DNA REPAIR MECHANISM

- The multiplicity of repair mechanisms that have evolved in organisms ranging from bacteria to humans tells the importance of repair mechanism keeping mutation at a tolerable level.
- E. coli cells possess five well-characterized mechanisms for the repair of defects in DNA:
  a. Light-dependent repair or photo-reactivation,
  b. Excision repair,
  c. Mismatch repair,
  d. SOS response.
- Mammals seem to possess all of the repair mechanisms found in E. coli except photoreactivation.

LIGHT-DEPENDENT REPAIR

- Light-dependent repair or photo-reactivation of DNA in bacteria is carried out by a light activated enzyme called DNA photolyase.
- When DNA is exposed to ultraviolet light, thymine dimers are produced by covalent cross-linkages between adjacent thymine residues.
- DNA photolyase recognizes and binds to thymine dimers in DNA, and uses light energy to cleave the covalent cross-links.
- Photolyase will bind to thymine dimers in DNA in the dark, but it cannot catalyze cleavage of the bonds.
- In the presence of light (Blue light) it cleaves the dimer bond and restores the original form.
- Photolyase also splits cytosine dimers and cytosine-thymine dimers.
- Thus, when ultraviolet light is used to induce mutations in bacteria, the irradiated cells are grown in the dark for a few generations to maximize the mutation frequency.
EXCISION REPAIR

- Excision repair of damaged DNA involves at least three steps.
- There are two major types of excision repair: base excision repair systems remove abnormal or chemically modified bases from DNA, whereas nucleotide excision repair pathways remove larger defects like thymine dimers.

**Base excision repair**

- Step 1 - It can be initiated by any of a group of enzymes called DNA glycosylases that recognize abnormal bases in DNA.
- Step 2 - Each glycosylase recognizes a specific type of altered base, such as deaminated bases, oxidized bases, and so on.
- Step 3 - The glycosylases cleave the glycosidic bond between the abnormal base and 2-deoxyribose, creating apurinic or apyrimidinic sites (AP sites) with missing bases.
- Step 4 - AP sites are recognized by enzymes called AP endonucleases, which act together with phosphodiesterases to excise the sugar-phosphate groups at these sites.
- Step 5 - DNA polymerase then replaces the missing nucleotide according to the specifications of the complementary strand, and
- Step 6 - DNA ligase seals the nick.
Nucleotide excision repair

- Excinuclease activity requires the products of three genes, **uvrA**, **uvrB**, and **uvrC** (designated uvr for UV repair).
- A trimeric protein containing two UvrA polypeptides and one UvrB polypeptide recognizes the defect in DNA, binds to it, and uses energy from ATP to bend the DNA at the damaged site.
- The UvrA dimer is then released, and the UvrC protein binds to the UvrB/DNA complex.
- The UvrC protein cleaves the fourth or fifth phosphodiester bond from the damaged nucleotide(s) on the 3’ side and the eighth phosphodiester linkage from the damage on the 5’ side.
- The **uvrD** gene product, DNA helicase II, releases the excised dodecamer. In the last two steps of the pathway, DNA polymerase I fills in the gap, and DNA ligase seals the remaining nick in the DNA molecule.

- Nucleotide excision repair in humans occurs through a pathway similar to the one in *E. coli*, but it involves about four times as many proteins. Protein XPA (for xeroderma
pigmentosum protein A) recognizes and binds to the damaged nucleotide(s) in DNA. It then recruits the other proteins required for excinuclease activity.

MISMATCH REPAIR

- The mechanism by which the 3′ → 5′ exonuclease activity built into DNA polymerases proofreads DNA strands during their synthesis, removing any mismatched nucleotides at the 3′ termini of growing strands.
- Another postreplication DNA repair pathway, mismatch repair, provides a backup to this replicative proofreading by correcting mismatched nucleotides remaining in DNA after replication.
- Mismatches often involve the normal four bases in DNA. For example, a T may be mispaired with a G.
- Because both T and G are normal components of DNA, mismatch repair systems need some way to determine whether the T or the G is the correct base at a given site.
- The repair system makes this distinction by identifying the template strand.
- In bacteria, this distinction can be made based on the pattern of methylation in newly replicated DNA. In E. coli, the A in GATC sequences is methylated subsequent to its synthesis.
- Thus, a time interval occurs during which the template strand is methylated, and the newly synthesized strand is unmethylated. The mismatch repair system uses this difference in methylation state to excise the mismatched nucleotide in the nascent strand and replace it with the correct nucleotide by using the methylated parental strand of DNA as template.

- In E. coli, mismatch repair requires the products of four genes, mutH, mutL, mutS, and mutU.
- The MutS protein recognizes mismatches and binds to them to initiate the repair process.
- MutH and MutL proteins then join the complex.
- MutH contains a GATC-specific endonuclease activity that cleaves the unmethylated strand at hemimethylated (that is, half methylated) GATC sites either 5′ or 3′ to the mismatch.
- The incision sites may be 1000 nucleotide pairs or more from the mismatch.
- The subsequent excision process requires MutS, MutL, DNA helicase II (MutU), and an appropriate exonuclease.
- After the excision process has removed the mismatched nucleotide from the unmethylated strand, DNA polymerase III fills in the large—up to 1000 bp—gap, and DNA ligase seals the nick.
- Homologues of the E. coli MutS and MutL proteins have been identified in fungi, plants, and mammals—a indication that similar mismatch repair pathways occur in eukaryotes.
SOS RESPONSE

- 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 (UV mutable) 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, in so doing, increases the frequency of replication errors.

- Two key regulatory proteins—LexA and RecA—control the SOS response.
- Both are synthesized at low levels in the cell in the absence of damaged DNA.
- Under this condition, LexA binds to the DNA regions that regulate the transcription of those genes that are activated/expressed 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 which are under replication process.

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.

When the error-prone repair system is operative, mutation rates increase sharply.

**References and further Readings:**

1). Principles of Genetics by Snustad

2). Principles of Genetics by Tamrin

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