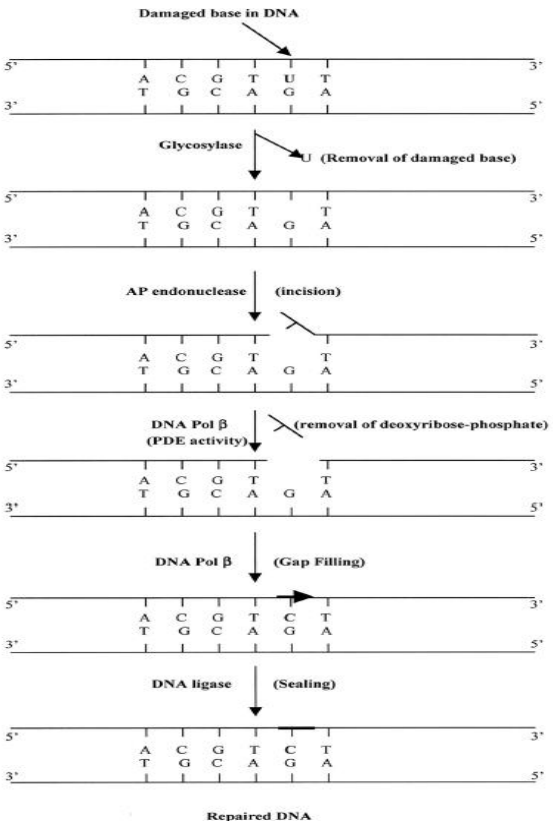
The gene (s) encoding PRE have been preserved throughout evolution. Activity of this repair system has been detected in both human cells in culture and in other eukaryotes. Conservation of the genetic components over millions of years suggests that this repair system is an important one to all organisms.

(2) Base excision Repair

- Consist of DNA glycosylases and AP endonuclease
- The DNA glycosylases are specific
  - Uracil glycosylase
  - Hypoxanthine DNA glycosylase
  - Etc…

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 by DNA Pol
6. Sealing DNA segments by DNA ligase.



(3) Nucleotide Excision Repair

- Used by the cell for bulky DNA damage
- Non-specific DNA damage by Chemical adducts & UV photoproducts

First identified in 1964 in *E. coli*.

- Steps for NER
  - Recognition of DAMAGE
  - Excision of DAMAGE
  - Replacement of excised DNA

