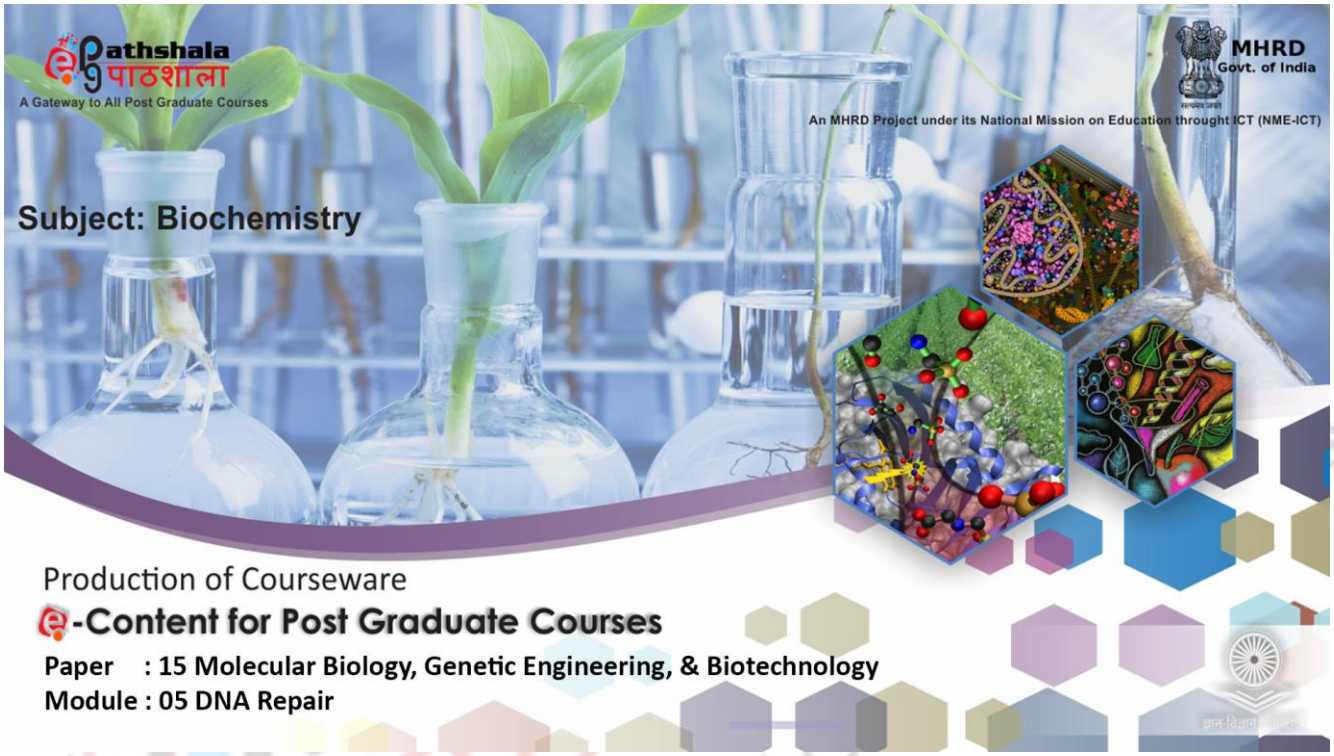

Production of Courseware

e-Content for Post Graduate Courses

Paper : 15 Molecular Biology, Genetic Engineering, & Biotechnology

Module : 05 DNA Repair

athshala पाठशाला

MHRD Govt. of India

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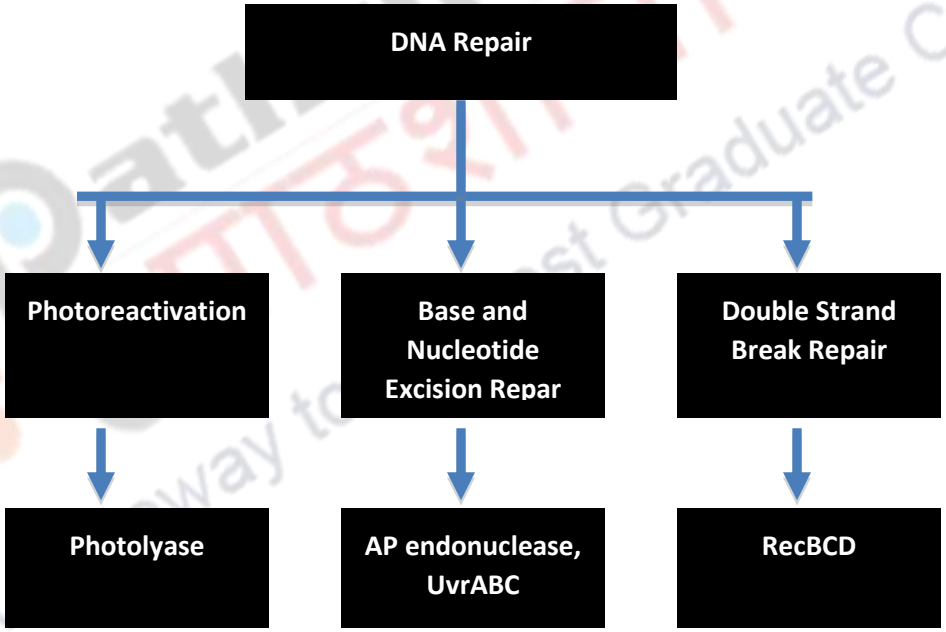
Description of Module	
Subject Name	Biochemistry
Paper Name	Molecular Biology, Genetic Engineering and Biotechnology
Module Name/Title	6 DNA Repair

DNA Repair

1. **Objective:**
   (a) Understanding of different mode of DNA repair.
   (b) How photoreactivation repairs DNA.
   (c) Base and Nucleotide excision Repair pathway.
   (d) Double strand break repair pathway.

2. **Concept map:**



3. **Description**

Maintenance of chromosomal integrity requires the repair of the damage part of the DNA before it is transferred to daughter cells after cell division. Cells with defective DNA repair system are unable to repair the damage site of the DNA, which results in permanent alteration of DNA bases leading to gene mutation. Consequences of mutation of essential gene of the cell are lethal for its survival. The presence of great variety of repair mechanisms in simple organisms like *E. coli* identifies its great physiological importance. These systems involves the action of a single enzyme which can eliminate the wrongly incorporated nucleotides with the correct one or a series