Genome Editing and Engineering
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Module - 02
Breakage and Repair of Genomic DNA
Lecture - 05
Repair of Genomic DNA

Welcome to module 2 of the course Genomic Editing and Engineering, where we are discussing about the Breakage and Repair of Genomic DNA. In the last class, we discussed about the various methods by which or the phenomena by which the DNA of a cell gets broken. Today we are going to discuss, how once this DNA is broken the cell repairs it. So, briefly we will be discussing today about the repair of genomic DNA.

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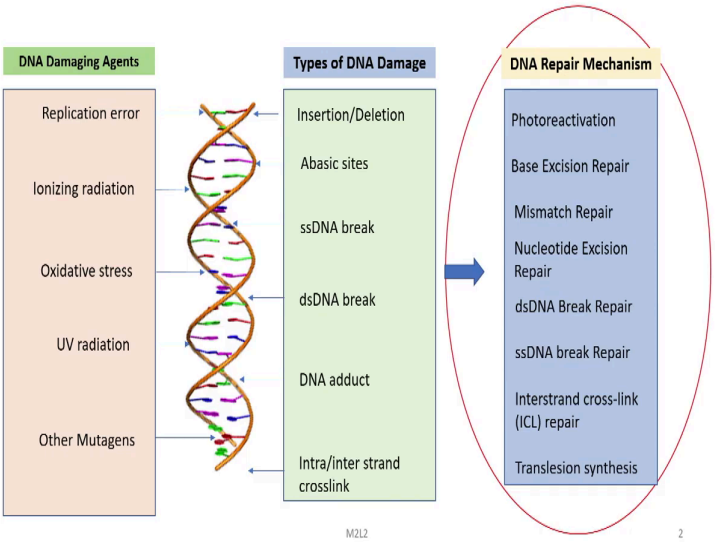

So, this figure is now familiar to you. We spoke about the DNA damaging agents in the last lecture and how they create different type of DNA damages. Now once the DNA damages has been inflicted, there are several DNA repair mechanisms in this inside the cell, which takes care of the broken DNA and if this DNA is left unrepaired, it will lead to mutagenesis and cause several genetic disorders.

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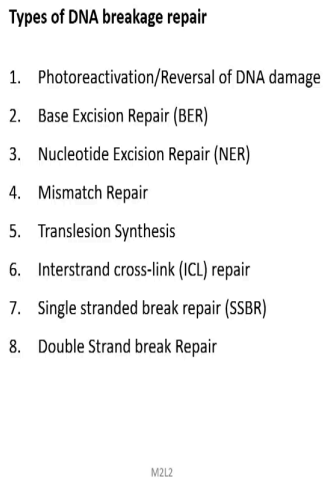

So, what are the different types of DNA breakage repair systems inside the cell? So, we have classified them into 8 main types, which may be; photo reactivation or reversal of DNA damage or base excision repair, nucleotide excision repair, mismatch repair, translation synthesis, interstrand cross-link repair or ICL repair, single stranded break repair and double stranded break repair. In this lecture we will be discussing the first seven repair mechanisms.

The double stranded break repair will be discussed in detailed separately. Let us start with the first type of repair that is used by the cell whenever a DNA is broken; this is the photo reactivation or reversal of DNA damage. So, what is photo reactivation? It is a process by which UV-inactivated organisms restore your function by repairing U- induced damages within the energy of near-UV light which is 310 to 410 nanometers and this is done with the help of an enzyme called photolyase.

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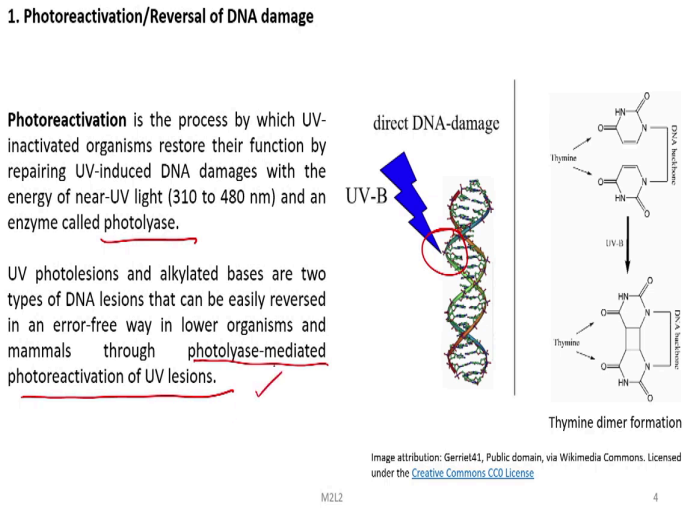

UV photolesions and alkylated bases are two types of DNA lesions that can be easily reversed in an error free way in lower organisms and mammals through photolyase-mediated photoreactivation of UV lesions. So, once the DNA is damaged by UV light it will create certain lesions and these lesions are repaired with the help of these enzyme.

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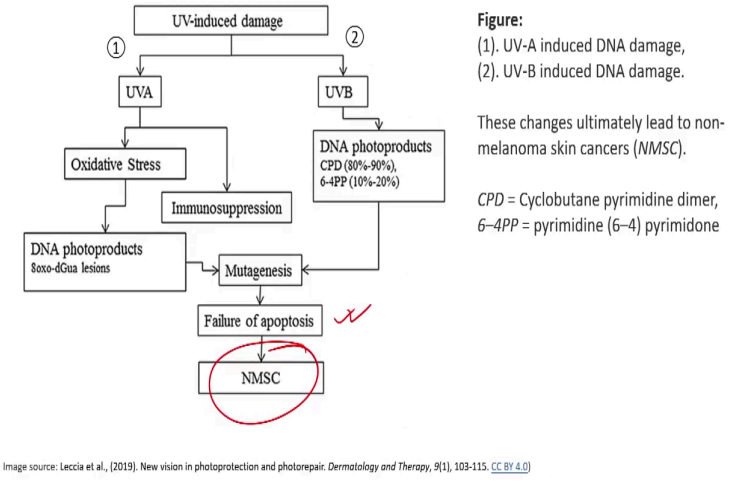

Now, what are the different kinds of UV induced damage. So, depending on the type of the UV; so, you have UV-A induced DNA damage as you can see in the left arm marked by the number 1, or you may have UV-B induced DNA damage in the right arm represented by the

number 2. Once UV-A induced damage occurs, it leads to oxidative stress and DNA photo products like 8oxo-dGua lesions are formed. UV-A induced damage may also lead to immunosuppression in certain cases and whenever there is a DNA photoproduct as an outcome of this damage it leads to the mutagenesis.

Similarly, due to UV-B induced damage certain DNA photo products are produced like cyclobutane pyrimidine dimer or 6-4PP and similar to the UV-A induced DNA photo products they also lead to mutagenesis inside the cell and when there is mutagenesis due to any of these UV induced damages, it leads to failure of apoptosis and which may lead to some kind of cancers.

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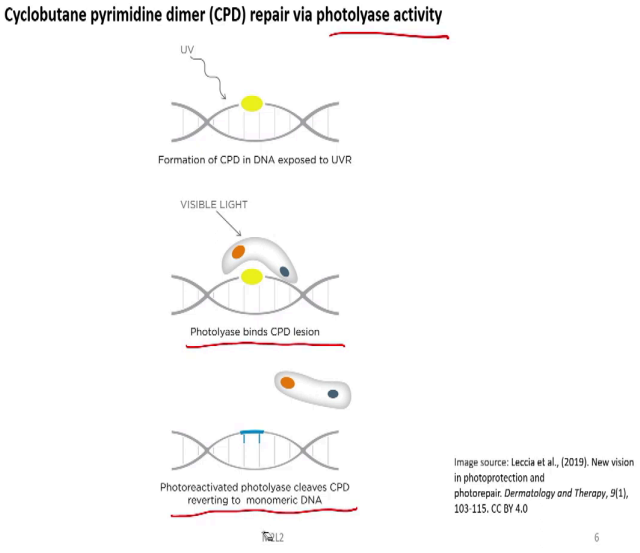

We will discuss this in detail in a stepwise manner. So, let us first discuss the CPD or cyclobutane pyrimidine dimer repair via photolyase activity. So, as you can see that whenever a UV ray is hitting the DNA, there is a formation of cyclobutane pyrimidine dimer or CPD dimer in the DNA. Now in the presence of visible light the photolyase binds to this CPD lesion and repairs it and thereby reverting it back to the monomeric DNA.

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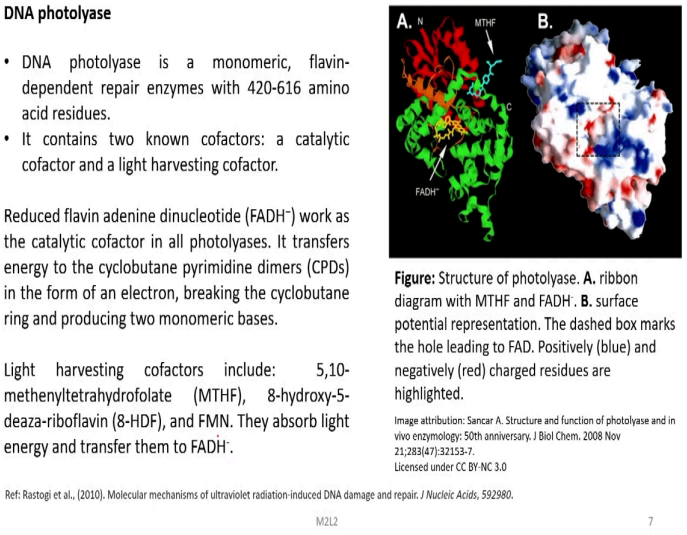

What is the nature, structure and function of this particular enzyme which carries out this kind of reversal reaction in the presence of visible light? So, DNA photolyase is basically a monomeric flavin dependent repair enzyme with 420 to 616 amino acid residues varies from species to species. It contains two known cofactors: a catalytic co factor and a light harvesting co factor. Reduced flavin adenine dinucleotide FADH minus work as the catalytic co factor in all photolyases.

It transfers energy to the cyclobutane pyrimidine dimers in the form of an electron, breaking the cyclobutane ring and producing two monomeric bases and you can see the location of the reduced FADH over here. This is the ribbon structure of photolyase DNA photolyase and here you can see the surface potential representation.

There is a dashed box over here which marks the whole leading to FAD. The positive blue colours and the negative red colours charged residues are represented in this figure in a colour code. Now going back to the structure of these enzyme, the light harvesting co factor include: 5,10-MTHF, 8-hydroxy-5-deaza-riboflavin or 8-HDF, and FMN. They absorb light energy and transfer them to FADH reduced.

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Figure: Photoreactivation:

1. UVR exposure causes pyrimidine lesion (thymine dimer)
2. Photoreactivating enzyme "photolyase" detects this damage.

The antenna molecules of photolyase (such as MTHF/8-HDF/FMN) trap light energy and transfer it to the catalytic cofactor FADH, which becomes excited and transfers energy to the pyrimidine dimer in the form of e-, splitting the CPD into two monomeric units, and then electron is transferred back to the flavin molecule.