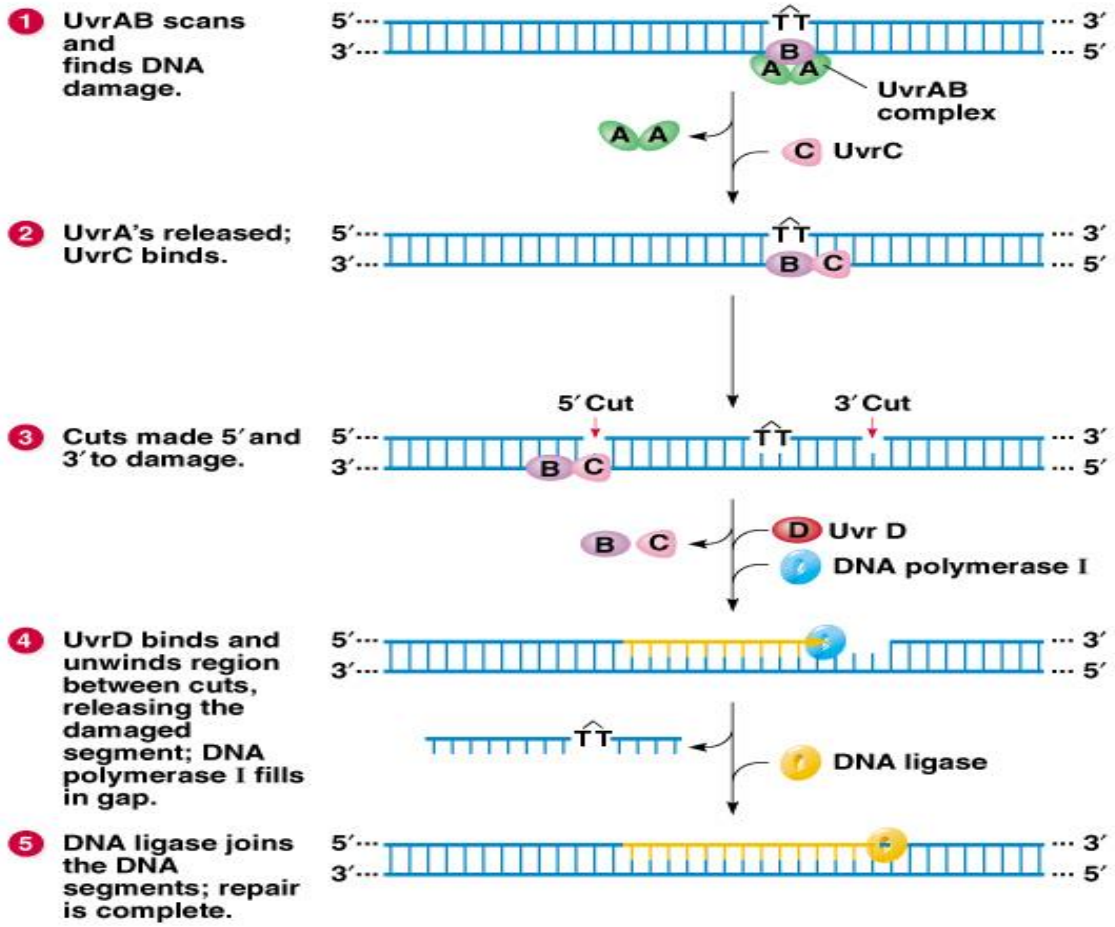
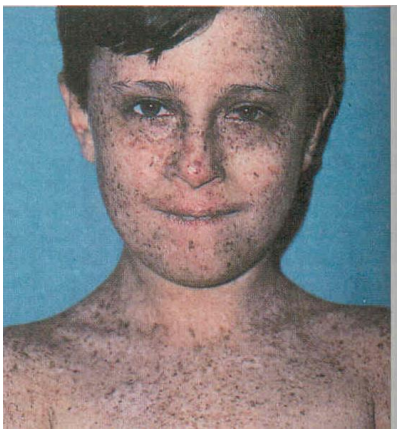


Figure: Nucleotide excision repair (NER) of pyrimidine dimmer and other damage-induced distortions of DNA

- Defects cause
- Xeroderma Pigmentosum
  - 1874, when Moriz Kaposi used this term for the first time to describe the symptoms observed in a patient.13 XP patients exhibit an extreme sensitivity to sunlight and have more than 1000-fold increased risk to develop skin cancer, especially in regions exposed to sunlight such as hands, face, neck