of enzymes acting at the damaged site to excise and repair the damage segment of DNA. This module is dedicated to examine different DNA repair pathways how cells excise and replace damaged segment of DNA with correct nucleotides.

3.1 Direct reversal of DNA damage

Damage on DNA at a particular site can be repaired by a single catalytic reaction. Reports show that enzyme identifies and reverse certain types of damage on DNA. One of such kind of DNA damage by UV irradiation is the formation of pyrimidine dimers which can be repaired to their monomeric forms by a process called **photoreactivation**. In that process, an enzyme known as **DNA photolyase** catalyse the reversal reaction to restore the original DNA sequence**.** This protein with molecular weight of 55- to 65-kD is active in its monomeric form and prevalent in many prokaryotes and eukaryotes but not in humans. Two prosthetic groups are present in photolyase: a light-absorbing cofactor and $FADH^-$. The mechanism of photoreactivation in the *E. coli* enzyme involves the excitation of light absorbing cofactor (N^5,N^{10}-methenyltetrahydrofolate) by absorbing UV–visible light (300–500 nm), followed by transfer of the excitation energy to the $FADH^-$. Finally, an electron is transferred from $FADH^-$ to the pyrimidine dimer, thereby splitting it. $FADH^-$ at the end is reduced by resulting pyrimidine anion to regenerate the enzyme.