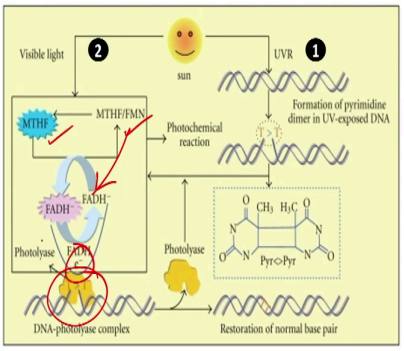

Image source: Rastogi et al., (2010). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *J Nucleic Acids, 592980.*
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Let us see the photo reactivation reaction a little bit of more detail. Whenever there is UV exposure this causes pyrimidine lesion. For example, the thymine dimer formation which we have discussed now and then. Whenever these thymine dimer formation takes place, the photolyase or the photo reactivating enzyme detects these damages. The antenna molecule of the photolyase such as the MTHF/8-HDF and FMN.

Here you can see them, trap the light energy and transfer it to the catalytic cofactor FADH which becomes excited and it transfers energy to the pyrimidine dimer in the form of an electron. Thereby splitting the CPD into two monomeric units, and then the electron is transferred back to the flavin molecule and in this way, the damage is repaired.

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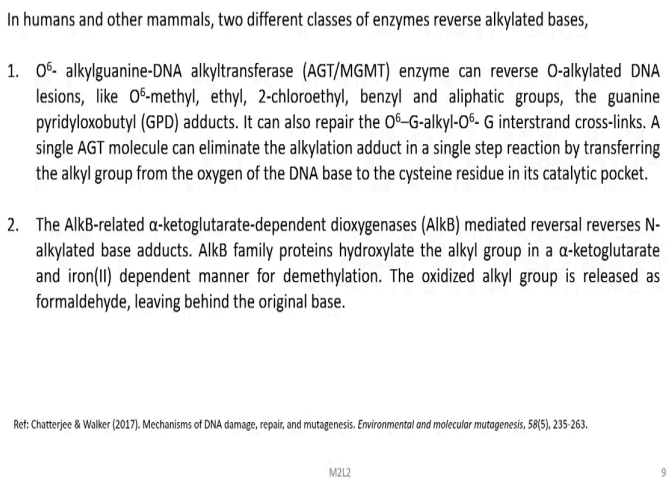

What happens in humans and other mammals? In these higher organisms, two different classes of enzymes reverse the alkylated bases. These are O6-alkyl guanine-DNA alkyl transferase or AGT/MGMT. Then O-alkylated DNA lesions reversed with this AGT MGMT and like O6-methyl, ethyl, 2-chloroethyl, benzyl aliphatic groups.

Let us now discuss how similar lesions are repaired in humans and other mammals. In them there are two different classes of enzymes which reverses the alkylated basis. In the first case O6-alkylguanine-DNA alkyl transferase enzyme can reverse O-alkylated DNA lesions, like O6-methyl, ethyl, 2-chloroethyl, benzyl and aliphatic groups. The guanine pyridyloxobutyl adducts or the GPD adducts. It can also repair the O 6 G-alkyl-O-6-G intra strand cross-links.

A single AGT molecule can eliminate the alkylation adduct in a single step reaction by transferring the alkyl group from the oxygen of the DNA base to the cysteine residue in its catalytic pocket. In a second case, the AlkB related alpha-ketoglutarate-dependent dioxyegenases AlkB mediates reversal, and it reverses the N-alkylated base adducts. AlkB family proteins hydroxylate an alkyl group in a alpha-ketoglutarate and iron (II) dependent manner for demethylation. The oxidized alkyl group is released as formaldehyde, leaving behind the original base.

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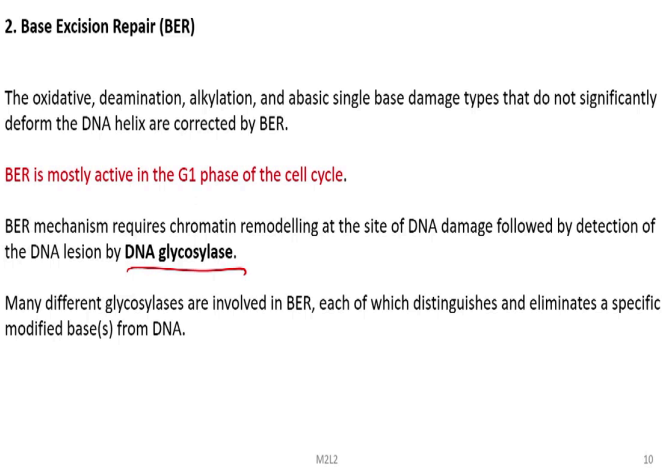

Let us now go to another type of DNA damage repair, which is known as the base excision repair or BER or BER. The oxidative de amination alkylation and abasic single base damage types, that do not significantly deform the DNA helix are corrected by base excision repair. Base excision repair is mostly active in the G1 phase of the cell cycle. Base excision repair mechanism requires chromatin remodelling at the site of DNA damage followed by detection of the DNA lesion by DNA glycosylase.

Many different glycosylases are involved in base excision repair, each of which distinguishes and eliminates a specific modified base or bases from the DNA.

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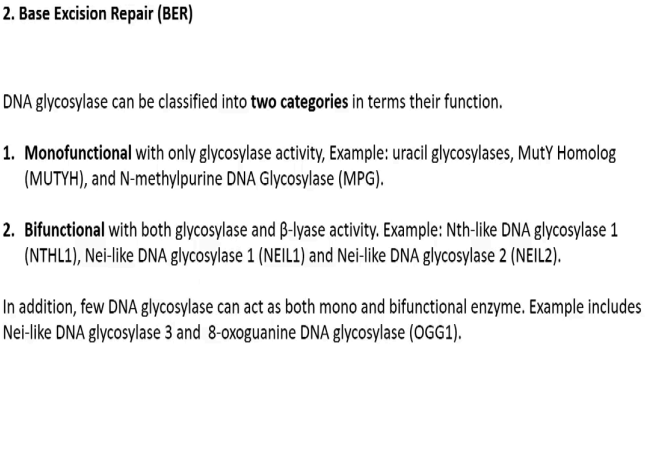

This DNA glycosylase can be classified into two categories in terms of their function. The first here the mono functional and the second are the bifunctional. The mono functional with only glycosylase activity, example uracil glycosylases, MutY Homolog and N-methylpurine DNA glycosylase.

Bifunctional have both glycosylase and beta-lyase activity and examples are DNA glycosylase 1, Nei-like DNA glycosylase 1 and Nei-like DNA glycosylase 2. In addition, a few DNA glycosylase connect as both mono and bifunctional enzymes. Examples include Nei-like DNA glycosylase 3 and 8-oxo guanine DNA glycosylase.

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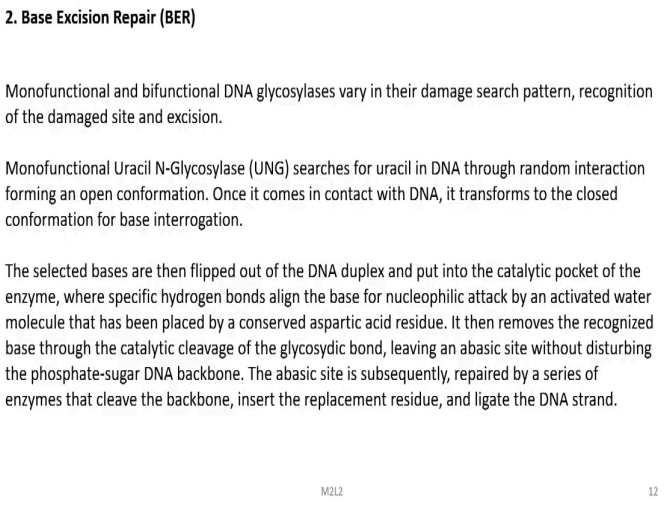

Mono functional and bifunctional DNA glycosylases vary in their DNA damage search pattern, recognition of the damage site and excision. Mono functional Uracil N-Glycosylase or UNG searches for uracil in DNA through a random interaction forming an open conformation. Once it comes in contact with DNA, it transforms to the closed conformation for base interrogation.

The selected bases are then flipped out of the DNA duplex and put into the catalytic pocket of the enzyme, where specific hydrogen bonds align the base for nucleophilic attack by an activated water molecule that has been placed by a conserved aspartic acid residue. It then removes the recognized base through the catalytic cleavage of the glycosidic bond, leaving on abasic site without disturbing the phosphate-sugar DNA backbone.

The abasic site is subsequently, repaired by a series of enzymes that cleave the backbone, insert the replacement residue, and ligate the DNA strand. So, many functions being carried out at the same time.

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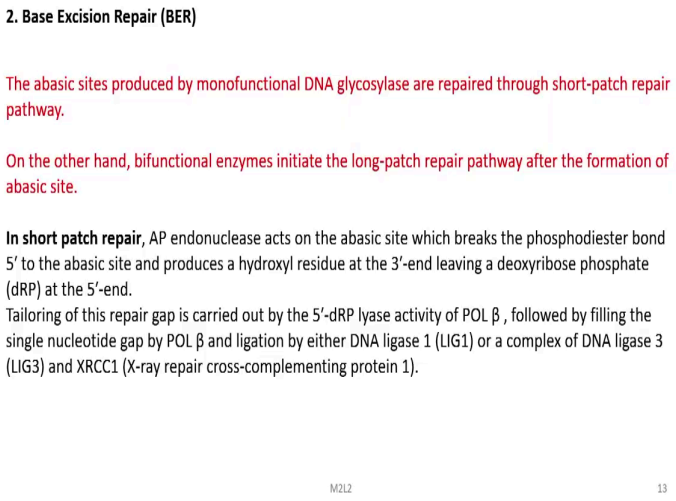

The abasic sites produced by mono functional DNA glycosylases are repaired through short patch repair pathway. On the other hand, bifunctional enzymes initiate the long-patch repair pathway after the formation of abasic sites. In short patch repair, AP endonuclease act on the abasic site which breaks the phosphodiester bond 5′ to the abasic site and produces a hydroxyl residue at the 3′ end leaving a deoxy ribosphosphate at the 5′ end.

Tailoring of these repair gaps is carried out by the 5′ dRP lyase activity of POL beta, followed by filling the single nucleated gap by POL beta and ligation by either DNA ligase 1, LIG1 or a complex of DNA ligase 3, LIG3 and XRCC1 which is X-ray repair cross complementing protein 1.