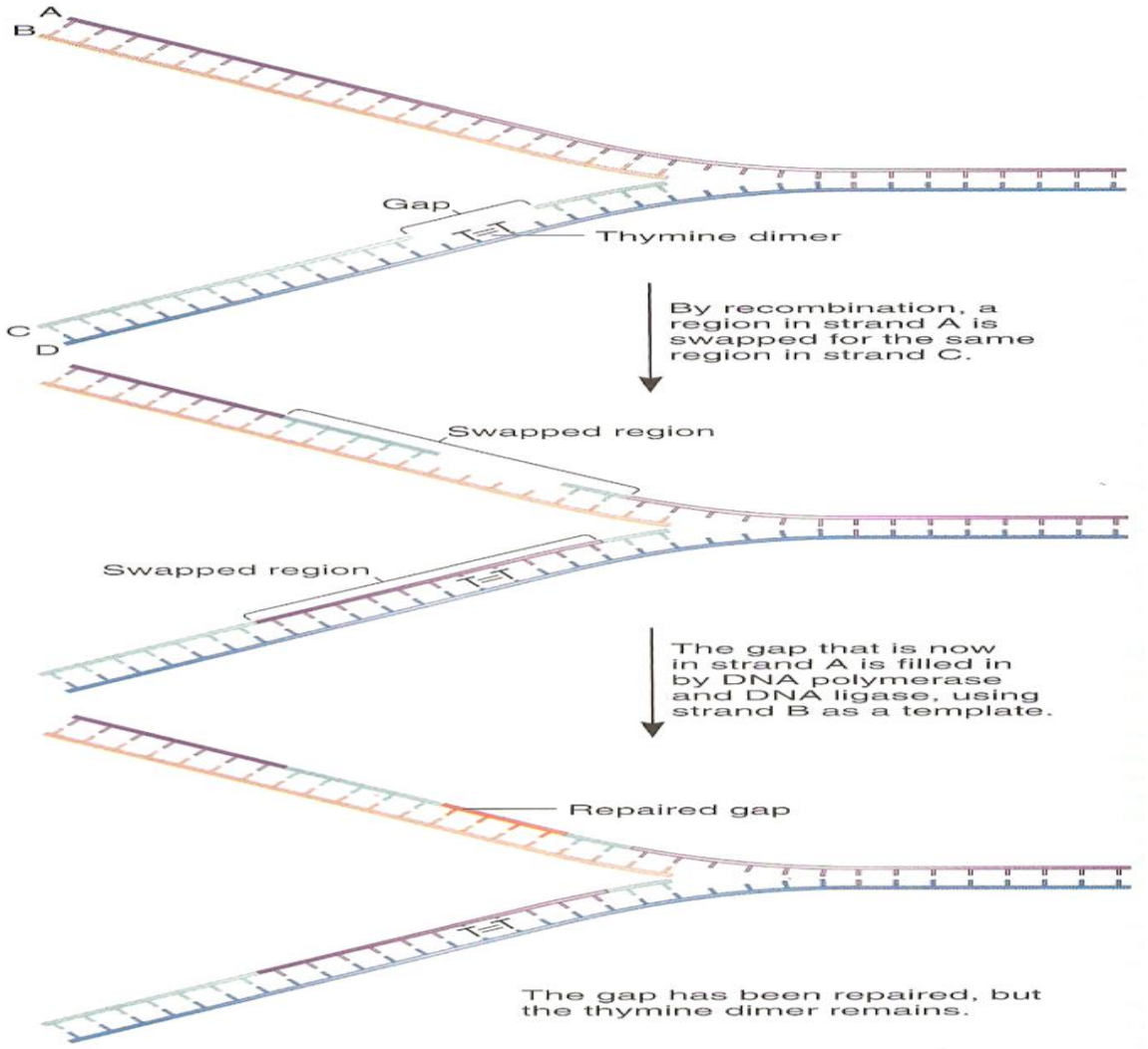
- Cockayne Syndrome
- Trichothiodystrophy

(4) Recombination repair

Another type of dark repair of UV damage that occurs in *E.coli* involves both replication and recombination and has thus been called postreplication recombination repair.

The process clearly involves replication of the damage chromosomes (or DNA strands) followed by recombination. A simplified model of postreplication recombination repair showed that, when DNA molecules containing thymine dimers are replicated, gaps are formed in the nascent complementary strands opposite the dimers because DNA polymerase cannot use the distorted strands as templates.

After a lag due to the thymine dimer blockage, replication is reinitiated at secondary initiation sites beyond the dimer. This results in progeny double helices with thymine dimers in one strand and gaps in the complementary strand. If these two "sister" chromosomes recombine such that the dimers and gaps end up in one chromosome and the intact, undamaged segments end up in the other chromosome, the latter will be functional and produce a viable cell.



(5) MisMatch Repair

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.