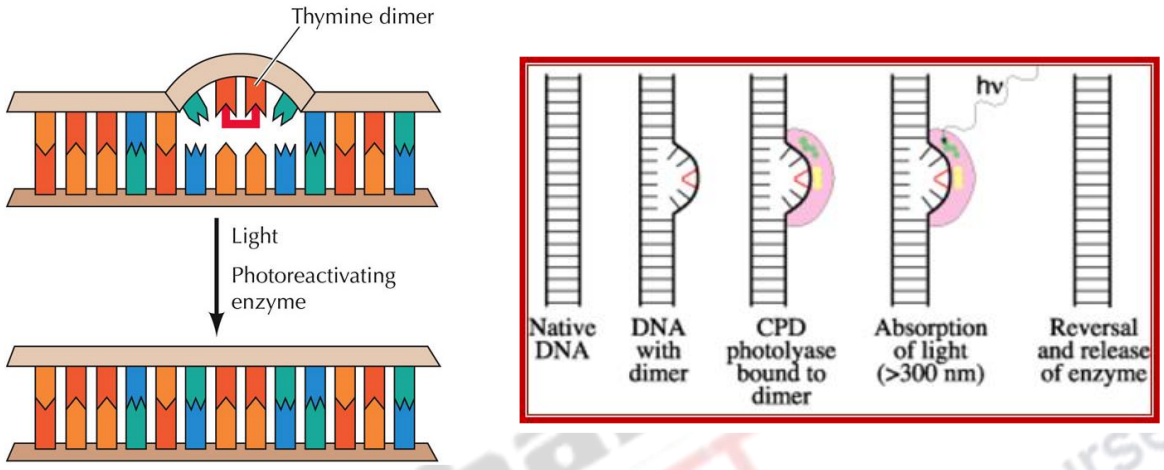


Figure 1: Mechanism of photoreactivation and DNA repair. Model in the left side show how thymine dimmer can be repaired in one catalytic step by the enzyme photolyase. Right side model shows the steps of photoreactivation and catalysis.

3.2 Replication error can be corrected by DNA mismatch repair

Any misincorporation of nucleotides during replication which elude the proofreading activity of DNA polymerases can be replaced with a correct nucleotide by a repair mechanism known as mismatch repair (MMR). One per 10^5 nucleotides is mistakenly incorporated during replication by DNA polymerase, the proofreading activity of which increases the fidelity of DNA replication by 100 fold with the removal of mispaired nucleotides before further extension of the DNA strand. Moreover, up to four nucleotide insertions or deletions which is arised from the slippage of one DNA strand relative to the other in the active site of DNA polymerase, can also be corrected by DNA mismatch repair mechanism. Slippage of DNA strand over other happens at particular region of DNA which contain the sequence motif rich in $[CA]_n$ or $[A]_n$. All these different kind of incidents which leads to misincrporation of nucleotides can produce permanent mutation if not repaired. Therefore, cells with impaired mismatch repair system will have high mutation rate and if that happens in essential gene will be lethal to cell survival. Reports show that defects in human mismatch repair system result in frequent incidence of cancer, most notably hereditary nonpolyposis colorectal cancer syndrome. Several organs are highly affected in this syndrome which may be the most common inherited predisposition to cancer. In *E. coli*, three different proteins carry out the mismatch repair process. A protein known as MutS in its homodimmeric form binds to unpaired bases or mismatch base pair of DNA, followed by

binding of another homodimmeric protein MutL. The resulting heterotetramer complex ($MutS_2MutL_2$) translocates in both direction of the DNA strands in an ATP-dependent process which cause the double stranded DNA to form a mismatch-containing loop that is closed by the $MutS_2MutL_2$ complex. Correcting the errors in the replication process, MMR system has to distinguish parental strand from daughter strand, which contains the misincorporated nucleotide. In *E. coli*, mismatch repair system can distinguish the two DNA strands because newly replicated DNA remains hemimethylated until methyltransferases have had sufficient time to methylate the daughter strand. After replication newly systhesized strand is not methylated by deoxyadenine methylase (Dam) which take some time to methylate the adenine residue in GATC sequence. $MutS_2MutL_2$ complex when encounters a nearby hemimethylated GATC palindrome (since parental strand is already methylated), recruits another enzyme MutH**,** an endonuclease, which makes a nick at the 5'-end of the unmethylated GATC sequence. The hemimethylated GATC site may be located on either side of the mismatch over nearly 1000 bp distant from it. A helicase UvrD, which is a member of nucleotide excision repair system, unwinds and seperates the nicked part of the daughter strand from the parental strand. Defective daughter strand is then digested completely by an exonuclease. DNA polymerase III then synthesizs that segment of daughter strand with correct nuleotides and finnaly DNA ligase seals the nick. Homologs of MutS and MutL are found in eukaryotic cells, however, homologue of MutH is absent and therefore eukaryotic cell must use some other cue than methylation status to differentiate the daughter and parental strands.