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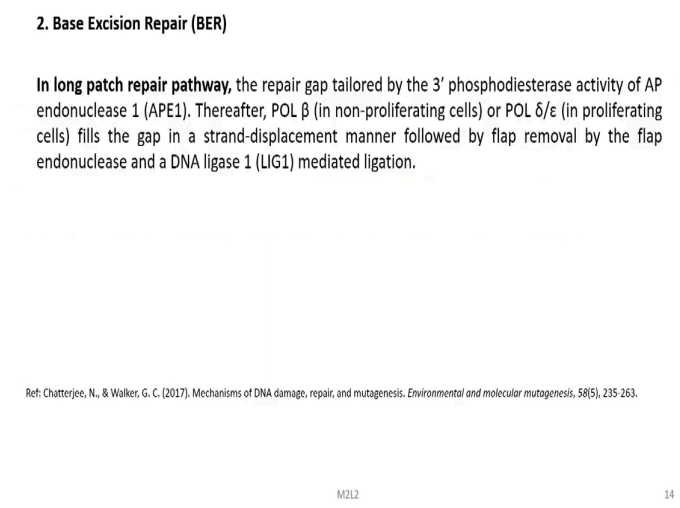

In the long patch repair pathway, the repair gap tailored by the 3´ phosphodiesterase activity of AP endonuclease 1. Thereafter POL b or POL delta/epsilon fills the gap particularly in proliferating cells in a strand displacement manner followed by flap removal by the flap endonuclease and a DNA ligase 1 mediated ligation.

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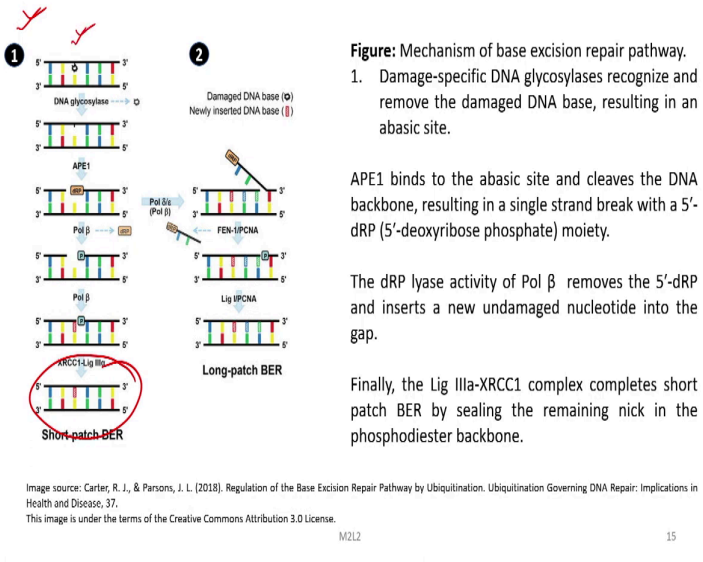

So, this figure shows the mechanism of base excision repair pathway. In figure 1 on the left side you can see, damage specific DNA glycosylase recognize and remove the damaged DNA base, resulting in an abasic site. So, this is the DNA glycosylase. So, APE1 binds to

this abasic site and cleave the DNA backbone and you can see here the outcome. This results in a single strand break with a 5′ dRP or 5 prime deoxy ribose phosphate moiety. The dRP lyase activity of Pol beta removes the 5′ dRP and inserts a new undamaged nucleotide into the gap.

Finally, the Lig IIIa-XRCC1 complex completes short patch base excision repair by sealing the remaining nick in the phosphodiester backbone. So, here you can see the damage and finally, the damage is being healed at the end of these pathway.

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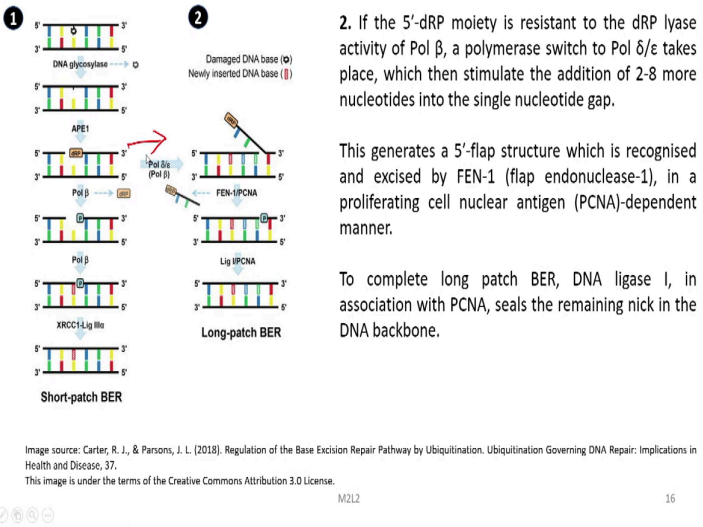

Image source: Carter, R. J., & Parsons, J. L. (2018). Regulation of the Base Excision Repair Pathway by Ubiquitination. Ubiquitination Governing DNA Repair: Implications in Health and Disease, 37.

Let us go to the second case where if the 5′ dRP moiety is resistant to the dRP lyase activity of Pol B, a polymerase switched to Pol delta/epsilon takes place. So, here this Pol B is ineffective that is the reason why these switched to this side of this reaction arm where Pol delta and epsilon will be helping it in repairing the damage. This generates a 5′ flap structure which is recognized and excised by FEN-1 or flap endonucleuase 1.

So, this we will be removed by this FEN-1, in a proliferating cell nuclear antigen dependent manner. To complete the patch base excision repair DNA ligase I, here it is Lig III, here it is ligase I, in association with the PCNA, seals the remaining nick in a DNA backbone, and this is getting resolved or repaired through the long patch base excision repair.

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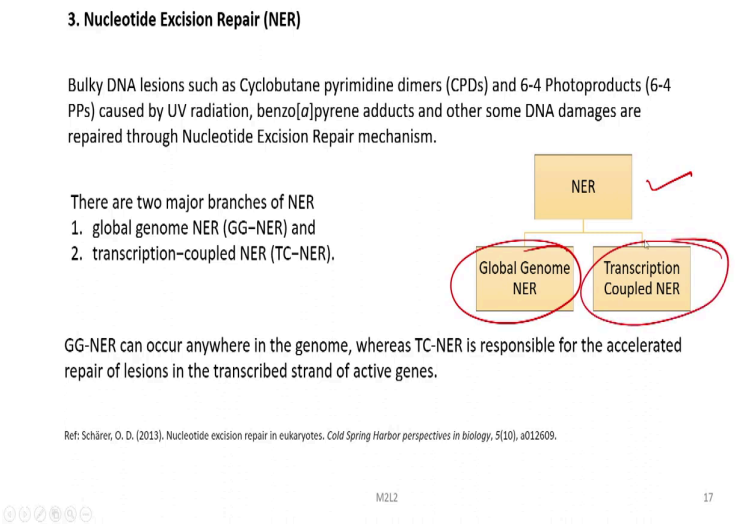

Let us now go to the third type of DNA damage repair which is called as the nucleotide excision repair or NER. Bulky DNA lesions such as CPDs or cyclobutane pyramidine dimers and 6-4 photo products, 6-4 PPs resulting out of UV radiation, benzo pyrene adducts and some other DNA damages are repaired by the nucleotide excision repair mechanisms.

The two major branches of NER: one is the global genome NER. The other another one is the transcription coupled NER, briefly they are known as GG-NER and TC-NER. We will discuss their mechanisms one by one. GG-NER can occur anywhere in the genome, whereas TC-NER is responsible for the accelerated repair of lesions in the transcribed strand of active genes.

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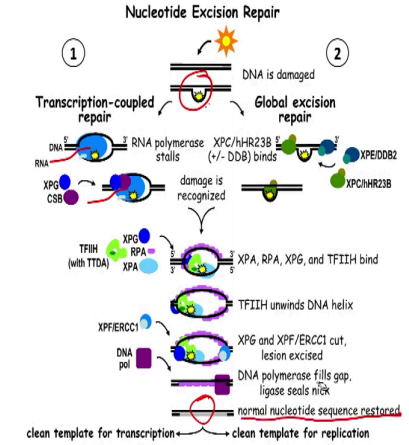

Figure: The DNA damage is recognized differently depending on whether **(1)** the DNA is transcriptionally active (transcription-coupled repair) or **(2)** transcriptionally inactive (global excision repair).
After the initial recognition step, the damage is repaired in a similar manner with the final outcome being the restoration of the normal nucleotide sequence.

XPA-XPG= Xeroderma pigmentosum (XP) genes A to G
TFIIH= Transcription factor IIH, a multi-functional protein

So, you can see here the transcription couple repair in the left arm and you can see the global excision repair in the right arm, and the players involved in the two process differ from one another in the starting towards the end they merged to the same pathway. So, let us see this figure kinley. The DNA damage that is happening over here is recognized differently depending on whether DNA is transcriptionally active or transcriptionally inactive.

If the DNA is transcriptionally active, transcription couple repair will take place. If it is inactive, the global excision repair will take place. After the initial recognition step the damage is repaired in a similar manner as already told to you with the final outcome being restoration of the normal nucleotide sequence. So, here you can see the damage in of the strands and finally, this damage is taken care of and the normal nucleotide sequence is restored.

So, we will not go into details of the various factors and enzymes which are involved in this mechanism and those are shown here in this figure, for your interest you may study this pathway to understand the mechanism in detail.

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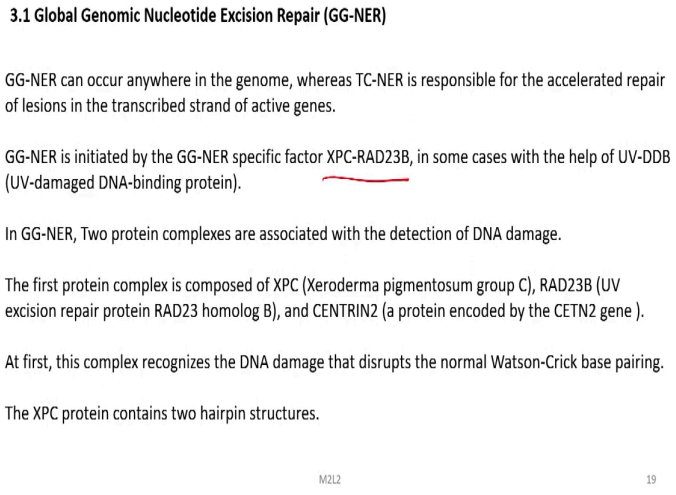

As already told to you, the global genomic nucleotide excision repair can occur anywhere in the genome, whereas TC-NER is responsible for the accelerated repair of lesions in the transcribed strand of active genes. GG-NER is initiated by the GG-NER specific factors XPC-RAD23B, in some cases with the help of UV-DDB or UV damaged DNA binding protein. In GG-NER, two protein complexes are associated with the detection of DNA damage.

The first protein complex is composed of XPC, xeroderma pig pigmentosum group C, RAD23B UV excision repair protein, RAD23 homolog B, and CENTRIN2 a protein encoded by the CETN2 gene. At first, this complex recognizes the DNA damage that disrupts the normal Watson-Crick base pairing. The XPC protein contains two hair pin structures.

