Genome Editing and Engineering
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Module - 02
Breakage and Repair of Genomic DNA
Lecture - 04
Breakage of Genomic DNA

Welcome to module 2 of the course Genome Editing and Engineering. And in this lecture, we are going to discuss about Breakage of Genomic DNA.

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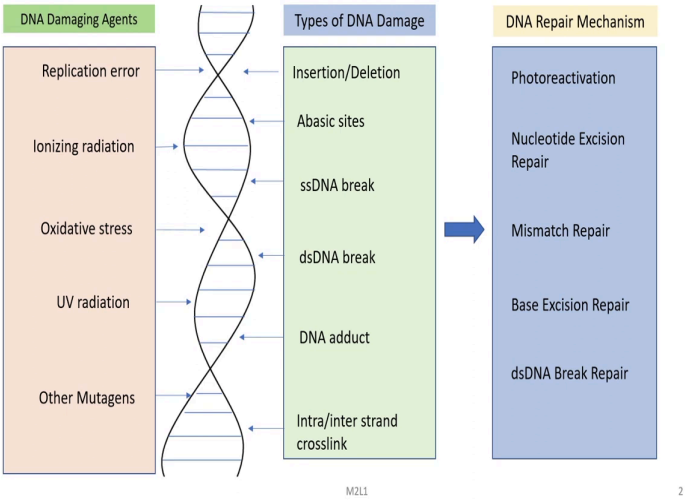

There are various DNA damaging agents and they cause different types of DNA damage and whenever a DNA is damaged inside the cell that is detected by the cells inherent system and certain repair mechanisms are activated to repair the damage caused by the damaging agents.

We will discuss about these various DNA damaging agents in our lecture today as well as what kind of damage these agents caused to the DNA molecules. In the next lecture we will discuss about the various DNA repair mechanisms that are activated as a result of the DNA damage that occurs due to these damaging agents.

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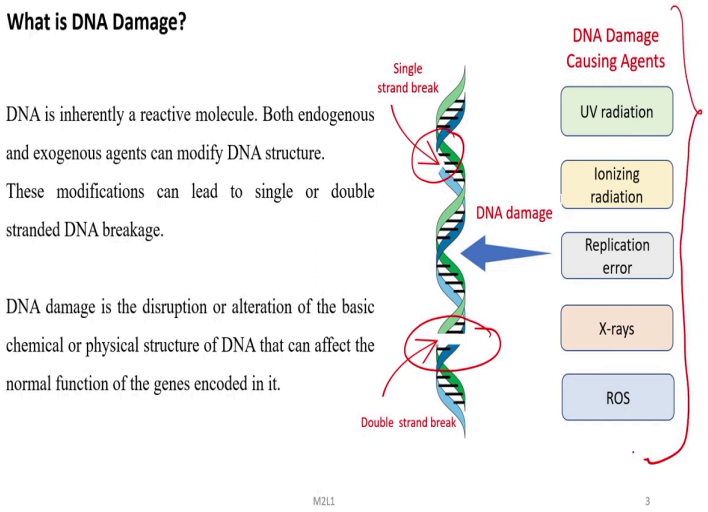

Before that let us discuss what do you mean by DNA damage and why DNA damage occurs DNA is inherently reactive molecule both endogenous and exogenous agents can modify DNA structure. These modifications can lead to a single or double stranded DNA breakage. In brief DNA damage is the disruption or alteration of the basic chemical or physical structure of DNA that can affect the normal function of the genes encoded in it.

So, you can see if the breakage happens in one single strand we call it as a single strand break if the breakage happens in both the strands we call this as a double strand break and there are many agents which can cause both the single strand break and the double strand break in DNA molecules.

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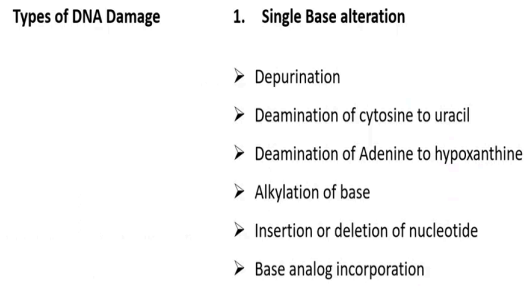

Let us start with simple things what are the different types of DNA damage? DNA damage can happen at the level of a single base, we call them as single base alteration which can be depurination reaction or it can be deamination of cytosine to uracil or adenine to hypoxanthine and it can be as simple as the alkylation of a base and there can be insertion or deletion of a single nucleotide. And in certain cases, incorporation of some of the base analogs into the particular site.

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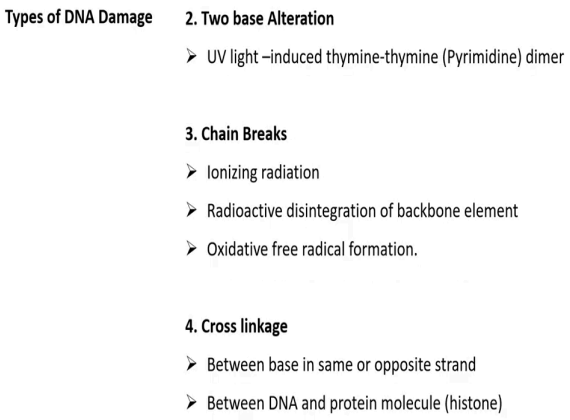

Other kinds of DNA damage may involve more than one base and they can be two base alterations. So, these are induced by UV light which induce thymine-thymine dimer formation. Or it can be little bit extensive which can be chain breaks which happens due to ionizing radiations or radioactive disintegration of the backbone element or due to the action of oxidative free radical formation.

And beyond this there can be cross linkage of the DNA strands. So, some linkages may happen between bases in the same or in the opposite strand or between the DNA and the protein molecule the histone molecules onto which the DNA wraps around.

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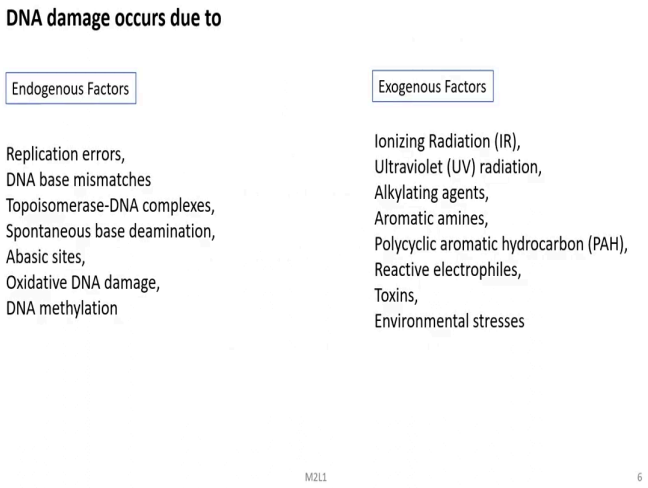

Now let us discuss the various factors which causes DNA damage as already told they may be endogenous factors or they may be exogenous factors the endogenous factors are inherent within the cellular environment and they can be due to some of the molecular events that take place inside the cell. For example, due to replication errors or DNA based mismatches, topoisomerase DNA complexes, spontaneous based deamination or formation of abasic sites, oxidative damage or DNA methylation.

And exogenous factors are all the factors which are external to the cell and they act from outside and they can be certain radiations like ionizing, ultraviolet or they can be certain chemicals like alkylating agents or aromatic amines or polycyclic aromatic hydrocarbons or reactive electrophiles or any toxins and also certain other environmental stresses.

