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Topic DNA REPAIR (PART 2)

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M.Sc IInd semester and B.Sc 2nd Year

Single-strand damage

When only one of the two strands of a double helix has a defect, the other strand can be used as a template to guide the correction of the damaged strand. In order to repair damage to one of the two paired molecules of DNA, there exist a number of excision repair mechanisms that remove the damaged nucleotide and replace it with an undamaged nucleotide complementary to that found in the undamaged DNA strand.

Base excision repair

Excision repair: A process whereby cells remove part of a damaged DNA strand and replace it through DNA synthesis using the undamaged strand as a template

The **discovery** of BER is credited to Tomas Lindahl

Received the 2015 Nobel Prize in Chemistry for his contributions to the mechanism of this DNA **repair** model

BER mainly repairs non-bulky lesions produced by alkylation, oxidation or deamination of bases.

Repairs damaged DNA throughout the cell cycle.

It is responsible primarily for removing small, non-helix-distorting base lesions from the genome.

BER is important for removing damaged bases that could otherwise cause mutations by mispairing or lead to breaks in DNA during replication.

The **repair** process takes place in five core **steps**: (1) **excision** of the **base**, (2) incision, (3) end processing, and (4) **repair** synthesis, including gap filling and ligation

Proteins involved in base excision repair

- **DNA glycosylases:** recognize and remove damaged bases from DNA by cleaving the base–sugar (N-glycosylic) bond, and downstream base excision repair enzymes restore the correct nucleotide. Leaving an AP (apurinic/apyrimidinic) site.
- **AP endonucleases:** AP endonucleases: Apurinic/apyrimidinic (AP) endonucleases play inportant roles in the repair of damaged or mismatched nucleotides in DNA to create a nick in the phosphodiester backbone of the AP site created after DNA glycosylase removes the damaged base. There are two kinds of AP endonucleases in humans, APE1 and APE2. APE1 is considered to be the major AP endonuclease in human cells. It exhibits robust AP-endonuclease activity, which accounts for >95% of the total cellular activity. Mg^{2+} is requested in its active site in order to carry out its role in base excision repair. APN1 is the homolog of this enzyme in the yeast.
- **End processing enzymes:** End processing enzymes: Polynucleotide kinase-phosphatase (PNKP) promotes formation of hydroxyl on its 3' end and a phosphate on its 5' end during BER. Its phosphatase domain removes phosphates from 3' ends and the kinase domain phosphorylates 5' hydroxyl ends. Together, these activities are ready for single-strand breaks with damaged termini for further ligation.
- **DNA polymerases:** Enzymes synthesize DNA molecules from deoxyribonucleotides, the building blocks of DNA. These enzymes are essential for DNA replication and usually work in pairs to create two identical DNA strands from a single original DNA molecule. During this process, DNA polymerase "reads" the existing DNA strands to create two new strands that match the existing ones.
- **Flap endonuclease:** A class of nucleolytic enzymes that act as both 5'-3' exonucleases and structure-specific endonucleases on specialised DNA structures. The later role functions during the biological processes of DNA replication, DNA repair, and DNA recombination. The endonuclease activity of FENs was initially identified as acting on a DNA duplex which has a single-stranded 5' overhang on one of the strands (termed a "5' flap", hence the name flap endonuclease). FENs catalyse hydrolytic cleavage of the phosphodiester bond at the junction of single- and double-stranded DNA.
- **DNA ligase:** DNA ligase: It is used for both DNA repair and DNA replication. DNA ligase catalyzes the formation of a phosphodiester bond to facilitate the joining of DNA strands together. It usually plays a role in repairing single-strand breaks in duplex DNA in living organisms, but

some forms (such as DNA ligase IV) may specifically repair double-strand break.