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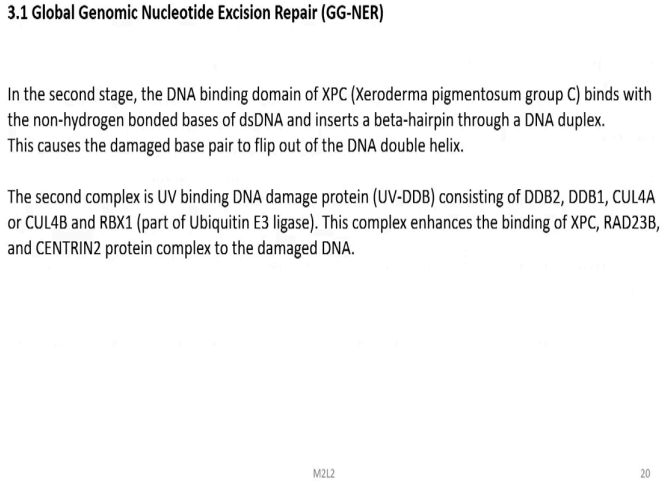

In the second stage, the DNA binding domain of XPC binds with the non-hydrogen bonded basis of double stranded DNA and inserts a beta-hairpin through a DNA duplex. This causes the damaged base to flip out of the DNA double helix. The second complex is UV binding DNA damage protein consisting of DDB2, DDB1, CUL4A or CUL4B and RBX1. This complex enhances the binding of XPC, RAD23B and CENTRIN2 protein complex to the damaged DNA.

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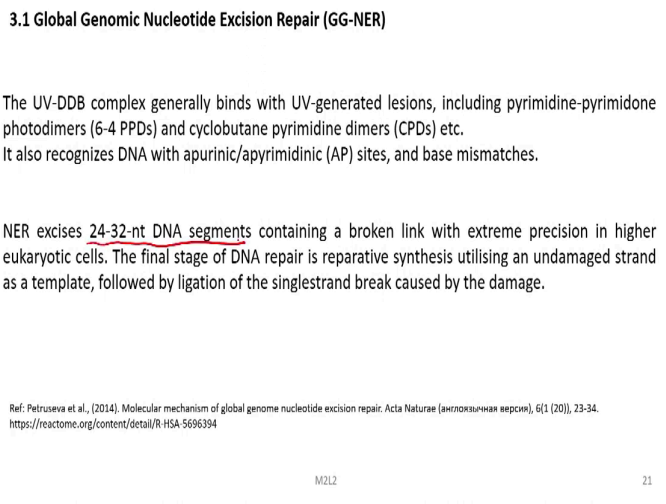

Global genomic nucleotide excision repair involves the UV-DDB complex which generally binds with UV generated lesions, including pyrimidine-pyramidone photo dimers or 6-4 PPDs and cyclobutane pyrimidine dimers or CPDs. It also recognizes DNA width apurinic or apyrimidinic or AP sites, and base mismatches. NER excises 24 to 32 nucleotide DNA segments containing a broken link with extreme precision in higher eukaryotic cells.

The final stage of DNA repair is reparative synthesis utilizing an undamaged strand as a template, followed by ligation of the single strand break caused by the damage.

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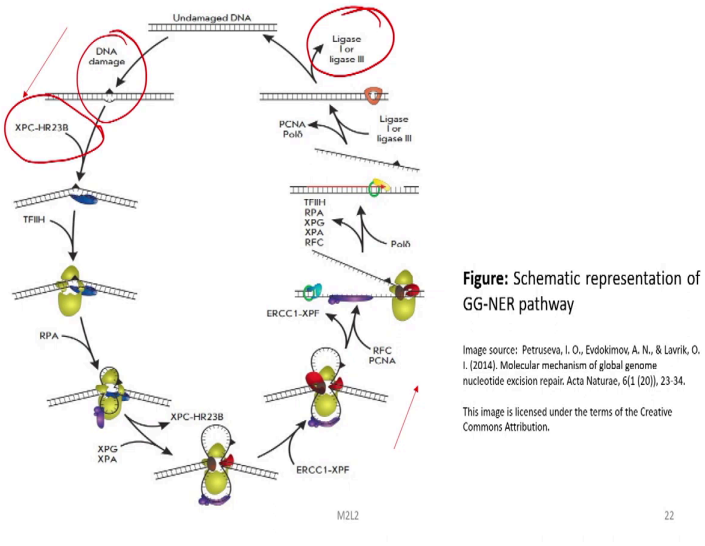

Figure: Schematic representation of GG-NER pathway

Image source: Petruseva, I. O., Evdokimov, A. N., & Lavrik, O. I. (2014). Molecular mechanism of global genome nucleotide excision repair. Acta Naturae, 6(1 (20)), 23-34.

So, this is the schematic representation of GG-NER pathway wherever a DNA damage occurs as already discussed to XPC-HR23B detects it. And these follows a cyclic pathway and at the end of which the DNA is repaired and the final nick sealing is done with the help of ligase I or ligase III.

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Protein	Function in NER
XPC-HR23B	Recognition of a distorted DNA structure
DDB	Recognition of damage, interaction with chromatin
XPA	Structural function, binding to a damaged strand
RPA	Binding to single-stranded DNA
TFIIH	ATPase, minor helicase activity 3'→5'- DNA-helicase
XPF	Endonuclease, catalyzes formation of single-strand break in DNA on the 5′ side of the damage
XPG	Endonuclease, catalyzes formation of single-strand break in DNA on the 3′ side of the damage
PCNA	Factor ensuring processivity of DNA polymerases
Polδ	DNA polymerase
Polε	DNA polymerase
Ligase I	Ligation of a single-strand break
Ligase III	Ligation of a single-strand break
RFC	ATP-dependent connection of PCN A

Ref: Petruseva IO, Evdokimov AN, Lavrik OI. Molecular mechanism of global genome nucleotide excision repair. Acta Naturae. 2014 Jan;6(1):23-34. PMID: 24772324; PMCID: PMC3999463.

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There are various proteins involved in this pathway. The details of which are listed in this table and these proteins have various carry out different functions. For example, this XPC-HR23B is used for the recognition of the distorted DNA structure as already told to you. Then DDB recognizes damage and it interact with the chromatin. XPA has a structural function binding to a damage strand. RPA it binds to a single stranded DNA then, TFIIH it has ATPase activity minor helicase activity 3′ to 5′ DNA helicase activity.

XPF have endonuclease and catalyzes the formation of single stranded break in DNA on the 5′ side of the damage. XPG it is an endonuclease which catalyzes the formation of single strand break in DNA on the 3′ side of the damage. PCNA is a factor which ensures processivity of the DNA polymerases, Pol delta Pol epsilon they are DNA polymerases ligases III and ligase I participates in the ligation of the single strand breaks and RFC is ATP dependent connection of the PCNA.

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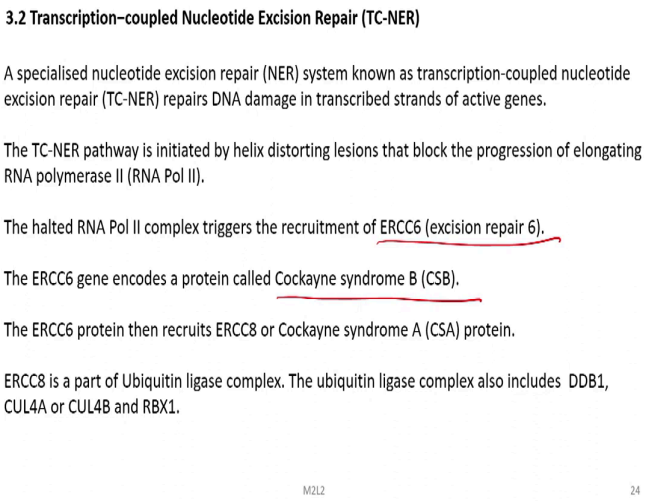

Let us now examine the transcription coupled nucleotide excision repair or the TC-NER. This is a specialized nucleotide excision repair system known as transcription coupled nucleotide excision repair, which repairs DNA damage in the transcribed strands of active genes. The TC-NER pathways limit initiated by helix distorting relations that block the progression of elongating RNA polymerase II.

The halted RNA II complex triggers the equipment of ERCC6 or excision repair 6. ERCC gene encodes a protein called Cockayne syndrome B, CSB, the ERCC6 protein recruits ERCC8 or CSA protein. ERCC8 is a part of ubiquitin ligase complex. The ubiquitin ligase complex also includes DDB1, CUL4A or CUL4B and RBX1.

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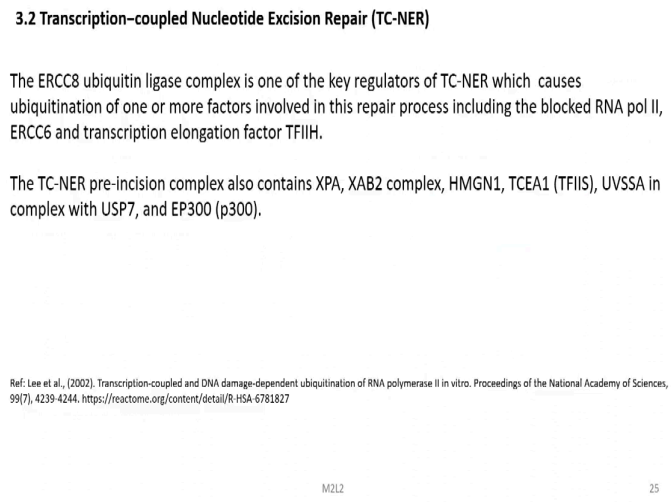

The ERCC8 ubiquitin ligase complex is one of the key regulators of TC-NER which causes ubiquitination of one of the more factors involved in this repair process including the blocked RNA Pol II, ERCC6 and transcription elongation factor TFIIH. The TC-NER pre-incision complex also contains XPA, XAB2 complex, HMGN1, TCEA1, UV SSA in complex with USP7, and EP300.

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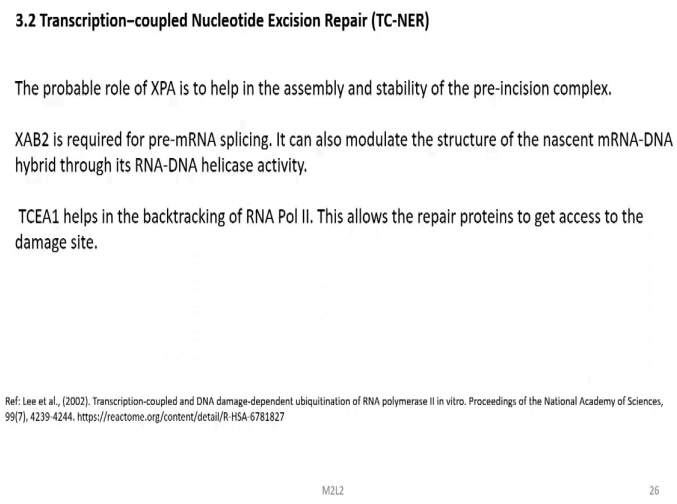

The probable role of XPA is to help in the assembly and stability of the pre-incision complex. XAB 2 is required for pre-mRNA splicing and also modulate the structure of

nascent mRNA-DNA hybrid through its RNA-DNA helicase activity. TCEA1 helps in the back tracking of the RNA Pol II. This allows the repair proteins to get access to the damaged site.