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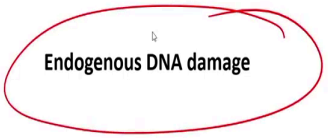

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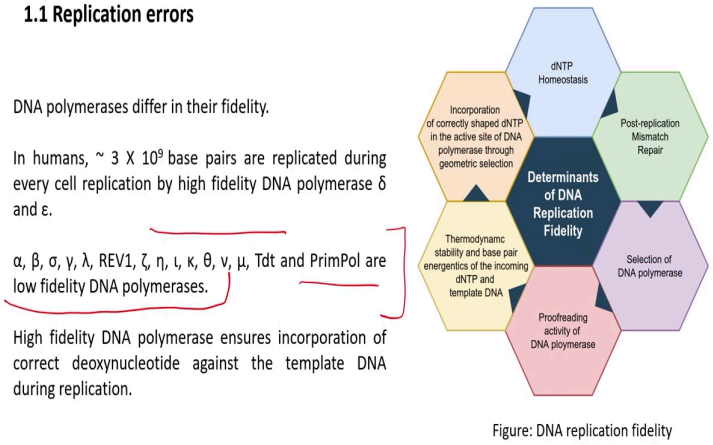

Figure: DNA replication fidelity

Let us discuss first about the endogenous DNA damage. Let us start with replication errors. So, we know that DNA synthesis involves the activity of DNA polymerases the DNA polymerases have varying fidelity inside the cell. In humans roughly around 3×10^9 base pairs are replicated during every cell replication by the high-fidelity DNA polymerase delta and epsilon alpha, beta, delta, gamma, lambda, REV 1 and many other such factors and PrimPol are the low fidelity DNA polymerases.

These high-fidelity DNA polymerases ensures the incorporation of correct deoxynucleotide against the template DNA during replication. And the low fidelity at just opposite to it and these contributes to the replication errors to a large extent. So, this figure sums up the DNA replication fidelity there are certain determinants of DNA replication fidelity which depends on the DNA polymerase that is being deployed for the process and the proofreading activity of the DNA polymerase if the proofreading activity is high then the fidelity will be high if the proofreading is low the fidelity will be low.

Then there are other things like the thermodynamic stability and base pair energetics of the incoming dNTP and template DNA and incorporation of correctly shaped dNTP in the active side of DNA polymerase through geometric selection. If that is not occurring then there will be replication errors and the dNTP homeostasis play a big role in this entire process and after replication there may be some kind of mismatch repair which we tell as the post replication mismatch repairs.

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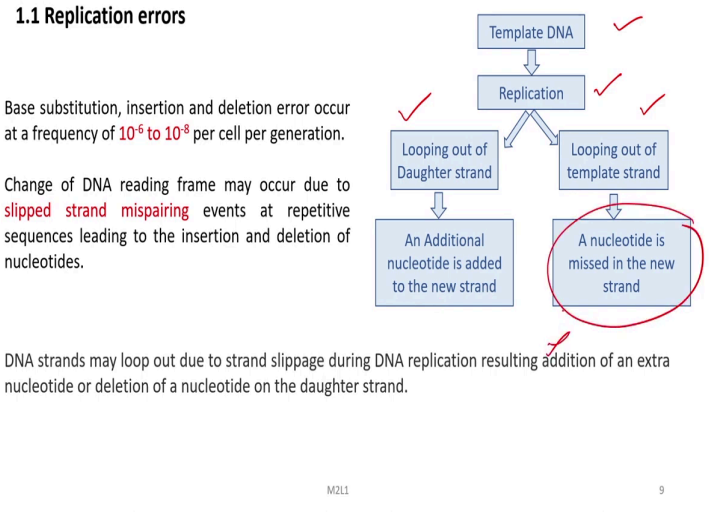

So, base substitution, insertion and deletion error occur at a frequency of around 10^{-6} to 10^{-8} per cell per generation change of the DNA reading frame may occur due to slip strand mispairing events at repetitive sequence leading to the insertion and deletion of the nucleotides. The DNA strands may loop out due to strand slippage during DNA replication resulting addition of an extra nucleotide or deletion of a nucleotide of the daughter strand.

So, if we have a template strand in which the replication occurs there may be looping out of the daughter strand or there may be looping out of the template strand. So, in the first case an additional nucleotide is added to the new strand. In the latter a nucleotide is missed in the new strand [FL].

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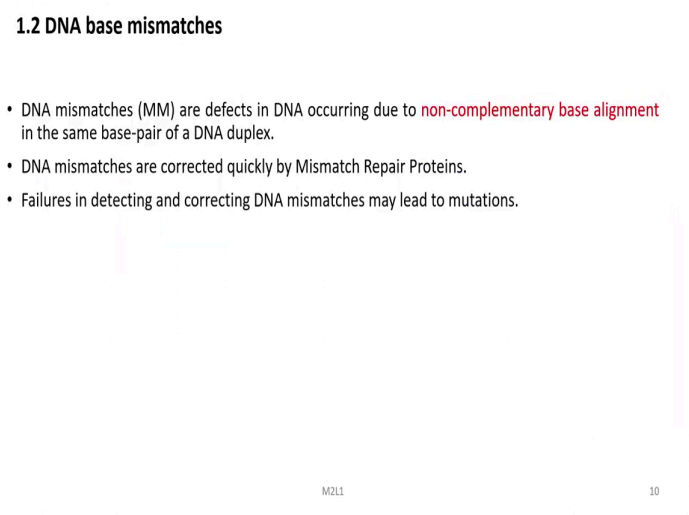

This is how the replication error occurs. Let us now discuss another type of DNA damage which is the DNA base mismatch. These are defects in DNA which occur due to non-complementary base alignment in the same base pair of a DNA duplex. These mismatches are corrected quickly by mismatch repair proteins. Failures in detecting and correcting the DNA mismatch will lead to the mutations.

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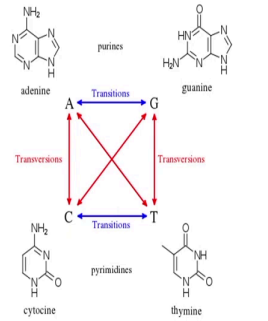
1.2 DNA base mismatches

A transition is when a purine nucleotide is changed into another purine (e.g., A to G) or a pyrimidine nucleotide is changed into another pyrimidine (e.g., C to T).

Transversions are any mutations in which a purine is replaced with a pyrimidine or vice versa.

Transversions (Tv's) are more likely to change the amino acid sequence of proteins than transitions (Ts's.)



Transition Vs Transversion

A transition happens when a purine nucleotide is changed into another purine A to G or a pyrimidine nucleotide is change to another pyrimidine C to T transversions are any mutations in which a purine is replaced with a pyrimidine or vice versa. Transversion are more likely to change the amino acid sequence of proteins than transitions and in this figure, you can see the A to G transition and A to C or G TT G to T transversion.

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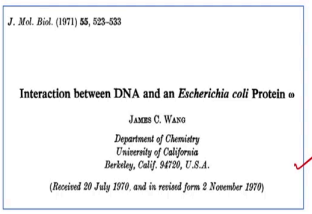
1.3 Topoisomerase mediated DNA damage



J. Mol. Biol. (1971) **55**, 523–533

Interaction between DNA and an *Escherichia coli* Protein ω

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James Wang discovered the first DNA topoisomerase in 1971 from E. coli as ω protein.