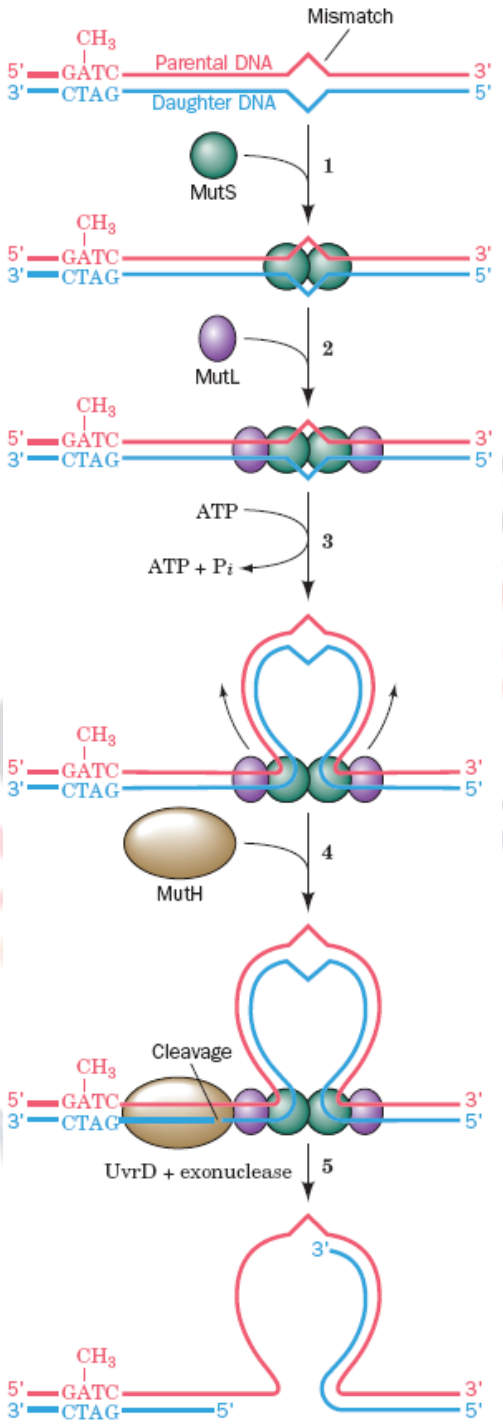


Figure 2: DNA mismatch repair system in *E. coli.* (**1**) Binding of MutS. (**2**) Binding of MutL. (**3**) The mismatch-containing DNA loops out. (**4**) Binding of MutH endonuclease. (**5**) Mismatched base is excised in unmethylated DNA strand.

3.3 Base excision repair

Not all types of DNA damage can be repaired by a single step, some repair pathway essentially involve a series of enzymes, each of which performs a specific job in the complete repair process. One of such repair process is known as base excision repair (BER), which is operative in an identical mechanism in almost all living cells. As the name implies, base excision repair pathway starts with removal of the damaged base by a variety of DNA glycosylases, which are present in the cell. DNA glycisylase cleaves the glycosidic bond linking deoxyribose to altered nucleotides resulting in the generation of a deoxyribose residue without any attached base, which is called apurinic or apyrimidinic sites (AP) or abasic sites. This situation can also be occasionally found after the spontaneous hydrolysis of glycosidic bonds. An enzyme, AP endonuclease, then cleaves off one side of deoxyribose residue, which along with some adjacent residues are removed by some exonucleases possibly associated with DNA polymerase. The gap generated by exonuclease action finally filled by DNA polymerase and the nick is sealed by DNA ligase. BER, which is found to be the most frequent type of DNA repair system, contains a glycosylase that identifies oxidized guanine nucleotide, 8-oxoguanine. Another example of such enzyme is uracil–DNA glycosylase, which excises uracil residues, which arise from cytosine deamination as well as the occasional misincorporation of uracil instead of thymine into DNA. Abasic sites in mammalian DNA have cytotoxic effect because mammalian topoisomerase I is trapped irreversibly in its covalent complex with DNA. In addition to that, since the sugar moiety at abasic site is devoid of a glycosidic bond, which can convert it to its linear form whose aldehyde group is very reactive and can cross-link to other cell components. This rationalizes why AP sites are tightly bound to UDG in solution. AP endonuclease enhances UDG activity, the following enzyme in BER pathway; however, two enzymes do not interact in the absence of DNA. This further suggests that an abasic site is always bound to UDG thereby protecting the cell from the AP site's cytotoxic effects. It is very likely that other DNA damage-specific glycosylases function in an identical pathway.