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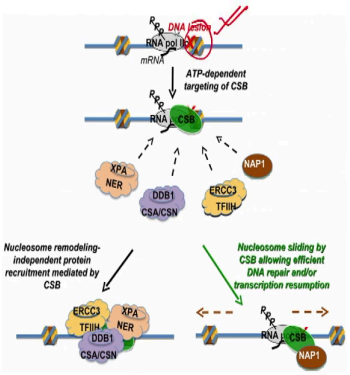

Figure: Model of CSB's Distinct Activities in Transcription-Coupled DNA Repair.

Top: The targeting of CSB to lesion-stalled transcription is an important and early stage in the transcription-coupled DNA repair process . CSB has two distinct actions once recruited. Protein factor recruitment is one action that occurs independently of nucleosome repositioning. Components of the nucleotide excision repair complex, the CSA/CSN E3 ligase complex, and the TFIIH transcription elongation complex are all recruited. CSB also recruits NAP1L1 and NAP1L4 to lesion-stalled transcription sites.

So, this figure is a model of CSBs distinct activities in transcription coupled DNA repair. So, you can see here on the top the targeting of CSB to lesion stalled transcription. So, due to this lesion here as already discussed earlier, the RNA polymerase two cannot progress further and therefore, it is stalled. CSB has two distinct actions once recruited. In this step you can see the recruitment of the CSB.

Protein factor recruitment is one action that occurs independently of nucleosomes repositioning. Components of the nucleotide excision repair complex the CSA/CSN E3 ligase complex, and the TFIIH transcription elongation complex are all recruited. At this stage CSB also recruits and NAP1L1 and NAP1L4 to lesion stalled transcription sites.

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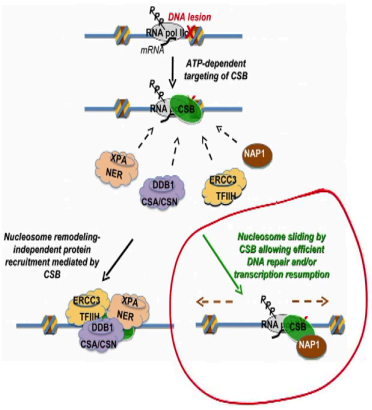

Lower right: nucleosome repositioning is required for a second action. CSB moves nucleosomes in combination with NAP1-like histone chaperones to allow effective DNA repair and/or continuation of transcription following repair.

CSB: Cockayne syndrome complementation group B
CSA: Cockayne syndrome complementation group A
NAP: Nucleosome Assembly Protein

In the low right, we can see the nucleosomes repositioning which is required for a second action. CSB moves nucleosomes in combination with NAP1-like histone chaperons to allow effective DNA repair and or continuation of transcription following the repair.

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4. Mismatch Repair

The mismatch repair (MMR) mechanism fixes short insertion and deletion loops (IDLs) within repetitive DNA sequences that have resulted from strand slippage events and base mismatches produced during DNA replication.

MMR pathway is highly conserved across prokaryotes and eukaryotes and mainly associated with DNA replication increasing replication fidelity by 100 fold.

https://reactome.org/content/detail/R-HSA-5358508
Ref: Chatterjee, N., & Walker, G. C. (2017). Mechanisms of DNA damage, repair, and mutagenesis. *Environmental and molecular mutagenesis*, *58*(5), 235-263.

Let us move to the next type of repair which is known as the mismatch repair. The mismatch repair mechanism fixes short insertion and deletion loops within repetitive DNA sequences that have resulted from strand slippage events and base mismatches produced during the DNA replication. Mismatch repair pathway is highly conserved whether in eukaryotes or

prokaryotes and mainly associated with DNA replication increasing replication fidelity by around 100 folds.

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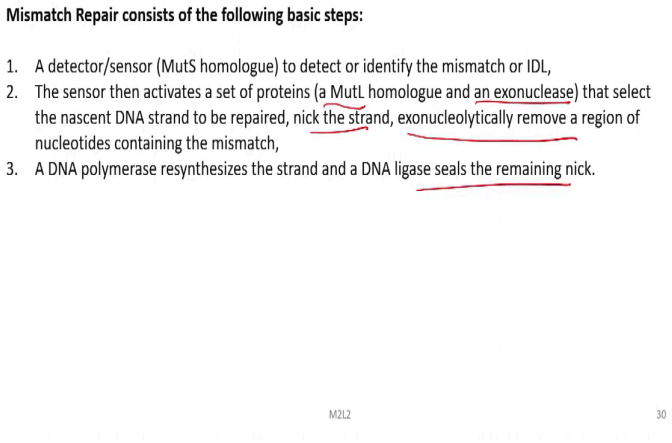

Mismatch repair consist of the following basic steps. In the first step, a detector or sensor MutS homologue is employed to detect or identify the mismatch. The sensor then activates a set of proteins a MutL homologue and an exonuclease that select the nascent DNA strand to be repaired, nick the strand, exonucleolytically remove a region of the nucleotides containing the mismatch. A DNA polymerase re synthesize the strand and a DNA ligase finally seals the remaining nick.

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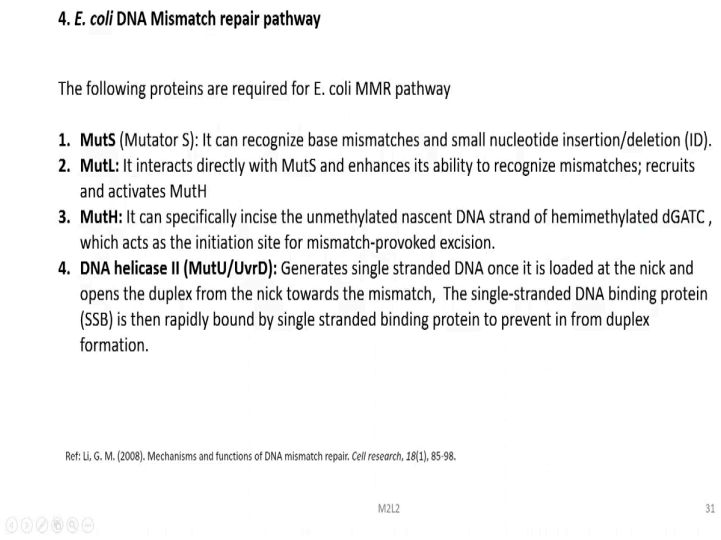

Let us study the *E. coli* DNA mismatch repair pathway. The following proteins are required for *E. coli* MMR pathway. MutS, MuL or mutator S, mutator L, MutH and finally, DNA helicase II. What does MutS do? As already told, it can recognize base mismatches and small nucleotide insertion or deletion. MutL interacts directly with the MutS and enhances its ability to recognize mismatches; recruits and activates MutH.

MutH can specifically in size the unmethylated nascent DNA strand of hemimethylated dGATC, which acts as the initiation site for the mismatch provoked excision. DNA helicase II generates single stranded DNA once it is loaded at the nick and opens the duplex from the nick towards the mismatch.

The single stranded DNA binding protein SSBs then rapidly bound by single stranded binding protein to prevent it from duplex formation. Let us now discuss the *E. coli* DNA mismatch pathway in little bit of details. The various proteins required for this MMR pathway in *E. coli* like MutS, MutL, MutH then DNA helicase II, endonucleases and DNA polymerase III holoenzyme and finally the DNA ligase.

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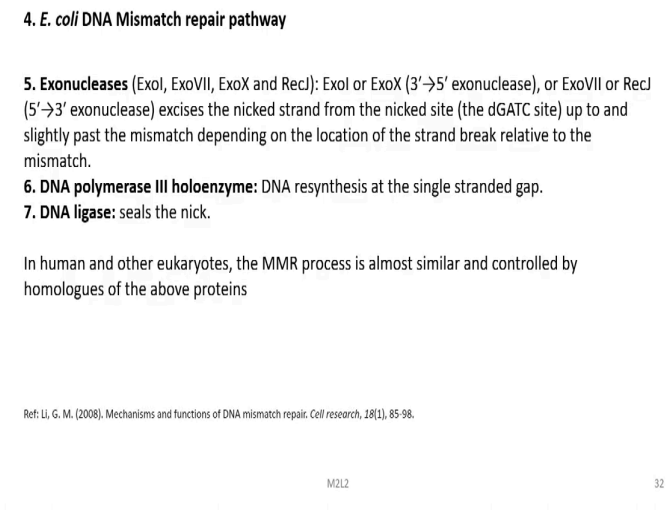

So, these proteins carry out various functions in this repair pathway for example, MutS recognizes the base mismatches and small nucleotide insertion deletions. The MutL interacts with directly with MutS and enhances its ability to recognize its mismatches; recruits and activates the MutH. The MutH can specifically incise the unmethylated nascent DNA strand of hemimethylated dGATC, which acts the initiation sites for mismatch provoked excision.

The DNA helicase II generates single stranded DNA once it is loaded at the nick and opens the duplex from the nick towards the mismatch. The single stranded DNA binding protein SSB is then rapidly bound by single stranded binding proteins to prevent in from duplex formation. In the fifth step, the exonucleases which are ExoI or ExoX and have 3′ to 5′ exonuclease activity or ExoVII or RecJ which has 5′ to 3′ exonuclease activity excises the nicked strand from the nicked site up to and slightly pass the mismatch depending on the location of the strand break relative to the mismatch.

DNA polymerase III holoenzyme resynthesizes at the single stranded gap while the DNA ligase seals the nick. In human and other eukaryotes, the MMR process is almost similar and controlled by homologues of the above proteins as discussed

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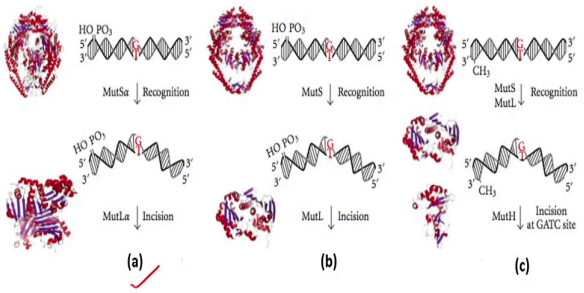

Figure: (a) Eukaryotic MMR: MutSα recognizes base-base mismatches and MutLα nicks the 3′- or 5′-side of the mismatched base on the discontinuous strand. **(b)** MMR in mutH-less bacteria: Mismatched bases are recognized by MutS and incision of discontinuous strand by MutL, **(c)** *E. coli* MMR: MutS recognizes mismatched bases, and MutL interacts with and stabilizes the complex. Then, MutH endonuclease is activated to incise the unmethylated GATC site to create an entry point for the excision reaction.

So, in this figure we can see the eukaryotic MMR the MutS alpha which recognizes base mismatches and MutL alpha it nicks the 3′ to 5′ side of the mismatched base of the discontinuous strand in figure a. In figures b, we can see the MMR in MutH its less bacteria mismatch bases are recognized by mass MutS and incision of the discontinuous strand by MutL.

In figure c we can see the E. coli MMR: MutS recognizes mismatched bases and MutL interacts with and stabilizes the complex. Then MutH endonuclease is activated to incise the unmethylated GATC site to create an entry point for the excise reaction.