Type I topoisomerase	Type II topoisomerase
Nicks single strand of the DNA double helix, relaxes and reanneals it.	Nicks both the strand of a DNA double helix, relaxes supercoils and reanneals.
Types: Type IA, Type IB, Type IC topoisomerases.	Types: Type IIA and type IIB topoisomerases.

There are other agents which causes DNA damage for example, topomerases topoisomerases are one of them James Wang discovered the first DNA topoisomerase in 1971 from E. coli as omega protein and this is the paper he published in journal of molecular biology.

There are two type of topoisomerases; type I and type II. The type I nicks single strand of the DNA double helix, relaxes and then reanneals it. The type II nicks both the strand of a DNA double helix, relaxes, supercoils and reanneals the molecule. So, here are some of the examples of the types IA IB IC and type IIA and IIB topoisomerases.

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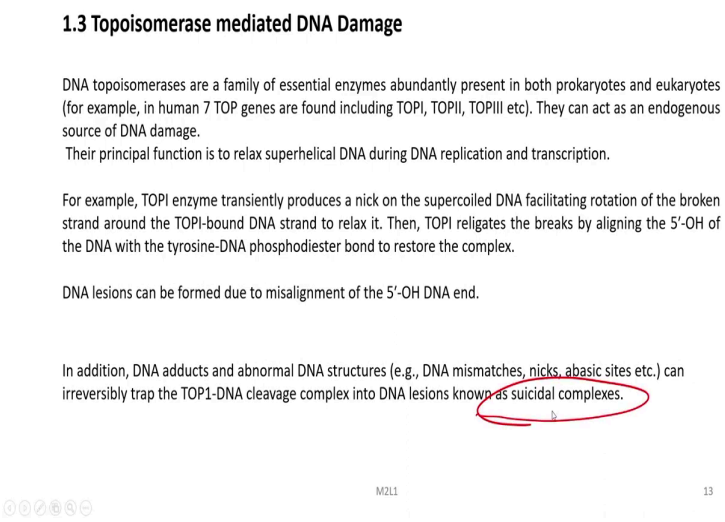

They are basically a family of essential enzymes and they are present abundantly in both prokaryotes and eukaryotes. They can act as endogenous source of DNA damage. Their principal function is to relax superhelical DNA during replication and transcription the TOP I enzyme transiently produces a nick on the supercoiled DNA facilitating rotation of the broken strand around the TOP I bound DNA strand to relax it then the TOP I relegates the breaks by realigning the 5′-OH of the DNA with the tyrosine DNA phosphodiester bond to restore the complex.

DNA lesions can be formed due to misalignment of the 5′-OH DNA ends in addition to these DNA adducts and abnormal DNA structures can irreversibly trap the top one DNA cleavage complex into DNA lesions which are known as suicidal complexes.

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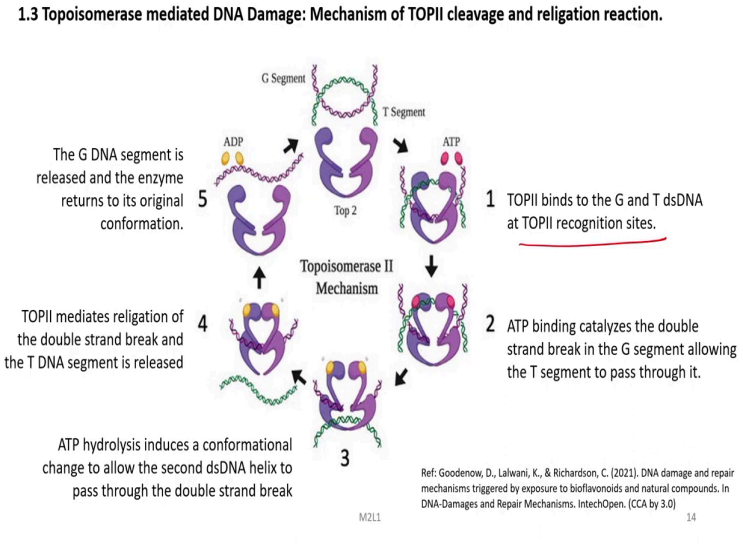

This figure briefly sums up the topoisomerase mediated DNA damage and we can start with this step 1 where the TOP II binds to the G and T double stranded DNA TOP II recognition sites. This is followed by the ATP binding that catalyzes double strand break in the G segment allowing the T segment to pass through it. In the 3rd step ATP hydrolysis occurs which induces a conformational change to allow the second double stranded helix to pass through the double stranded break.

In the 4th step TOP II mediates relegation of the double strand break and the T DNA segment is released and in the 5th step you can see the G DNA segment is released and the enzyme return to its original conformation. So, this is the mechanism by which TOP II cleavage and religation happens.

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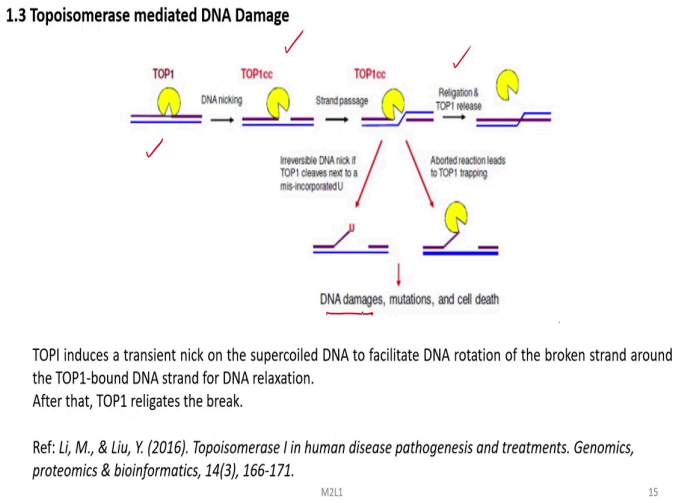

Let us look into the TOP I mediated DNA damage the TOP I is binding to the DNA in the first step as you can see then it nicks in one single strand. Then there is a strand passage step and then religation happens after which the TOP I is released. So, the TOP I induces a transient nick on the supercoiled DNA to facilitate DNA rotation of the broken strand around the TOP 1 bound DNA strand for DNA relaxation after that TOP 1 religates the breakage.

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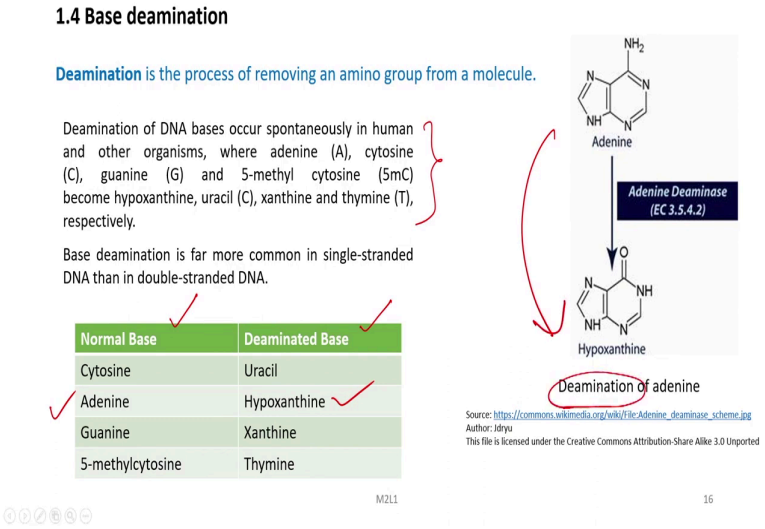

In certain cases, if these are left unrepaired the DNA would be damaged and these mutations will lead to cell death. Let us now discuss about the base deamination reaction this is

basically a process of removing an amino group from a molecule. Deamination of DNA bases occurs spontaneously in human and other organisms where adenine, cytosine, guanine and 5-methyl, cytosine became hypoxanthine, uracil, xanthine and thymine respectively. Base deamination is far more common in single stranded DNA than in double stranded DNA.