(6) SOS response

- SOS repair occurs when cells are overwhelmed by UV damage, this allows the cell to survive but at the cost of mutagenesis.
- SOS response only triggered when other repair systems are overwhelmed by amount of damage so that unrepaired DNA accumulates in the cell.
- 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 od 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*

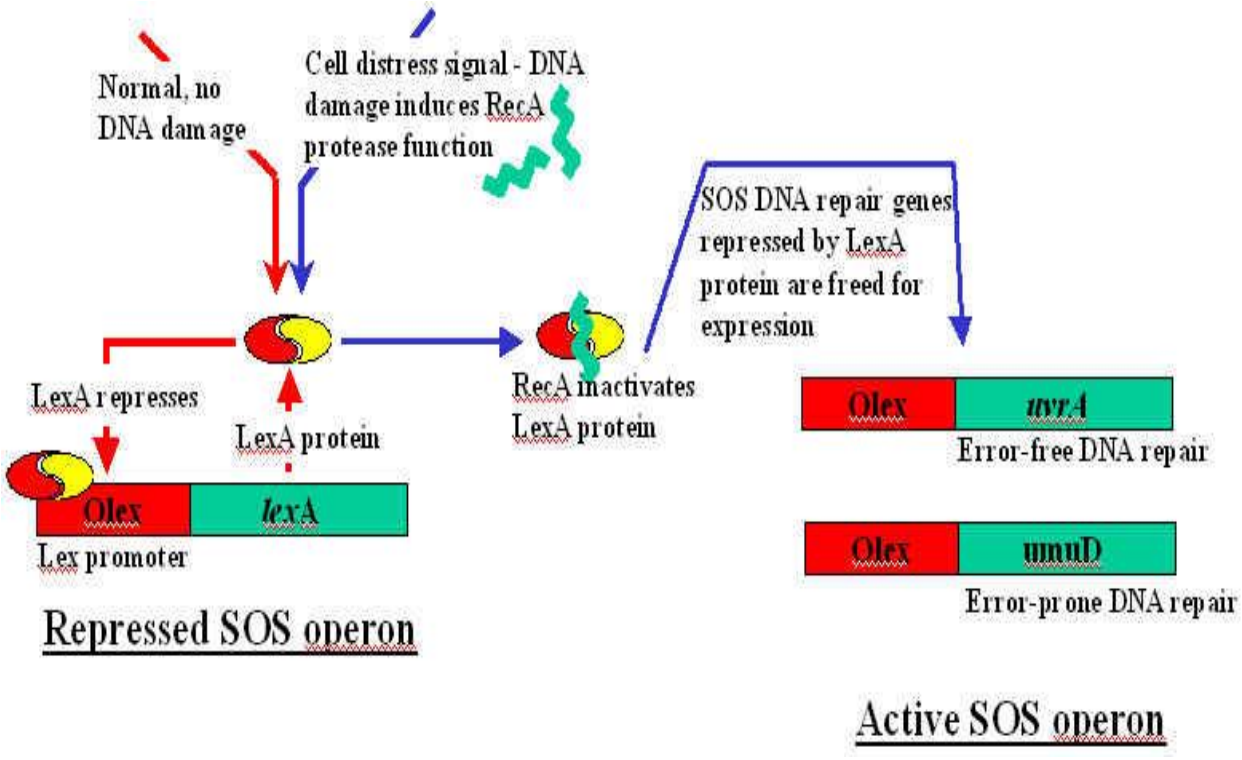
The SOS response in bacteria results when specific base pairing cannot occur. It allows the cell to survive otherwise lethal events, but usually at the cost of incurring new mutations.