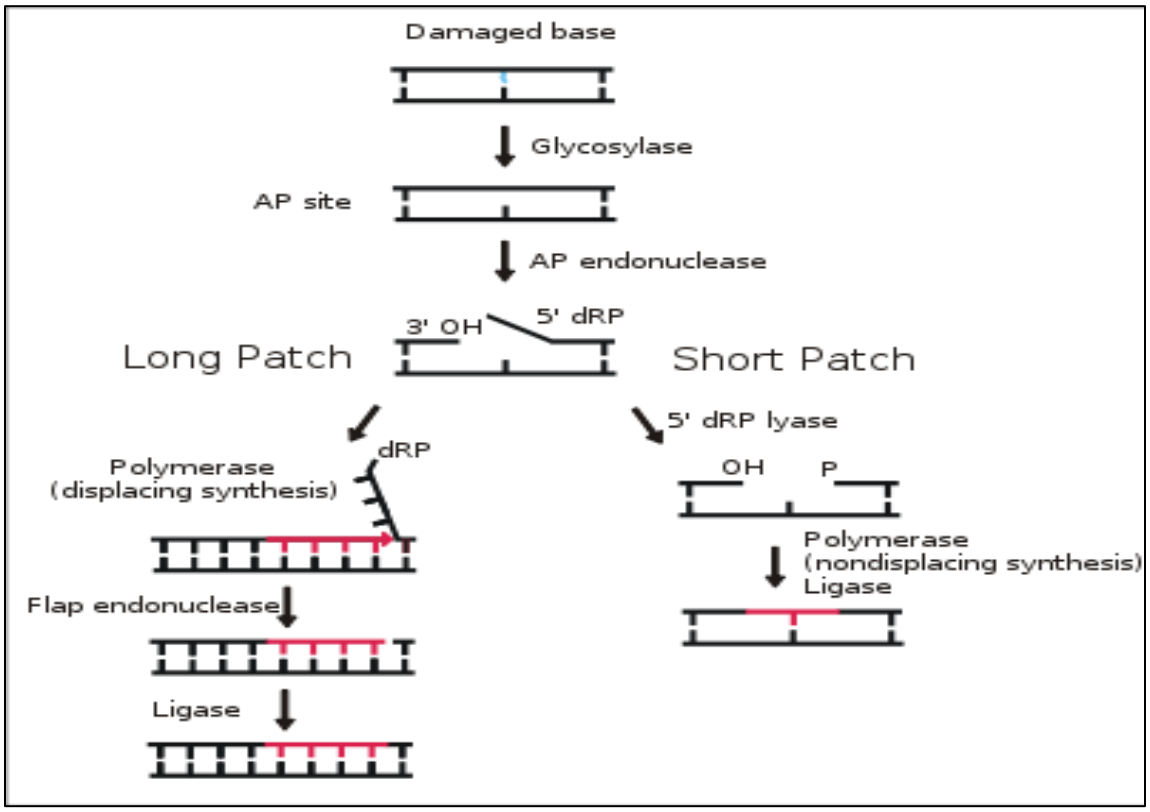

FIG: STEPS OF BASE EXCISION REPAIR

(1) Cells contain several DNA glycosylases, each of them exhibiting a specific substrate spectrum.

(2) After cleavage of the N-glycosylic bond by a DNA glycosylase, the damaged base is released and an apurinic/apyrimidinic (AP site) is created.

(3) An AP site can also occur spontaneously and represents damage itself.

(4) Bifunctional glycosylases have an intrinsic AP lyase activity, which cleaves the sugar-phosphate backbone 3′ to the AP site.

(5) The resulting fragmented sugar residue is removed by a phosphodiesterase activity, contributed by either an AP endonuclease or by DNA polymerase β

(6) The one-nucleotide gap is filled by Pol β and ligated.

(7) Pol β incorporates a nucleotide and its deoxyribophosphodiesterase (dRPase) activity removes the 5′ moiety. The remaining nick is sealed by ligation.

NUCLEOTIDE EXCISION REPAIR

Nucleotide excision repair (NER) is a particularly important **excision** mechanism that removes DNA damage induced by ultraviolet light (UV), environmental mutagens, and some cancer chemotherapeutic adducts from DNA.

Deficiencies in NER are associated with the extremely skin cancer-prone inherited disorder xeroderma pigmentosum.

UV radiation can make cytosine and thymine bases react with neighboring bases that are also Cs or Ts, forming bonds that distort the double helix and cause errors in DNA replication. The most common type of linkage, a **thymine dimer**, consists of two thymine bases that react with each other and become chemically linked

This pathway detects bases that have been modified with bulky chemical groups, like the ones that get attached to your DNA when it's exposed to chemicals in cigarette smoke

In **nucleotide excision repair** (**NER**), damaged bases are cut out within a string of nucleotides, and replaced with DNA as directed by the undamaged template strand.

This repair system is used to remove pyrimidine dimers formed by UV radiation as well as nucleotides modified by bulky chemical adducts.

The common feature of damage that is repaired by nucleotide excision is that the modified nucleotides cause a significant distortion in the DNA helix.

NER occurs in almost all organisms examined.

Nucleotide excision repair (NER). NER pathways can be classified into two groups, global genome repair (GGR) and transcription-coupled repair (TCR). The NER pathway consists of a series of reactions: recognition of DNA damage, unwinding double-stranded DNA in the neighborhood of the damage, excision of the damaged nucleotides, and filling the gap by DNA synthesis and ligation.