You can see here the name of the normal base and the name of the deaminated base which has already been discussed over here this table helps us in remembering them in a better way. And you can see here the action of adenine deaminase which has converted adenine into a hypoxanthine molecule by the process of deamination.

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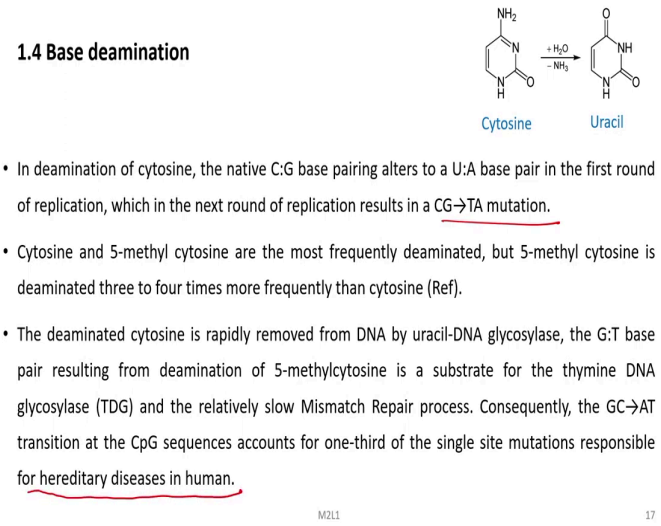

In deamination of cytosine the native C:G base pairing alters to a U:A base pair in the first round of replication which in the next round of replication results in a CG to TA mutation. Cytosine and 5-methyl cytosine are the most frequently deaminated, but 5-methyl cytosine is deaminated 3 to 4 times more frequently than cytosine. The deaminated cytosine is rapidly removed from DNA by uracil DNA glycosylase. The G:T base pair resulting from deamination of 5-methyl cytosine is a substrate for the thymine DNA glycosylase and a relatively slow mismatch repair process.

Consequently, the CG to AT transition at the CpG sequences accounts for one third of the single site mutations responsible for hereditary diseases in human. So, we cannot undermine base deamination reactions they play a huge role in human health particularly genetic diseases.

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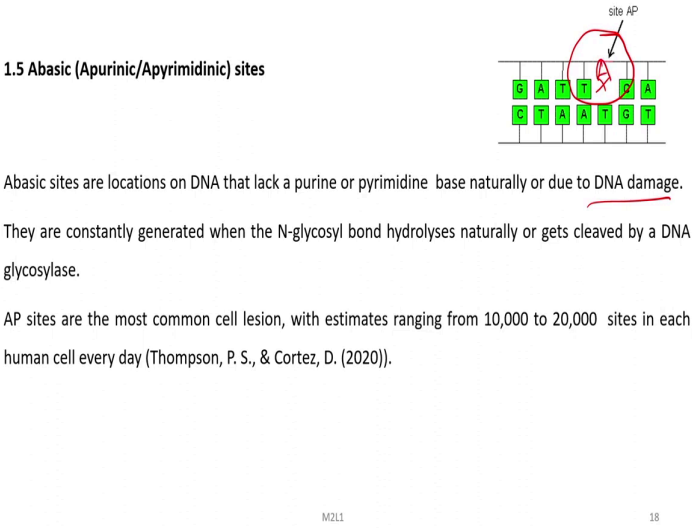

Now, let us discuss about abasic or sites where the base is removed from that site and this reaction can be removal of a purine or a pyrimidine. Accordingly, this is called as a apurinic or apyrimidinic site. So, abasic sites are locations on the DNA that lacks a purine or pyrimidine base naturally or due to DNA damage. And you can see here a site where there is no any base because that has been removed this should have been a because the opposite strand had T, but due to this abasic site reaction there is no any adenine over there.

They are constantly generated it when the n glycosyl bond hydrolyses naturally or gets cleaved by a DNA glycosylase. Asbasic sites or apurinic or apyrimidinic sites are the most common cell lesions which estimates ranging from 10000 to 20000 sites in each human cell every day and this has to be addressed by the cell frequently and you can just understand the level of repair work that goes on every day inside the human cell and if any of these are left behind that causes the DNA damage.

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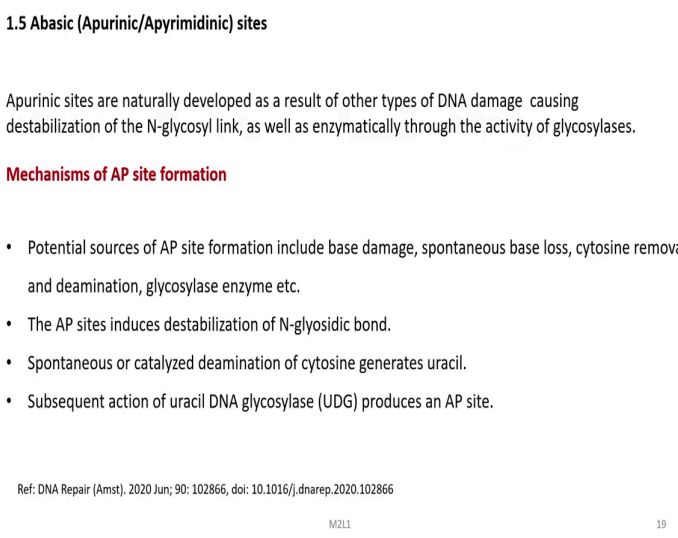

What are the mechanisms of AP site formation or abasic site formation? The potential sources of AP site formation include base damage, spontaneous base loss, cytosine removal and deamination and the involvement of glycosylase enzyme. The AP site induces destabilization of N-glycosidic bonds, spontaneous or catalyzed deamination of cytosine generates the uracil, subsequent action of uracil DNA glycosylase produces an AP site.

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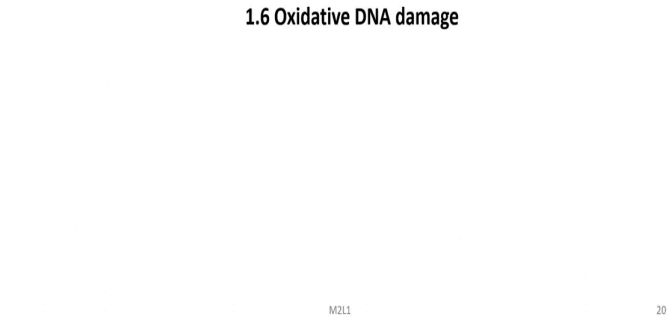

Let us now discuss about the oxidative DNA damage.