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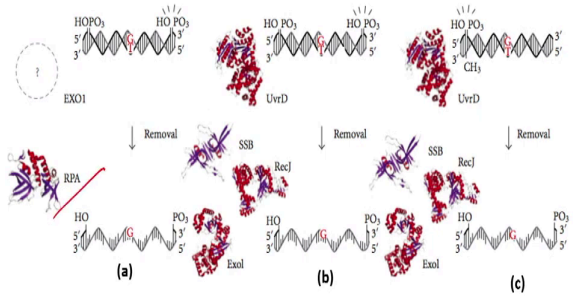

(a) Eukaryotic MMR: The resulting DNA segment is excised by the EXO1 exonuclease, in cooperation with the single-stranded DNA-binding protein RPA. **(b)** MMR in mutH-less bacteria: the error-containing DNA strand is removed by the cooperative functions of DNA helicases, such as UvrD, the exonucleases RecJ and ExoI, and the single-stranded DNA-binding protein SSB. **(c)** *E. coli* MMR: DNA helicase, a single-stranded DNA-binding protein, and several exonucleases are involved in the excision reaction.

Image source: Fukui, K. (2010). DNA mismatch repair in eukaryotes and bacteria. *Journal of nucleic acids, 2010.*
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Let us now examine the reaction in eukaryotes as well as in E. coli. So, you can see in figure a, the eukaryotic MMR where MutS alpha recognizes base mismatches and MutL alpha nicks the 3′ or 5′ side of the mismatched base on the discontinuous strand. In b you can see the MMR in MutH less bacteria where my mismatch bases are recognized by MutS and incision of discontinuous strand by MutL.

In figure c is the E. coli MMR, here MutS recognizes mismatched bases and MutL interacts weight and stabilizes the complex then MutH endonuclease is activated to incise the un methylated GATC site to create an entry point for the excision reaction. In figure a you can see the resulting DNA segment is excised by Exo 1 nuclease in cooperation with the single stranded DNA binding protein RPA.

In the figure b you can see the error containing DNA strand is removed by the cooperative function of DNA helicases, such as UvrD, the exonuclease RecJ and ExoI and the single stranded DNA binding protein SSB. In the case of E. coli MMR the DNA helicase a single stranded DNA binding protein and several exonucleases are involved in the excision reaction.

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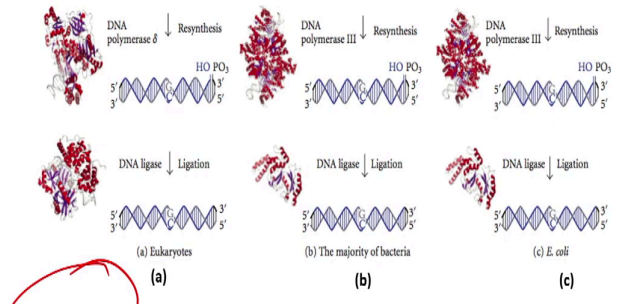

(a) Eukaryotic MMR: The DNA strand is resynthesized by DNA polymerase *δ* and DNA ligase 1. **(b)** MMR in mutH-less bacteria: DNA polymerase III and DNA ligase fill the gap to complete the repair. **(c)** *E. coli* MMR: DNA polymerase III and DNA ligase fill the gap to complete the repair.

In eukaryotes, as seen in figure a, the DNA strand is re synthesized by DNA polymerase delta and DNA ligase I will do the ligation reaction. Whereas in MutH less bacteria DNA polymerase III and DNA ligase does the job. In E. coli the DNA polymerase three and the DNA ligase fill up the gap to complete the repair. So, this is a comparison between the eukaryotic MMR and E. coli MMR and with MMR in MutH-less bacteria.

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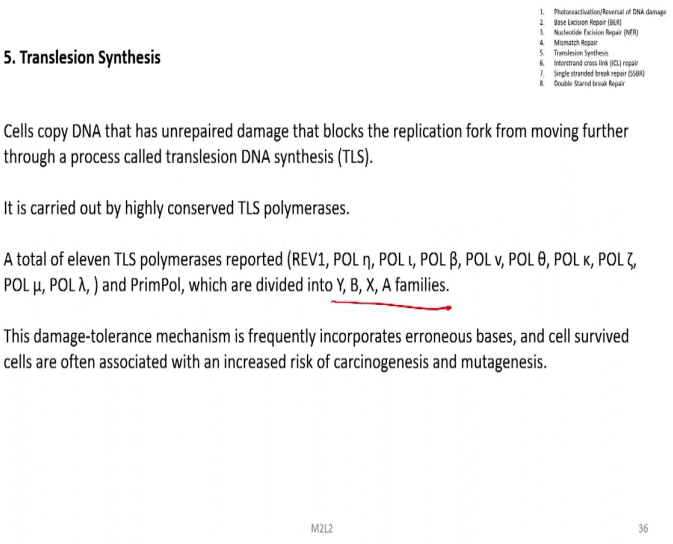

Let us now go to the fifth type of repair mechanism which is the translation synthesis. Cells copy DNA that has unrepaired damage that blocks the replication fork from moving further to

a process called translational translation DNA synthesis or TLS. It is carried out by highly conserved TLS polymerases. There are a total of eleven TLS poly polymerases reported, and these are divided into Y, B, X and A families.

This damage tolerance mechanism is frequently involved in incorporating erroneous bases and the cells survive as an outcome of these, but are often associated with an increased risk of carcinogens and mutagenesis.

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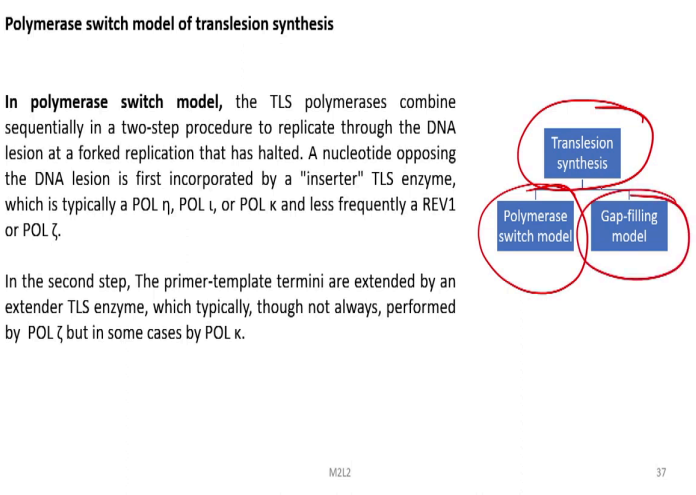

Let us discuss the polymerase switch model of translation synthesis. The other model is the gap filling model. In polymerase switch model the TLS polymerase combines sequentially in a two step procedure to replicate through the DNA lesion at a forked replication that has halted. A nucleotide opposing the DNA lesion is first incorporated by an inserter TLS enzyme, which is typically a POL n, POL L and POL K and less frequently REV1 or POL zeta.

So, there are two types of translational synthesis, one is the polymerase switch translational synthesis or the polymerase switch model. The other one is the gap filling model. In the polymerase switch model of translational synthesis there are two sequential steps to replicate through the DNA lesion and the forked replication which has been halted. A nucleotide opposing the DNA lesion is first incorporated by an inserted TLS enzyme and it has various members as you can see in this list.

In the second step, the primer template amino extended by an extender TLS enzyme, which typically, though not always is performed, but in some cases by another enzyme called POL K.

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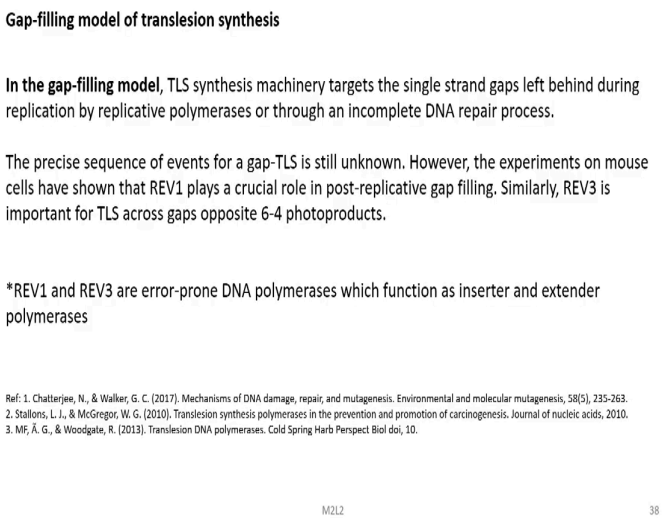

In the gap filling model of translation synthesis TLS synthesis machinery targets the single strand gaps left behind during replication by replicative polymerases or through an incomplete DNA repair process. The precise sequence of events for a gap TLS is still unknown. However, experiments on mouse cells have shown that REV1 plays a crucial role in post replicative gap filling. Similarly, REV3 is important for TLS across gaps opposite to 6-4 photo products. Both REV1 and REV3 are error prone DNA polymerases which function as inserter and extender polymerases.

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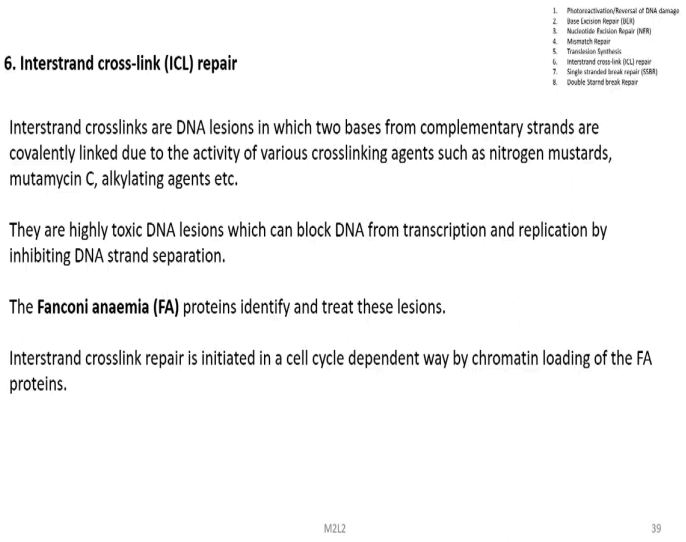

Let us now discuss the six type of DNA repair which is the interstrand cross link repair. Interstrand cross links are DNA lesions in which two bases from complementary strands are covalently linked due to the activity of various cross-linking agents such as nitrogen mustards, mutamycin C, alkylating agents etcetera. They are highly toxic DNA lesions and can block DNA from transcription and replication by inhibiting DNA strand separation.

The Fanconi anemia proteins identify and treat these lesions. Interstrand cross link repair is initiated in a cell cycle dependent way by chromatin loading of the FA proteins.