In nucleotide excision repair, the damaged nucleotide(s) are removed along with a surrounding patch of DNA. In this process, a helicase (DNA-opening enzyme) cranks open the DNA to form a bubble, and DNA-cutting enzymes chop out the damaged part of the bubble. A DNA polymerase replaces the missing DNA, and a DNA ligase seals the gap in the backbone of the strand

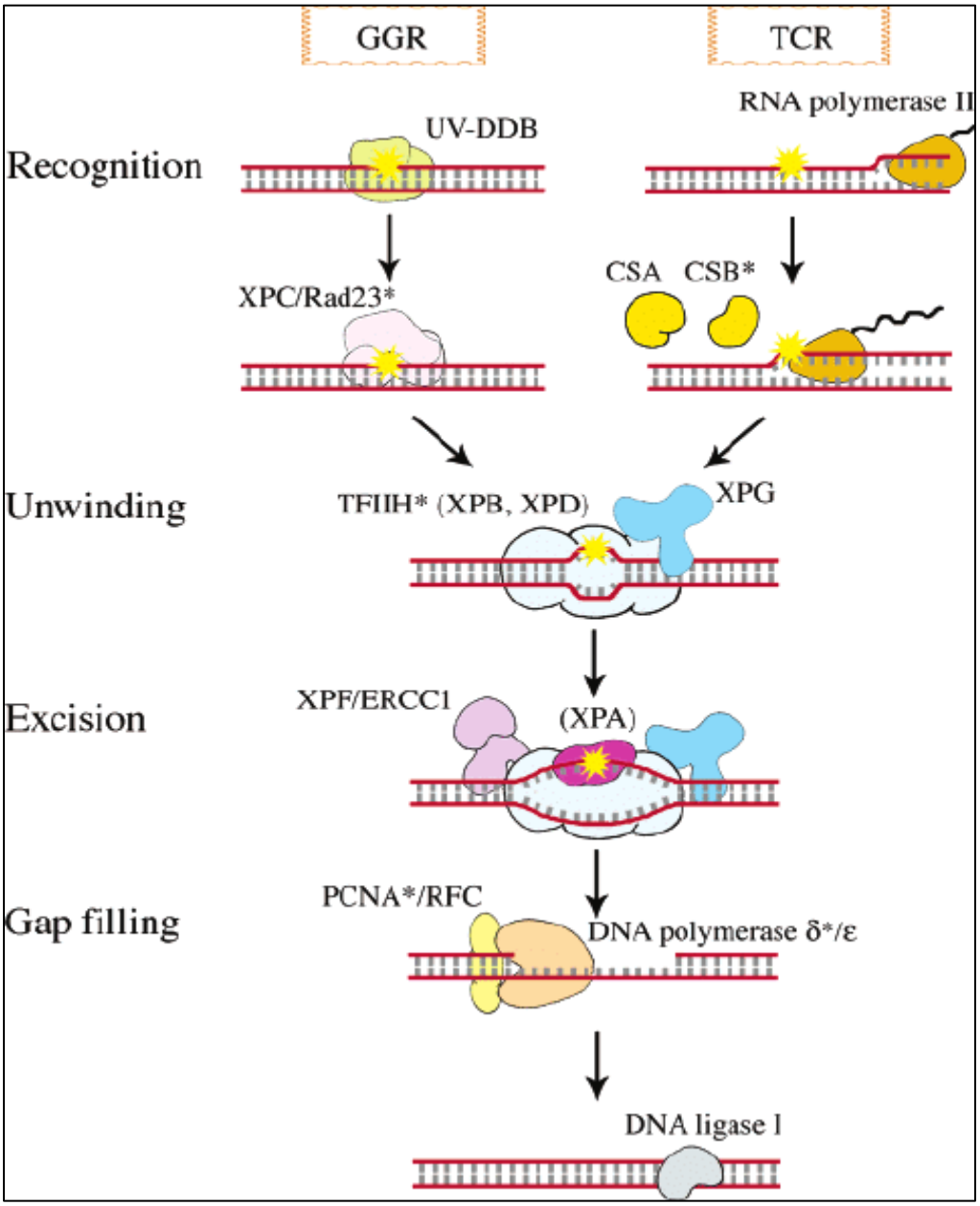

Figure Nucleotide excision repair pathway

Detail mechanism will be discussed in the next class

MISMATCH REAIR