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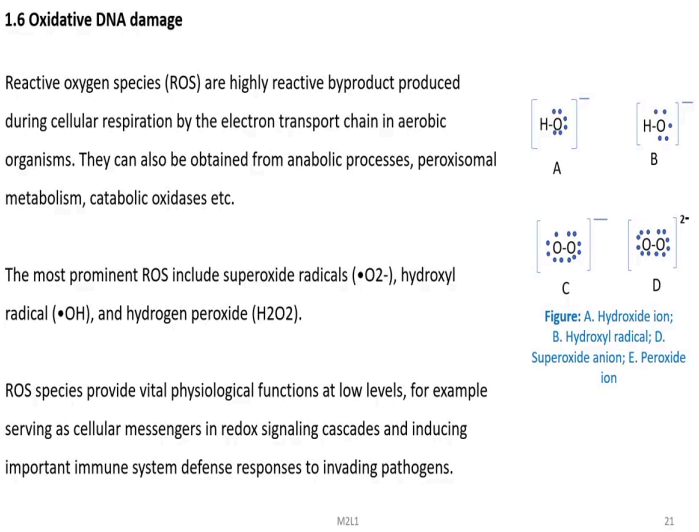

Reactive oxygen species are available inside the cell which occurs due to cellular respiration especially the electron transport chain in aerobic organisms. They can also be obtained from anabolic processes, peroxisomal metabolism, catabolic oxidases etcetera. The most prominent reactive oxygen species include superoxide radical, hydroxyl radical, and hydrogen peroxide.

ROS species provide vital physiological functions at low levels. For example, serving as cellular messengers in redox signalling cascades and inducing important immune system defence responses to invading pathogens.

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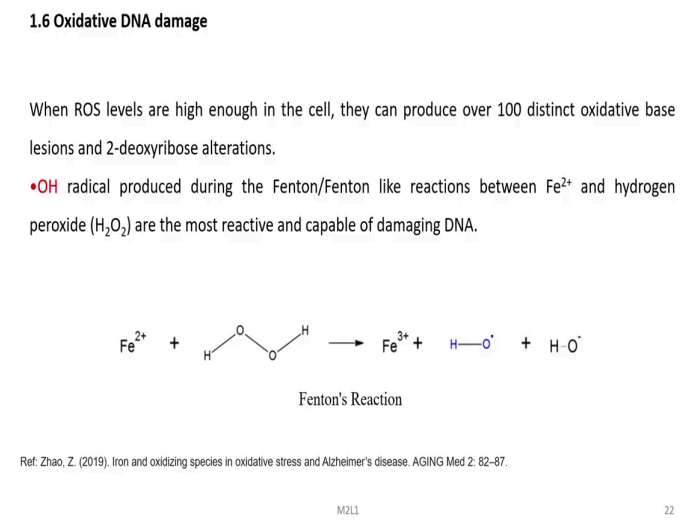

However, they are also harmful because they cause DNA damage. Especially when ROS levels are high enough in the cell, they can produce over 100 distinct oxidative base lesions and 2-deoxyribose alterations. The hydroxyl radical produced during the Fenton/Fenton like reactions between Fe^{2+} and hydrogen peroxide are the most reactive and capable of damaging DNA.

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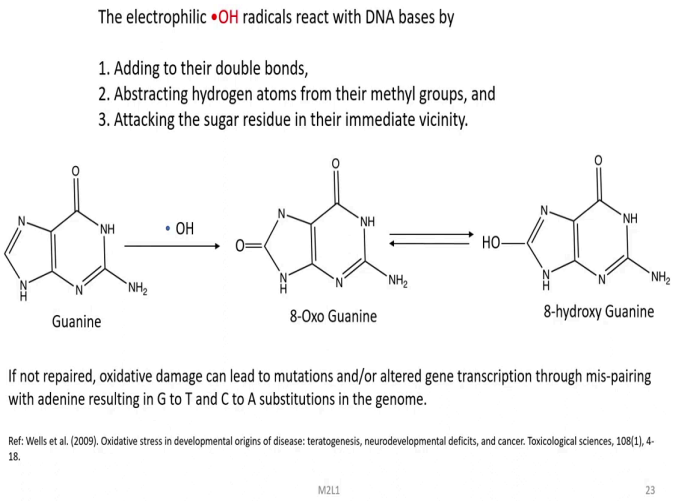

The electrophilic hydroxyl radical reacts with DNA bases by various ways. Number 1, by adding to their double bonds. Number 2, abstracting hydrogen atoms from their methyl groups. And number 3, attacking the sugar residue in their immediate vicinity. And you can see here the conversion of guanine into an intermediate 8-oxoguanine and finally, getting it converted to 8-hydroxyguanine. If this is not repaired oxidative damage can lead to mutations and or altered gene transcription through mis mispairing with adenine resulting in G to T and C to A substitutions in the cell in the genome.

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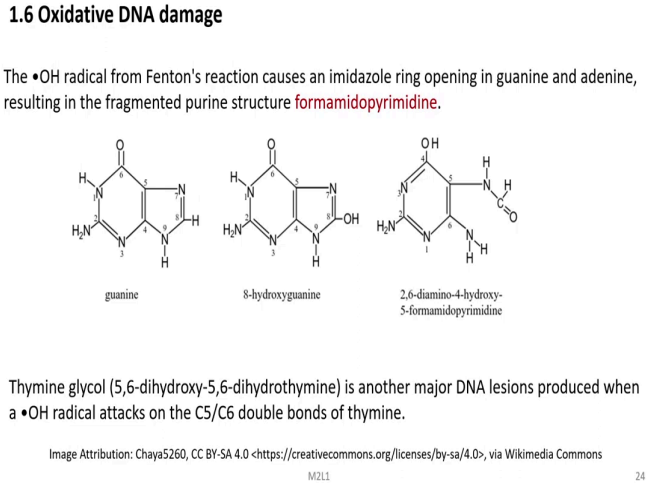

The hydroxyl radical from Fenton's reaction causes an imidazole ring opening in guanine and adenine resulting in the fragmented purine structure. You can see here the transformation of guanine. The thymine glycol is another major DNA lesion produced when a hydroxyl radical attack on the C5 C6 double bonds of thymine.

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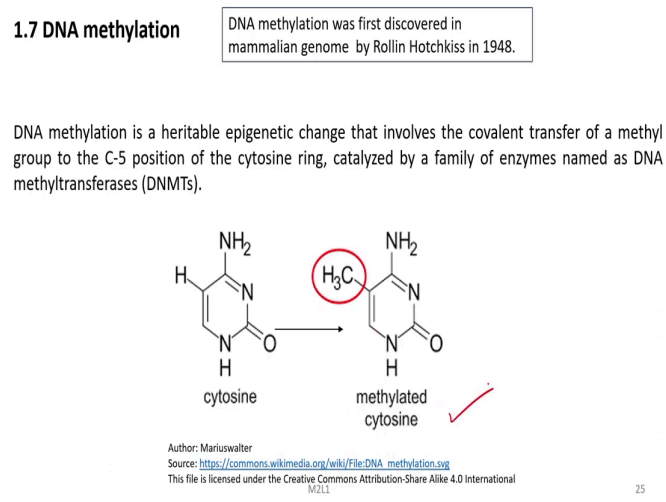

Next, we discuss about the DNA methylation, which was first discovered by mammalian in mammalian genome by Rollin Hotchkiss in 1948. DNA methylation is a heritable epigenetic change that involves the covalent transfer of a methyl group to the C-5 position of the

cytosine ring catalyzed by a family of enzymes named as DNA methyltransferases or DNMTs.

So, here you can see cytosine and upon the removal of the methyl group it gets converted into methylated. So, let us discuss about the oxidative DNA damage where the hydroxyl radical from Fenton's reaction causes an imidazole ring opening in guanine and adenine resulting in the fragmented purine structure form an aminopyrimidine. Thymine glycol is another major DNA lesion which is produced when a hydroxyl radical attacks on the C-5 C-6 double bonds of thymine.