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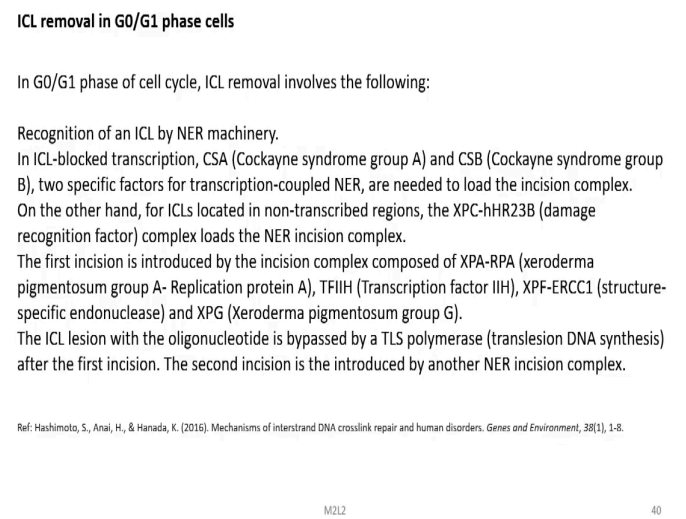

Let us discuss the ICL removal in G0/G1 phase cycle in G0/G1 phase of cell cycle ICL removes as described below. Recognition of an ICL by NER machinery takes place. In ICL block transcription, CSA and CSB two specific factors for transcription couple NER, are needed to load the incision complex. On the other hand, for ICLs located in non-transcribed regions, the XP-hHR23B damage recognition factor complex loads the NER incision complex.

The first incision is introduced by the incision complex composed of XPA-RPA TFIIH and XPG. The ICL lesion with the oligonucleotide is bypassed by TLS polymerases after the first incision. The second incision is introduced by another NER incision complex.

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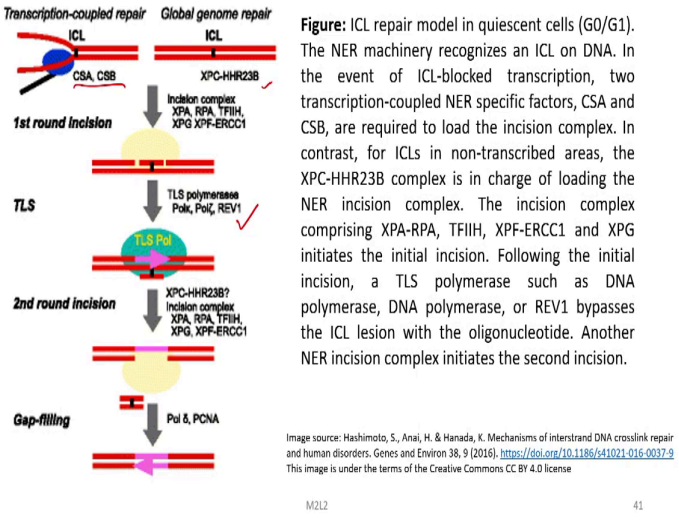

Figure: ICL repair model in quiescent cells (G0/G1). The NER machinery recognizes an ICL on DNA. In the event of ICL-blocked transcription, two transcription-coupled NER specific factors, CSA and CSB, are required to load the incision complex. In contrast, for ICLs in non-transcribed areas, the XPC-HHR23B complex is in charge of loading the NER incision complex. The incision complex comprising XPA-RPA, TFIIH, XPF-ERCC1 and XPG initiates the initial incision. Following the initial incision, a TLS polymerase such as DNA polymerase, DNA polymerase, or REV1 bypasses the ICL lesion with the oligonucleotide. Another NER incision complex initiates the second incision.

So, here we can see the ICL repair model in quiescent cells or G0/G1 cells a phase. The NER machinery recognizes an ICL on the DNA. In the event of ICL blocked transcription, two transcription couple NER specific factors CSA and CSB, are required to load the incision complex in contrast for ICLs in the non-transcribed areas, the XPC-HHR23B complex is in charge of loading the NER incision complex.

The incision complex comprising XPA-RPA, TFIIH, XPF-ERCC1, XPG initiates the initial incision. Following the initial incision, a TLS polymerase such as DNA polymerase, or REV1 bypasses the ICL lesion with the oligo nucleotide. Another NER incision complex initiates the second incision.

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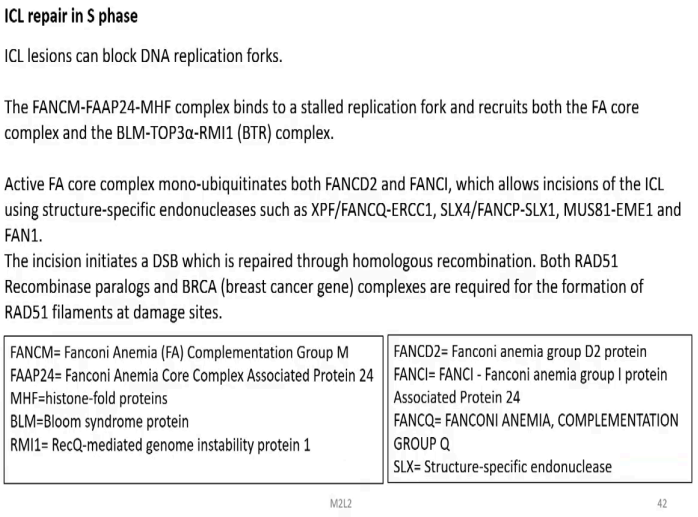

ICL repair in S phase: ICL lesions can block DNA replication forks. The FANCM- FAAP 24-MHF complex binds to a stalled replication fork and recruits both the FA core complex and BLM TOP3 alpha RM1 BTR complex. Active FA core complex mono ubiquitinates both FANCD2 and FANC1 which allows incisions of the ICL using unique structure specific endonucleases such as XPF FANCQ ERCC1, SLX4, FANCP- SLX1, MUS81, EME1 and FAN1.

The incision initiates a double strand break which is repaired through homologous recombination both RAD51 recombination paralogs and BRCA complexes are required for the formation of RAD51 filaments at damage sites. These double strand breaks mechanisms will be discussed in detail separately.

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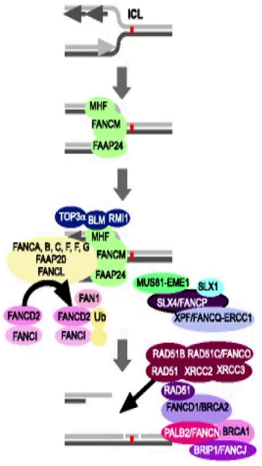

Figure: Model of ICL repair in S phase.
ICL lesions cause DNA replication forks to halt. The FANCM-FAAP24-MHF complex attaches to a stalled replication fork and recruits the FA core complex as well as the BLM-TOP3-RMI1. Activated FA core complex mono-ubiquitinates both FANCD2 and FANCI, allowing ICL incisions with structure-specific endonucleases including XPF/FANCQ-ERCC1, SLX4/FANCP-SLX1, MUS81-EME1, and FAN1. The incision creates a DSB, which HR repairs. The production of RAD51 filaments at damage sites requires both RAD51 paralogs (RAD51B, RAD51C/FANCO, RAD51D, XRCC2 and XRCC3) and BRCA complexes (BRCA1, BRCA2/FANCD1, PALB2/FANCN, and BRIP1/FANCJ).

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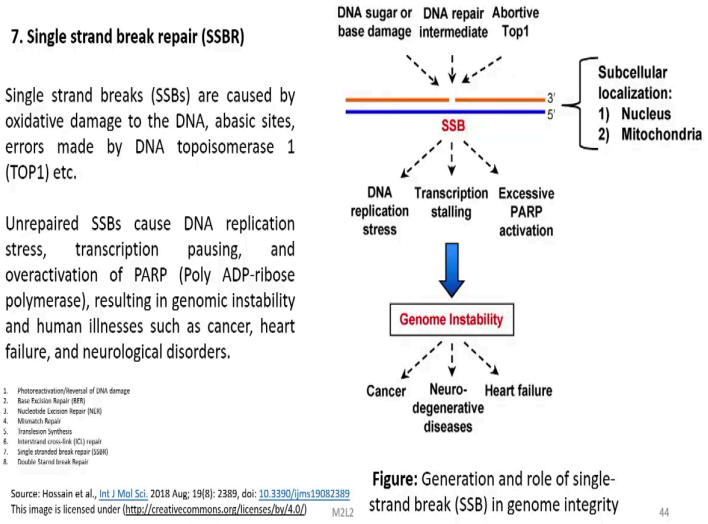

Figure: Generation and role of single-strand break (SSB) in genome integrity

Let us now go to the seventh repair mechanism the single strand break repair. These single strand breaks are caused by oxidative damage to the DNA, abasic sites, errors made by DNA topoisomerase I etcetera. Unrepaired SSBs cause DNA replication stress and transcription pausing, and over activation of PARP resulting in genomic instability and human illnesses such as cancer, heart failure and neurological disorders.

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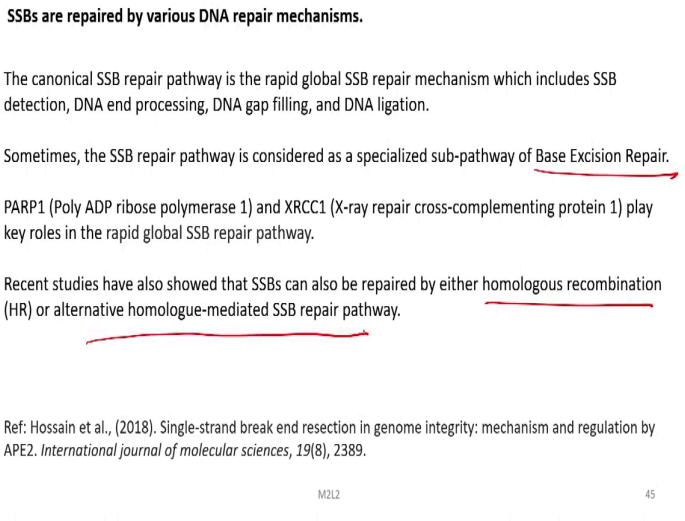

These SSBs are repaired by various DNA repair mechanisms. There is a canonical SSB repair pathway which is a rapid global SSB repair mechanism and includes SSB detection DNA and processing, DNA gap filling, and DNA ligation. Sometimes the SSB repair pathway is considered as a specialized sub pathway of base excision repair as discussed earlier. PARP1 poly ADP ribose polymerase 1 and X ray repair cross complementing protein 1 or XRCC1 play key roles in the rapid global SSB repair pathway.

Recent studies have also showed that SSBs can also be repaired by either homologous recombination or alternative homologous mediated SSB repair pathway.

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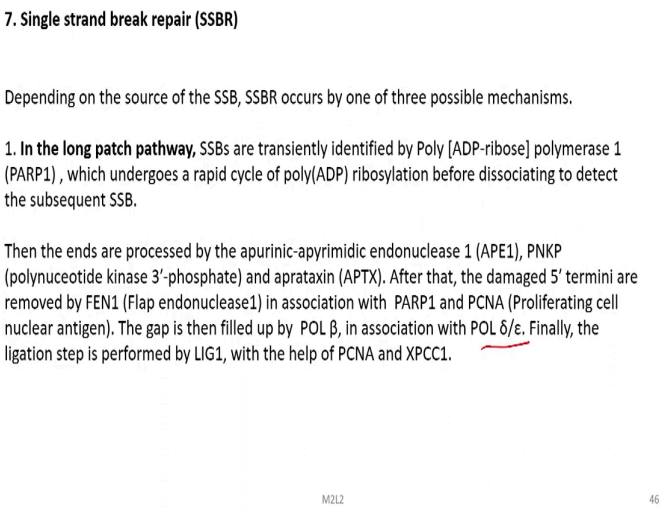

Depending on the source of the SSB, SSB repair occurs by one of the three possible mechanisms. The first one is in the long patch pathway, SSB are transiently identified by Poly ADP ribose polymerase 1 or PARP 1, which undergoes a rapid cycle of poly ADP ribosylation before dissociating to detect the subsequent SSB. Then the ends are processed by the apurinic apyrimidic endonuclease 1 APE 1 PNKP and APTX. After that, the damaged 5 prime termini are removed by FEN1 in association with PARP1 and PCNA.