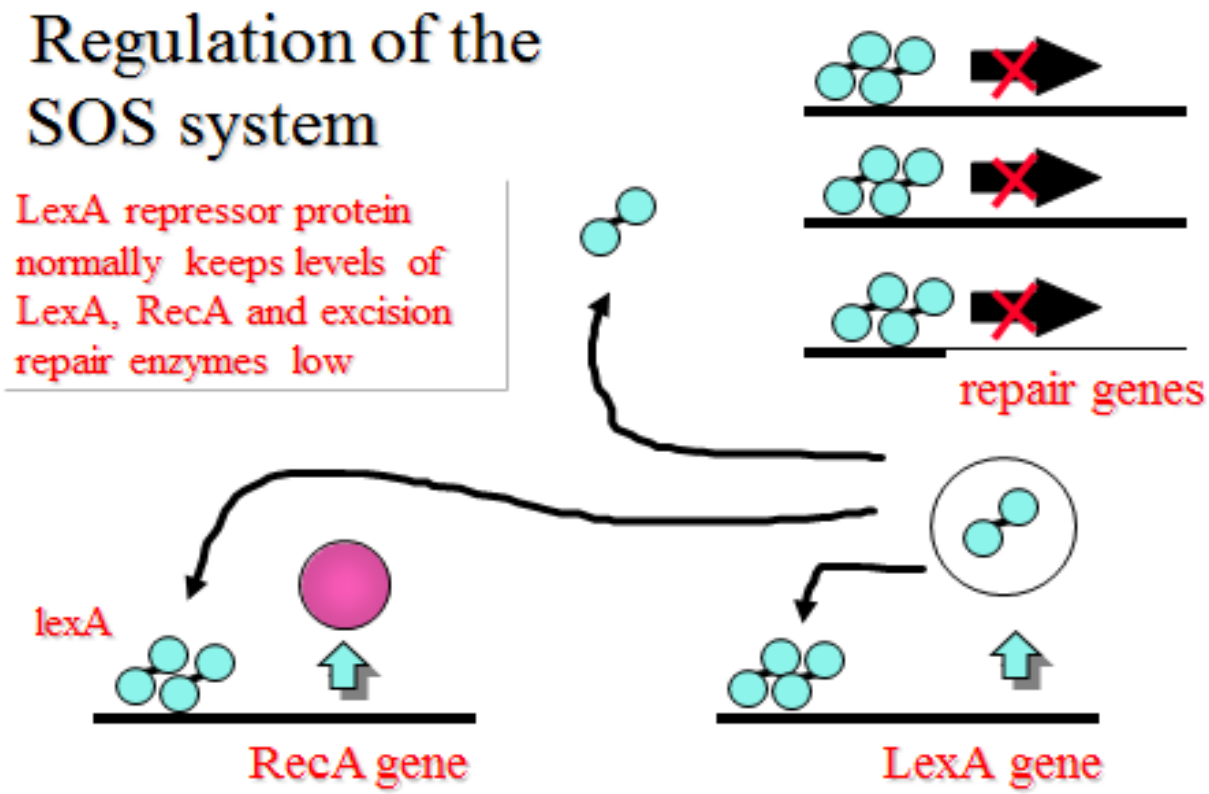
a) *E. coli* SOS is controlled by two genes, *lexA* and *recA*. (Mutants in either of these genes have their SOS response permanently turned on.)