Let us now discuss about the DNA methylation process, which was first discovered in mammalian genome by Rollin Hotchkiss in 1948. DNA methylation is a heritable epigenetic change that involves the covalent transfer of a methyl group to the C-5 position on the cytosine ring catalyzed by a family of enzymes named as DNA methyltransferases or DNMTs. And you can see here the addition of the methyl group to a cytosine to form the methylated cytosine.

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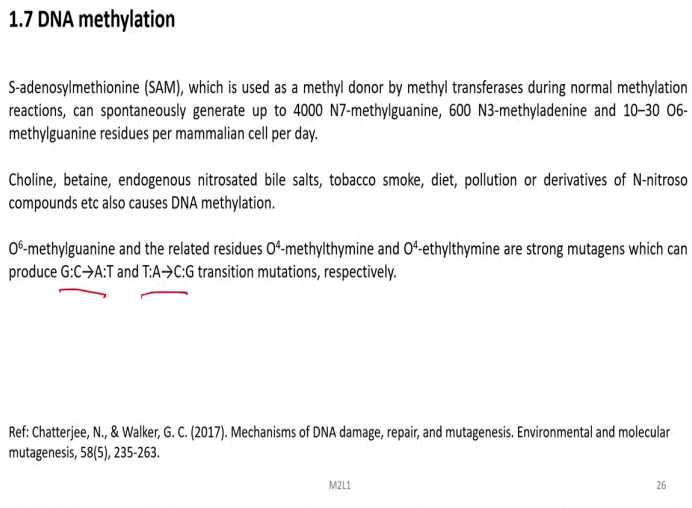

S-adenosylmethionine or SAM is used as a methyl donor by methyl transferases during normal methylation reactions. It can spontaneously generate up to 4000 N7-methylguanine 600 N3-methyladenine and 10 to 30 O6-methylguanine residues per mammalian cell per day. Choline, betaine, endogenous nitrosated bile salts, tobacco smoke, diet, pollution or derivatives of N-nitroso compounds etcetera also causes DNA methylation.

O6-methylguanine and the related residues O4-methylthymine and O4-ethylthymine are strong mutagens which can produce G:C to A:T and T:A to C:G transition mutations respectively.

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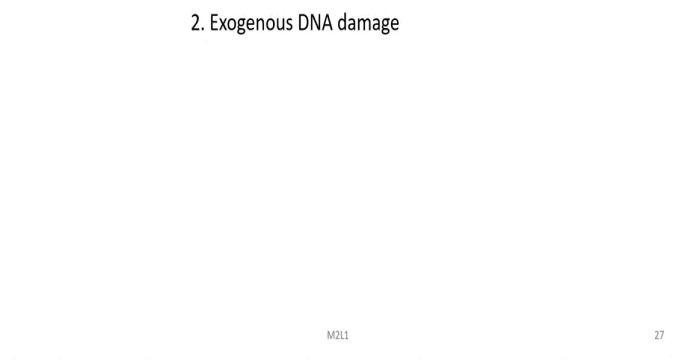

Now let us move on to the various factors which causes exogenous DNA damage.

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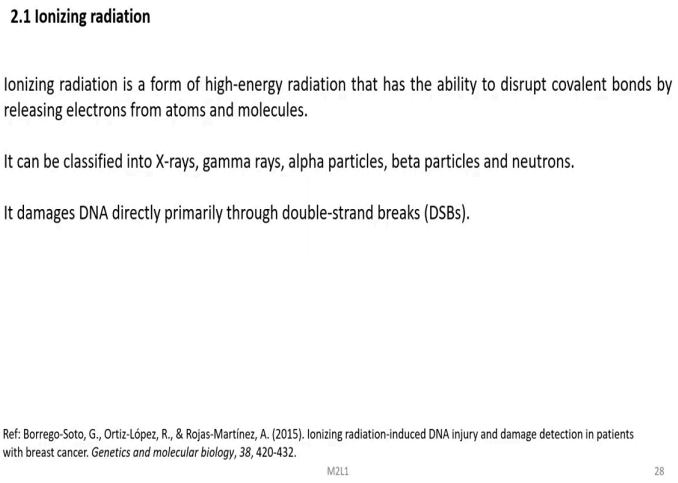

One of the most important agents which causes exogenous DNA damage are the ionizing radiations. Ionizing radiation is a form of high energy radiation that has the ability to disrupt

covalent bonds by releasing electrons from atoms and molecules. They can be classified into X-rays, gamma rays, alpha particles, beta particles and neutrons and damages DNA directly primarily through double strand breaks.

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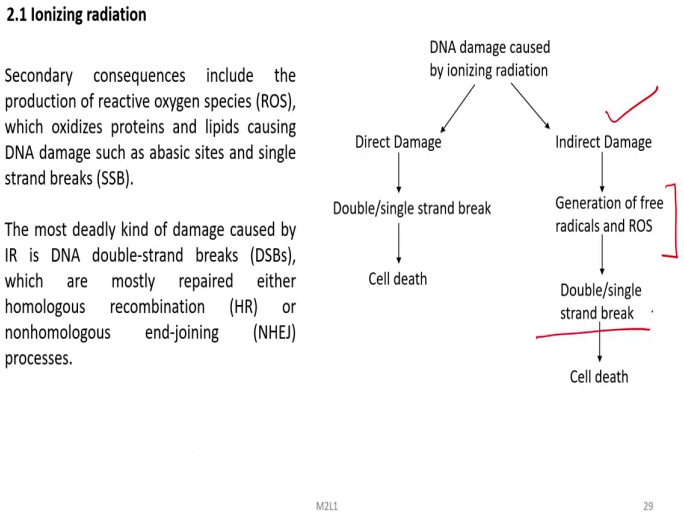

The secondary consequences include the production of reactive oxygen species which oxidizes proteins and lipids causing DNA damage such as abasic sites and single strand breaks. The most deadly kind of DNA damage caused by IR is double strand breaks which are mostly repaired either through homologous recombination or nonhomologous end-joining processes which we will discuss in later lectures. And briefly you can see here the type of DNA damage caused by the ionizing radiations. It can be direct damage or it can be indirect damage.

In the case of direct damage is it causes double or single strand breaks which leads to cell death if not repaired. In the case of indirect damage, it generates free radicals and reactive oxygen species and that leads to the formation of the double or single strand break and cell death similarly.

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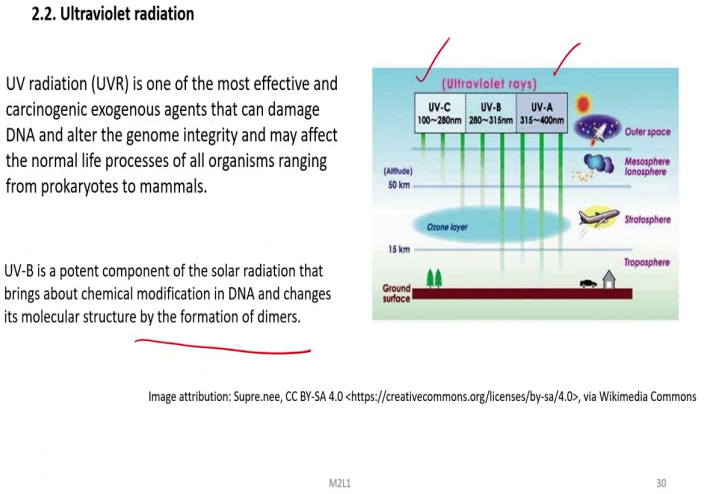

The next agent that we are discussing are the ultraviolet radiation. UVR is one of the most effective and carcinogenic exogenous agents that can cause damage to DNA and alter the genome integrity and may affect the normal life process of all organisms ranging from prokaryotes to mammals. Those in laboratory must be careful that while working in the laminar hood to create a sterile condition a UV radiation is used exposure to such radiation may actually be very very harmful because it causes DNA damage.