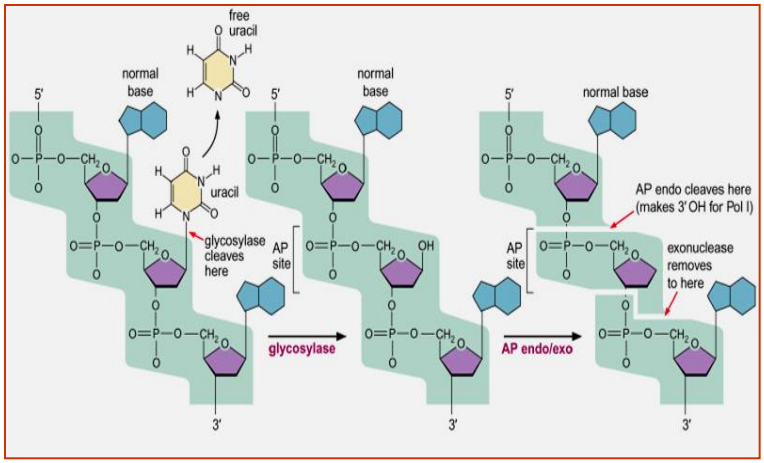


Figure 3: Base excision repair system in *E. coli.* The enzyme glycosylase catalyse the cleavage of N-glycosidic bond eliminating defective base. Another enzyme AP endonuclease catalyse the removal of sugar at the AP site.

3.4 Nucleotide excision repair

Apart from single base pair elimination at the damaged site of DNA, all cells also contain elaborate pathway of damage repair known as nucleotide excision repair (NER) to correct DNA lesions where DNA bases are displaced or substituted with a bulky group and also to correct pyrimidine dimmers to their monomeric form. Any kind of distortions in DNA helix rather than any mistakenly incorporated nucleotide, are recognized and responded by this repair system. There are reports showing that NER plays the major defense against two important carcinogens, sunlight and tobacco smoke in human. In bacteria like *E. coli*, NER is an ATP-dependent process where the actions of UvrA, UvrB, and UvrC proteins (the products of the *uvrA*, *uvrB*, and *uvrC* genes) are required to repair the helix distortion in DNA. This pathway is often referred to as the UvrABC endonuclease repair pathway, which is responsible for the cleavage of the damaged part of the DNA strand at the seventh and at the third or fourth phosphodiester bonds from the lesion's 5' and 3' sides, respectively. The DNA segment of 11- or 12-nt oligonucleotide long containing damage part is excised by UvrABC endonucleases and then displaced by a helicase UvrD. In this mechanism, a heterotrimeric complex consisting of $UvrA_2$ and UvrB recognizes a pyrimidine dimmer or other damage sites of the DNA. UvrA dissociates from the complex after damage site recognition with the expense of ATP. Endonuclease UvrC then joins UvrC and make an 5'-incision at a distance of 7 nuceotides from the damage site and another 3'-incision which is three to four nuceotides away from the damaged site. This step also requires energy from ATP hydrolysis. A helicase UvrD then helps unwind and release the single strand between two cuts. The gap is then filled by the actions of DNA polymerase I and DNA ligase seals the nick at the end.