The gap is then filled up by polymerase beta in association with delta and epsilon finally, ligation performed by LIG1, with the help of PCNA XPCC1.

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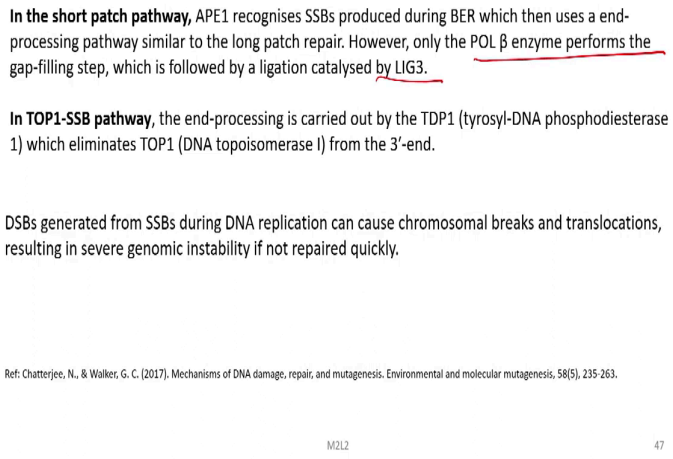

In a short patch pathway, APE1 recognizes SSBs produced during base excision repair which then uses a end processing pathway similar to the long patch repair. However, only the polymerase beta enzyme performs the gap filling step, which is followed by ligation catalyzed by LIG 3. In TPO1 SSB pathway, the end processing is carried out by the TDP1 which eliminates TOP1 from the 3´ end.

Double strand breaks generated from SSBs during DNA replication can cause chromosomal breaks and translocations, resulting in severe genomic instability if not repaired quickly and these are addressed by the double strand break pathways.

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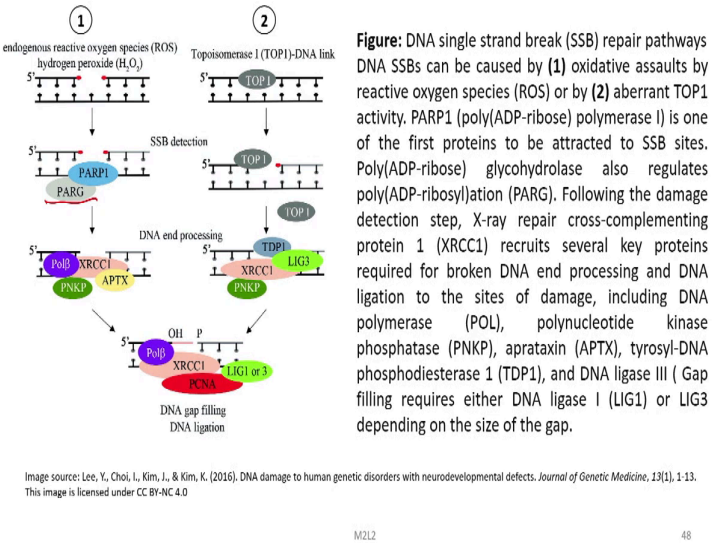

Figure: DNA single strand break (SSB) repair pathways DNA SSBs can be caused by **(1)** oxidative assaults by reactive oxygen species (ROS) or by **(2)** aberrant TOP1 activity. PARP1 (poly(ADP-ribose) polymerase I) is one of the first proteins to be attracted to SSB sites. Poly(ADP-ribose) glycohydrolase also regulates poly(ADP-ribosyl)ation (PARG). Following the damage detection step, X-ray repair cross-complementing protein 1 (XRCC1) recruits several key proteins required for broken DNA end processing and DNA ligation to the sites of damage, including DNA polymerase (POL), polynucleotide kinase phosphatase (PNKP), aprataxin (APTX), tyrosyl-DNA phosphodiesterase 1 (TDP1), and DNA ligase III (Gap filling requires either DNA ligase I (LIG1) or LIG3 depending on the size of the gap.

So, you can see here the left arm is having endogenous reactive oxygen species and hydrogen peroxide basically these are, oxidative assaults by these reactive oxygen species and in the right arm or pathway 2, aberrant TOP1 activity, PARP1 is one of the first proteins to be attracted to the SSB site. Poly ADP ribose glycohydrolase also regulates poly ADP ribosylation or PARG.

Following the damage detection step X ray repair cross complementing protein 1 XRCC1 recruits several key proteins required for broken DNA processing DNA end processing and DNA ligation to the sites of the damage, including DNA polymerase poly nucleotide kinase APTX, tyrosyl DNA phosphodiesterase 1 and DNA ligase 3. This gap filling requires either DNA ligase 1 or LIG 3 depending on the size of the gap.

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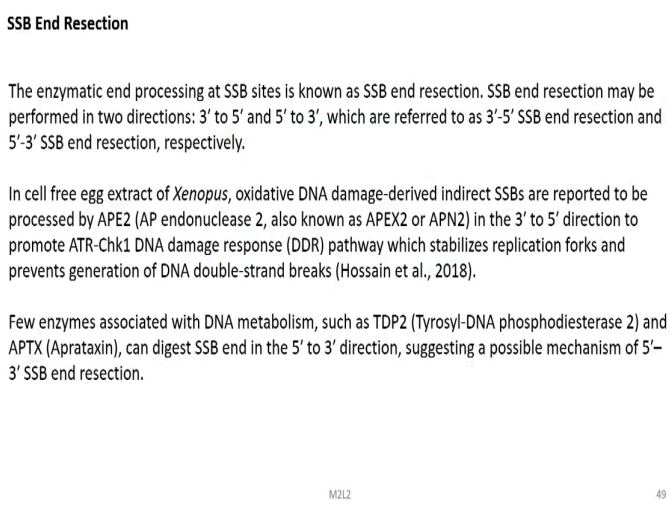

End resection is important in SSB repair. The enzymatic end processing at SSB site may be performed in two directions 3′ to 5′ or 5′ to 3′, which are referred to as 3′ to 5′ SSB resection and 5′ to 3′ SSB end resection respectively. In cell free egg extracts of Xenopus, oxidative DNA damage derived indirect SSBs are reported to be processed by APE2 in the 3′ to 5′ direction to promote ATR Chk 1 DNA damage response pathway which stabilizes replication forks and prevents generation of DNA double strand breaks.

Few enzymes associated with DNA metabolism, such as TDP 2 and APTX can digest SSB ends in the 5 prime to 3 prime direction, suggesting a possible mechanism of 5′ to 3′ SSB end resection.

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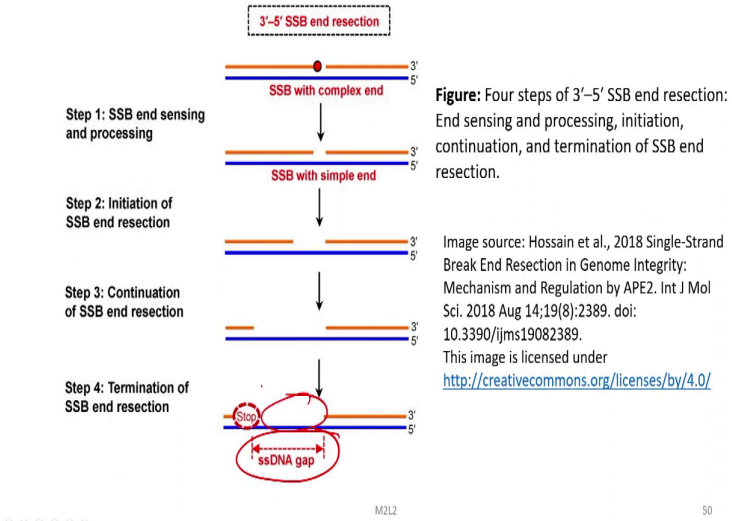

Figure: Four steps of 3′–5′ SSB end resection: End sensing and processing, initiation, continuation, and termination of SSB end resection.

Image source: Hossain et al., 2018 Single-Strand Break End Resection in Genome Integrity: Mechanism and Regulation by APE2. Int J Mol Sci. 2018 Aug 14;19(8):2389. doi: 10.3390/ijms19082389.
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So, in this figure four steps of 3′ to 5′ SSB end resection is being shown. In the first step SSB end sensing and processing takes place, in the second step initiation of the SSB end resection takes place. In the third step continuation of the SSB end resection happens and there is finally, a termination of the end resection at one particular point and as a result of these a SSB DNA gap is created.

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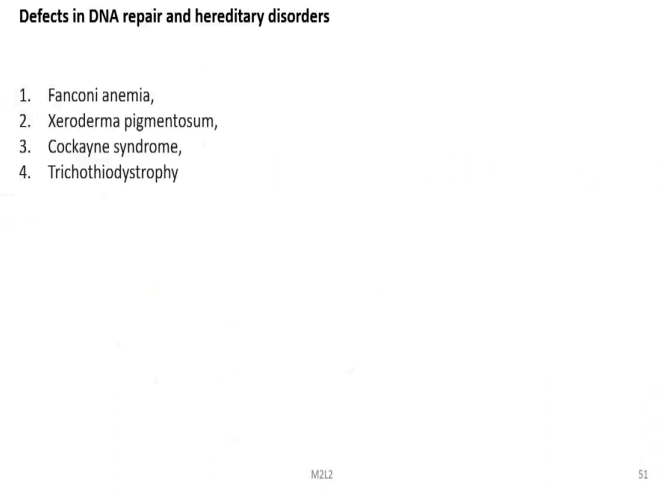

What happens if there are defects in DNA repair and all the seven different DNA repair mechanisms are failed to operate. This will lead to various kinds of genetic diseases as told in

the beginning of these lectures. Some of the diseases that may occur due to defects in the DNA repair mechanism are Fanconi anemia, Xeroderma pigmentosum, Cockayne syndrome, Tricothiodystrophy and there are many, these are some of the diseases and the list is endless.

As already told to you in the beginning of the lecture, if the DNA repair mechanisms fails, it may lead to many mutations and genetic diseases. The cell is quite rich in its repertory of DNA repair mechanisms and we have discussed in detail about seven different DNA damage repair mechanisms and we are going to discuss about the double stranded break repair next time in detail separately.

These are some of the examples of genetic diseases which may occur due to failure in the DNA repair mechanisms for example, Fanconi anaemia, Xeroderma pigmentosum, Cockayne syndrome, Tricothiodystrophy etcetera, but there are many other which you can find out as an small assignment.

Thank you for your patient hearing.