UV-B is a potent component of the solar radiation that brings about chemical modification in DNA and changes its molecular structure by the formation of dimmers. The other components of the ultraviolet are UVA and UVC.

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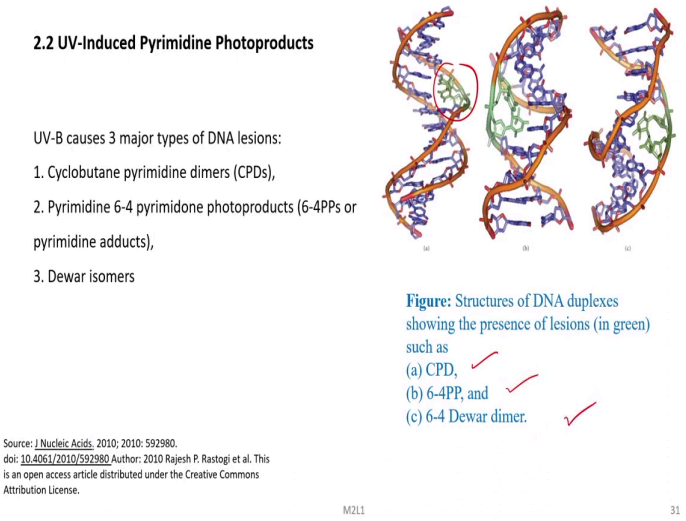

What are the UV induced pyrimidine photoproducts? So, particularly UV-B causes three major types of DNA lesions the formation of CPDs or cyclobutane pyrimidine dimers then pyrimidine 6-4 pyrimidone photoproducts or 6-4 PPs or dewar isomers. And you can see in this particular figure the structures of DNA duplexes showing the presence of the lesions in green such as CPD, 6-4 PP and 6-4 Dewar dimer.

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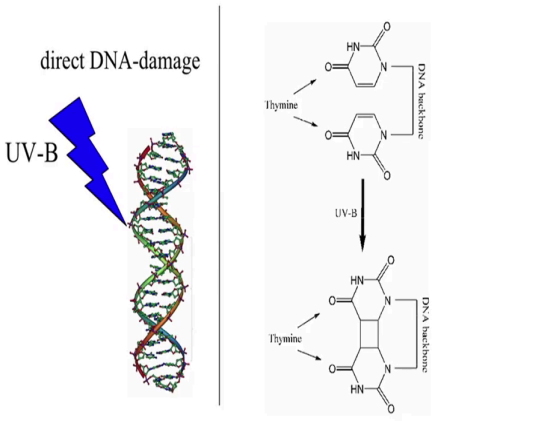

So, DNA can be damaged directly by UV-B and this figure you can see the UV induced direct DNA damage the UV-B photons are directly absorbed by the DNA possible reactions from the excited state leads to the formation of a thymine-thymine cyclobutane dimer.

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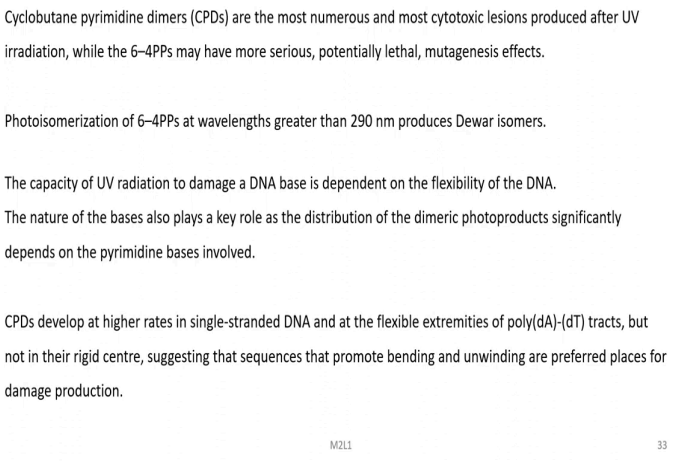

The CPDs are the most abundant and most cytotoxic lesions produced after UV irradiation while the 6-4 PPs may have more serious potentially lethal mutagenesis effect photoisomerization of 6-4 PPs at wavelength greater than 290 nanometer produces the Dewar isomers.

The capacity of UV radiation to damage a DNA base is dependent on the flexibility of the DNA. The nature of the bases also plays a key role as the distribution of the dimeric photoproducts significantly depends on the pyrimidine bases involved. CPDs develop at higher rates in single stranded DNA and at the flexible extremities of poly(dA)-(dT) tracts, but not in their rigid centre, suggesting that sequences that promote bending and unwinding are preferred places for DNA damage production.

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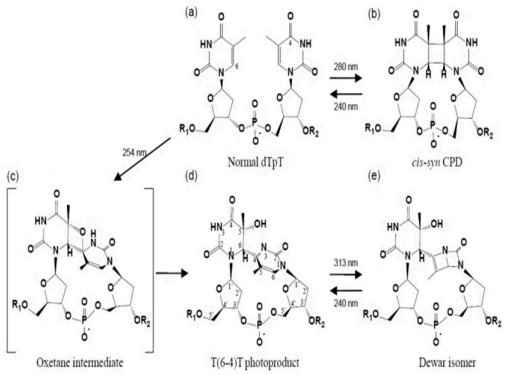

DNA photoproducts formed by ultraviolet radiation. (**a**) Normal dTpT; (**b**) *cis-syn* cyclobutane pyrimidine dimer (CPD); (**c**) Oxetane intermediate; (**d**) A dT(6-4)T photoproduct with atom numbering; and (**e**) Dewar isomer.

Image source: Yokoyama, H., & Mizutani, R. (2014); (CC BY 4.0)

Ref: Yokoyama, H., & Mizutani, R. (2014). Structural biology of DNA (6-4) photoproducts formed by ultraviolet radiation and interactions with their binding proteins. International journal of molecular sciences, 15(11), 20321-20338.

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Here you can see the various DNA photoproducts formed by ultraviolet radiation. Figure a, shows the normal dTpT, figure b, shows the cis-syn cyclobutane pyrimidine dimer or CPD and figure c, shows the oxetane intermediate the figure d, shows A dT(6-4)T photoproduct with atom numbering and e shows the Dewar isomer.

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2.2 UV-Induced Purine Photoproducts

UV-Induced Purine Photoproducts are the photoproducts that involve, at least, one adenine residue that undergoes photocycloaddition reactions with contiguous adenine or thymine upon exposure to UV-B radiation.
Photodimerization of adenine (A) involves the cycloaddition of N7-C8 double bond of the 5′-A across the C6 and C5 positions of the 3′-A and generates a very unstable azetidine intermediate.

This intermediate photoproduct is split into two different adenine photoproducts, adenine dimer (A=A) and Pörschke photoproduct, by opposing reaction pathways.

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What are UV induced purine photoproducts? UV induced purine photoproducts are the photoproducts that involve at least one adenine residue that undergoes photocycloaddition reaction with contiguous adenine or thymine upon exposure to UV-B radiation.