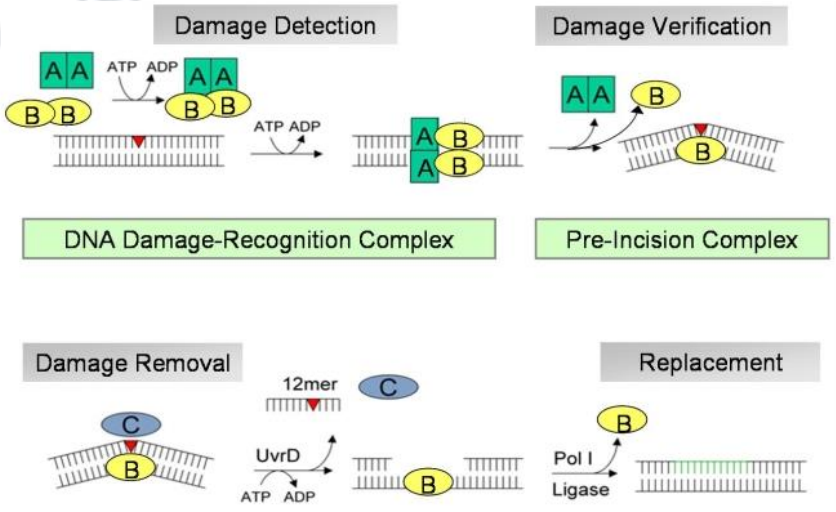


Figure 4: Nucleiotide excision repair system in *E. coli.* A homodimer of UvrA in association with a homodimer of UvrB identify the DNA lesion. UvrA falls off from UvrB after damage site recognition. UvrC protein binds to UvrB and make two incisions on both sides of damaged base generating a 12 nt long segment of DNA, which is removed by UvrD and rest of the job done by DNA pol I and ligase.

Defective NER causes genetic disorder like Xeroderma Pigmentosum and Cockayne Syndrome. A segment of 30 nucleotides long DNA can be excised out by eukaryotic NER system, which contains nearly 16 different proteins. The proteins are conserved in eukayotic system, however they don't show any sequence similarity with prokaryotic protein although they are doing the same kind of functions. This suggests that the two NER systems evolved in convergent pathway. Malfunction of any of these enzymes in human pathway have been identified through mutations that are manifested as two genetic diseases. Xeroderma pigmentosum (XP) is one of such diseases mainly inherited from ancestors to descendents and is associated with the impaired NER system in skin cells where the skin cells are unable to repair UV-induced DNA lesions. Individuals with autosomal recessive condition to XP are extremely sensitive to sunlight. Skin during infancy changes such as excessive freckling, dryness, and keratoses (a type of skin tumor, together with eye damage, such as opacification and ulceration of the cornea. Moreover, cells with impaired NER system often develop fatal skin cancers at nearly 2000-fold greater rate than normal and internal cancers at a 10- to 20-fold increased rate. Reports show that many individuals with XP also are prone to develop many physiological dysfunction including neurological degeneration and developmental deficits.

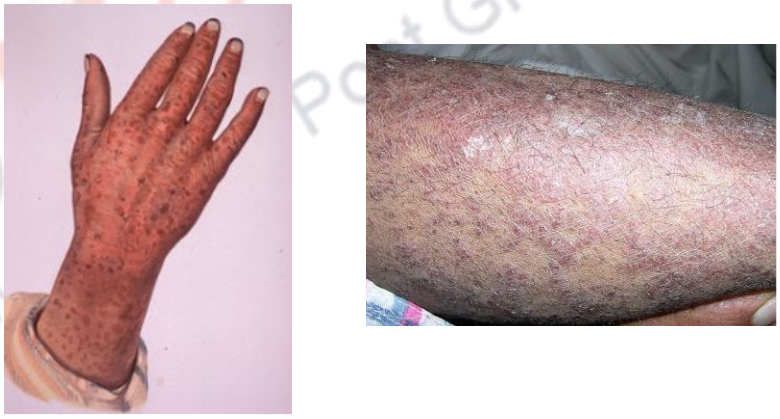


Figure 5: Individuals with impaired NER develop Xeroderma pigmentosum. Above pictures show the appearance of the skin of an individual suffering from Xeroderma pigmentosum.

Cockayne syndrome (CS) is another inherited disease, which is also associated with defective NER. This disease is developed from defects in same three genes that are defective in XP as well as in two additional genes. Individuals with CS develop hypersensitiveity to UV radiation and exhibit stunted growth. Other effects includes neurological dysfunction as a result of neuron demyelination, and the appearance of premature aging but, intriguingly, have a normal incidence of skin cancer. The defective proteins in CS normally recognize a halted RNA polymerase at a damaged or distorted DNA template. To resume halted transcription process the stalled RNA polymerase should be removed so that NER system can repair the damaged part of the DNA, which causes the helix distortion. Individuals with CS cannot repair which causes the cell to go though programmed cell death or apoptosis. The death of cells, which are transcriptionally active may account for the developmental symptoms of Cockayne syndrome.