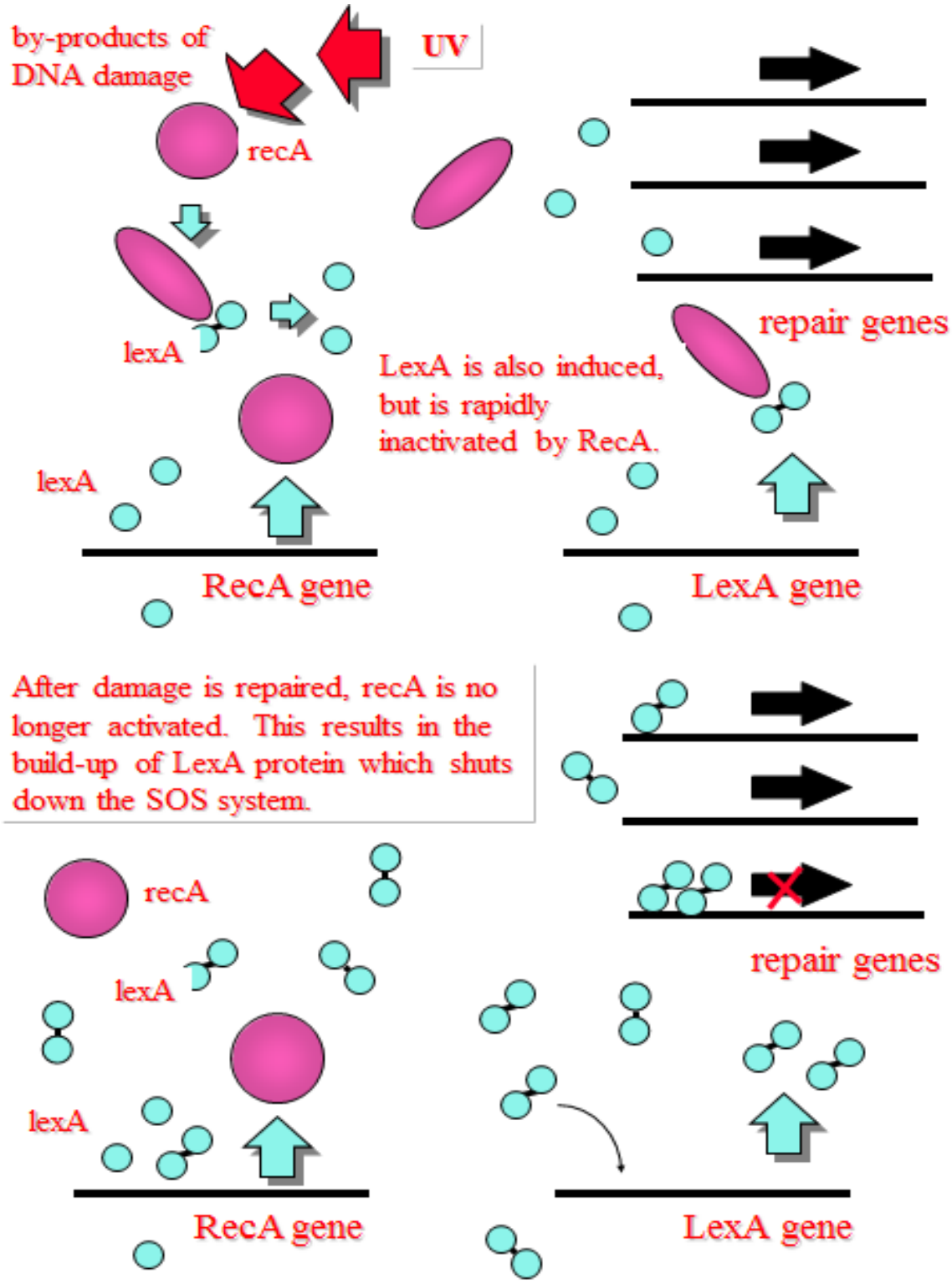
i. When no DNA damage is present, *LexA* represses transcription of about 17 genes with products involved in various types of DNA repair.
ii. Sufficient DNA damage activates the *RecA* protein, which stimulates *LexA* to autocleave, removing repression of the DNA repair genes.
iii. After damage is repaired, *RecA* is inactivated, and newly synthesized *LexA* again represses the DNA repair genes.

b) The SOS system is an error-prone bypass synthesis system.

i. Some lesions, like T^T dimers, are easily copied to give AA in the new DNA strand.
ii. Others, like C^C dimers, stall the SOS repair system, creating a delay during which C can be deaminated to a U (forming a C^U), creating a transition mutation.


Normal, no DNA damage
Cell distress signal - DNA damage induces RecA protease function
SOS DNA repair genes repressed by LexA protein are freed for expression
LexA represses
LexA protein
RecA inactivates LexA protein
Olex
lexA
Lex promoter
Repressed SOS operon
Olex
uvrA
Error-free DNA repair
Olex
umuD
Error-prone DNA repair
Active SOS operon


Regulation of the SOS system
LexA repressor protein normally keeps levels of LexA, RecA and excision repair enzymes low
repair genes
lexA
RecA gene
LexA gene



(7) Double strand break repair

DNA double-strand breaks (DSBs) are the most hazardous lesions arising in the genome of eukaryotic organisms, and yet occur normally during DNA replication, meiosis, and immune system development. The efficient repair of DSBs is crucial in maintaining genomic integrity, cellular viability, and the prevention of tumorigenesis.