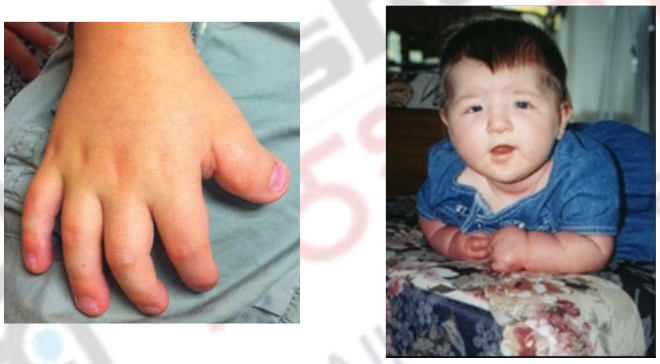


Figure 6: Above pictures show the appearance of the skin of a child suffering from Cockayne syndrome.

3.5 Double strand break repair

DNA damage often times causes double strand break, which should be repaired otherwise replication machinery will be stalled at the site of double strand break. The repair system, which fix this break and make a normal continuous DNA, requires the action of a multi-enzyme complex, RecBCD protein in *E. coli*. This complex is a 330-kD heterotrimeric product of genes recB, recC, and recD. The proteins have both helicase and nuclease activities and binds to double strand break site of DNA and then with its helicase function unwinds dsDNA with the expense of ATP. Its nuclease activity degrades the unwound single strands and move forward until they encounter a Chi site on DNA from its 3' end. Chi site is very specific sequence of GCTGGTGG occurs after every five kilobases in *E. coli* genome. Once RecBCD reaches chi sequence it stops trimming from 3'-end of the

strands and increases the rate of cleavage from 5'-end of the strands, thereby generating a 3'-end single-strand overhang to which recombination inducing protein RecA binds to form a filamentous structure. This fact also explains why regions containing Chi sequences have elevated rates of recombination. RecA, a recombination inducing protein, is recruited by RecB protein and is also nucleated to the 3′-overhang of the DNA strand. RecA in association with 3'-overhang of DNA strand scan both the strands of an undamaged DNA to identify the complementary sequence to that overhang and then direct to exchange strand forming a branch point. Using both stands of undamaged strands as template the gaps generated by the action of RecBCD are filled by DNA polymerase followed by the action of DNA ligase, which seals the nick.

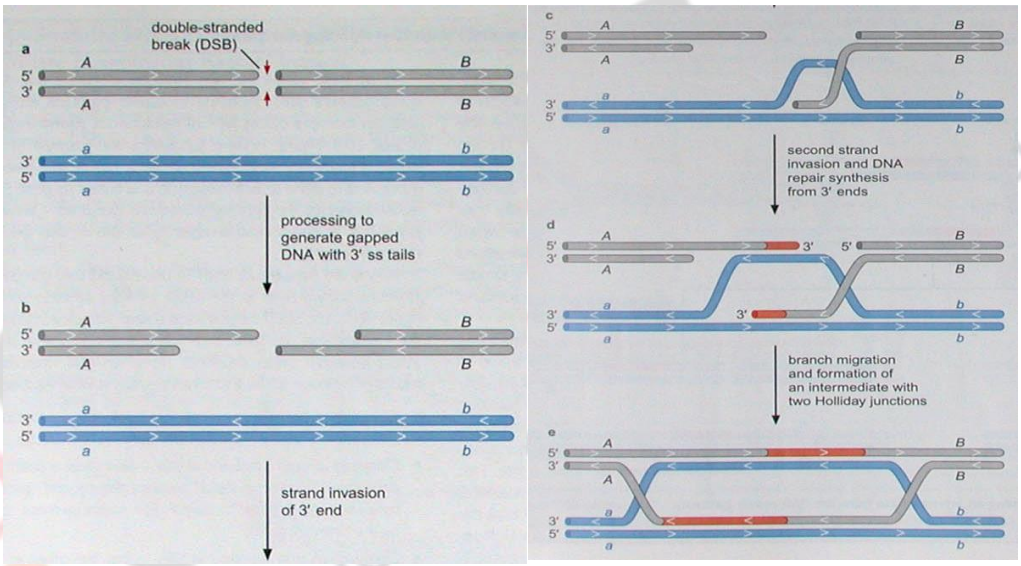


Figure 7: Steps of Double strand break repair pathway. At the beginning exonuclease processing generates gapped DNA with a single-stranded overhang. RecA protein binds to single-stranded region and help promote the strand invasion. DNA repair synthesis starts from 3′-end. Branch migration occurs and generates intermediates with two cross over points.

3.6 SOS Response

SOS Response is mutagenic in *E. coli*. DNA damaging agents in *E. coli* induce a complex system of cellular changes, which is known as the SOS response. In this condition, cell division is stopped and cell's ability to repair DNA damage is increased. A repressor protein called LexA and a DNA binding protein RecA, which is also a key player in homologous recombination, regulates the activity of the SOS response. RecA protein is also involved in DNA repair after replication. Under

normal physiological growth condition, LexA represses SOS gene expression. However, when DNA is not completely replicated due to DNA damage the resulting single strands bind to RecA to form a filamentous complex that activates LexA to cleave and thereby inactivate itself. Once the repression is removed due to the damage signal mediated by RecA different SOS genes start to express. In bacteria there are nearly forty different kinds of SOS genes can be expressed, which include recA and lexA as well as the nucleotide excision repair genes like uvrA and uvrB. Again in the absence of any DNA damaging agents, the DNA.RecA complex is no longer present, so the expression of the SOS genes are repressed by the newly synthesized LexA. The SOS repair system is error-prone and consequently mutagenic.

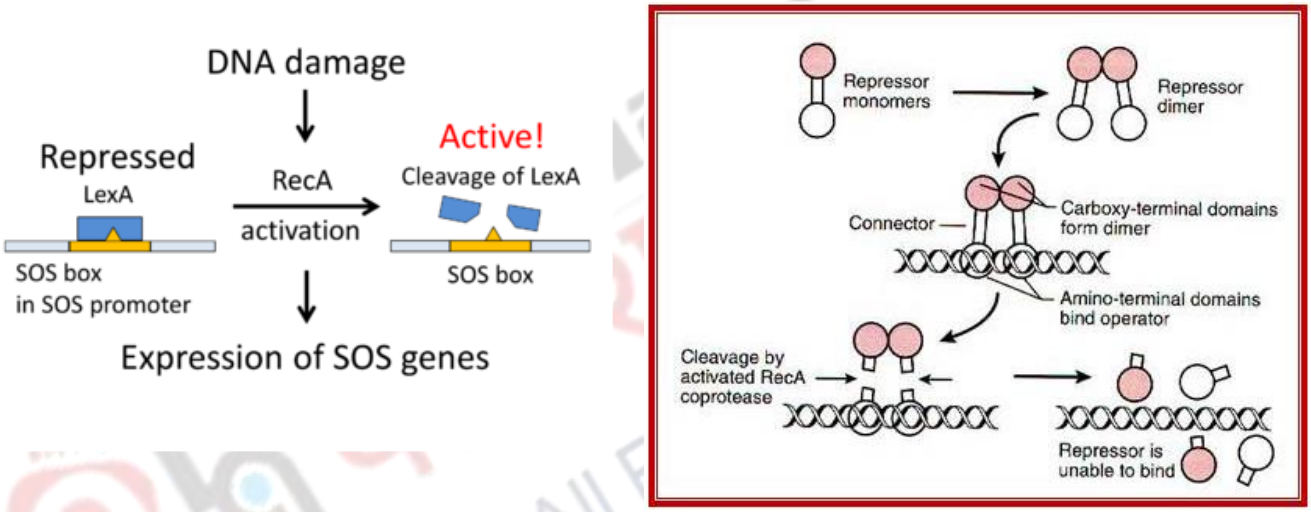


Figure 8: Expression of SOS genes. Picture in the left shows the overall process of SOS response. LexA binds to the SOS box and suppress the expression of SOS genes. However, different radiation induces and activates RecA protein, which facilitates the autocatalysis of LexA and thereby turn on the SOS response genes. Picture in left side shows that a dimeric repressor LexA activated under normal condition and binds to the operator site of the SOS genes through its amino terminal domain.

4. Summary

In this lecture we learnt about:

- Different kind of DNA repair process in cells
- Mechanism of DNA repair by different Pathway
- How SOS response induce repair system
- Impaired repair system leads to genetic disorder