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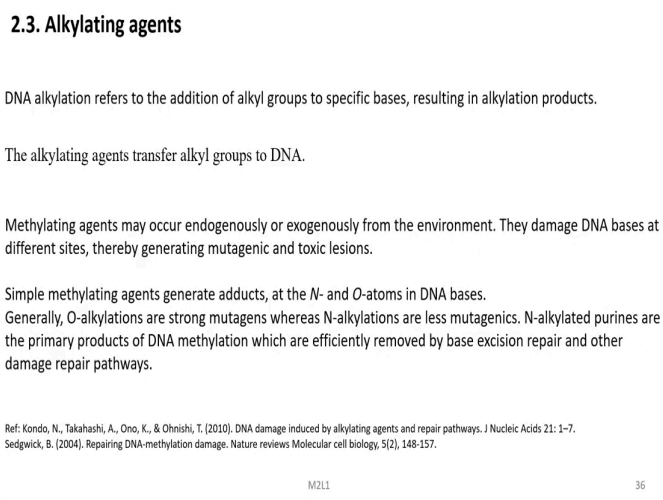

The next most important type of agents are alkylating agents. DNA alkylation is the addition of alkyl groups to specific bases resulting in DNA alkylation products. The alkylating agents transfer alkyl groups to the DNA. Methylating agents may occur endogenous or exogenously from the environment. They damage DNA bases at different sites and generates mutagenic and toxic lesions. Simple methylating agents generate adducts at the N and O atoms in the DNA bases.

Generally O alkylations are strong mutagens whereas, N alkylations are less mutagenic. N alkylated purines are the primary products of DNA methylation, which are efficiently removed by base excision repair and other damage pathways.

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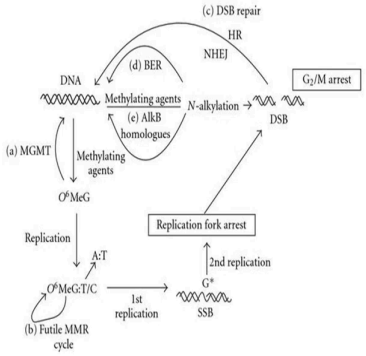

Figure: DNA damage pathways induced by methylating agents

(a) If left unrepaired, O^6MeG:C ambiguous pairs or O6MeG:T mismatch pairs can form during replication if not repaired. In the next round of replication, O^6MeG:T pairs can become A:T transition mutations. (b) O^6MeG:T and O^6MeG:C pairs are recognized by the mismatch repair (MMR) system, which creates a single-strand break (SSB), cause replication arrest, and finally leads to a double-strand break (DSB). (c) Homologous recombination (HR) and nonhomologous end joining (NHEJ) may play a role in the repair of DSBs. *N*-alkylations are repaired by either (d) base excision repair (BER), or (e) AlkB homologues, and if not repaired, DSBs occur.

So, once the DNA damage happens the DNA damage pathways are activated and the repair takes place. If left unrepaired O-6 methyl G:C ambiguous pairs or O-6 methyl G:T mismatch pairs can form during replication in the next round of replication the O-6 methylated G:T pair can become A:T transition mutations as shown in figure a.

In figure b, you can see that O-6 methylated G:T and O-6 methylated G:C pairs are recognized by the mismatch repair system which creates a single strand break causes replication arrest and finally, leads to a double stranded break. In figure c, we can see homologous recombination and nonhomologous end-joining playing a role in the repair of the double strand brakes. N-alkylations may play a role in the repair of DSBs as well.

N-alkylations are repaired by either base excision repair or AlkB homologues and if not repaired the DSBs will occur. We will have detailed discussion on the repair mechanism, but this is just to show that if the damages are left unrepaired then the various kind of DNA breakage like DSB or SSB may occur.

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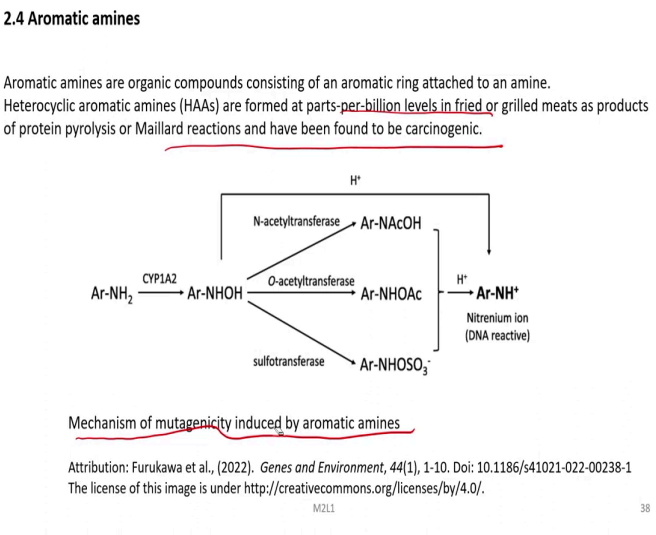

Another kind of agents which are responsible for DNA damage are the aromatic amines. The aromatic amines are organic compounds consisting of an aromatic ring attached to an amine. The heterocyclic aromatic amines or HAAs are formed at parts per billion level in fried or grilled meat products. So, the food we eat may actually have some kind of amines and they can be dangerous as well, but the lower concentration does not make them that unsafe. Or there may be protein pyrolysis or Maillard reactions and all of them has been found to be carcinogenic at a particular concentration and exposure.

So, let us look into the mechanism of mutagenicity induced by aromatic amines over here. So, these N-acetyltransferase or O-acetyltransferase or sulfotransferases converts this aromatic amines into some of the intermediate products which are finally, converted into nitrenium ion and these are having high reactivity particularly along with DNA molecules.

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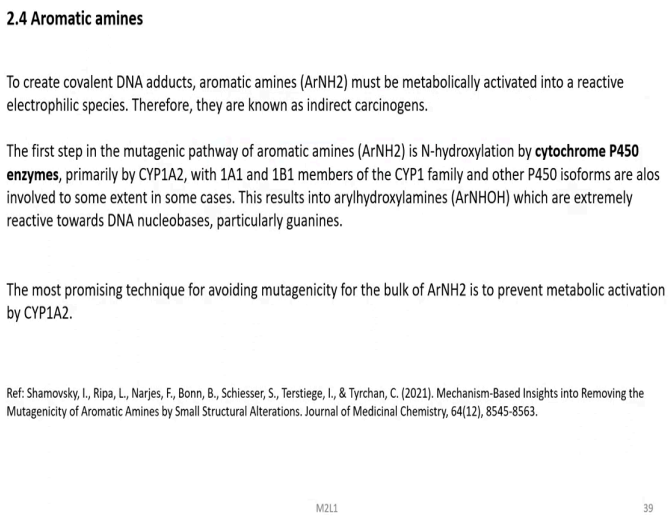

To create covalent DNA adducts aromatic amines must be metabolically activated into a reactive electrophilic species therefore, they are known as indirect carcinogens. The first step in the mutagenic pathway of aromatic amines is N-hydroxylation by cytochrome P450 enzymes primarily by CYP1A2 with 1A1 and 1B1 members of the CYP1 family and other P450 isomers are also involved to some extent in certain cases. This results into arylhydroxylamines, which are extremely reactive towards DNA nucleobases particularly guanines.

The most promising technique for avoiding mutagenicity for the bulk of aromatic amines is to prevent metabolic activation by CYP1A2.

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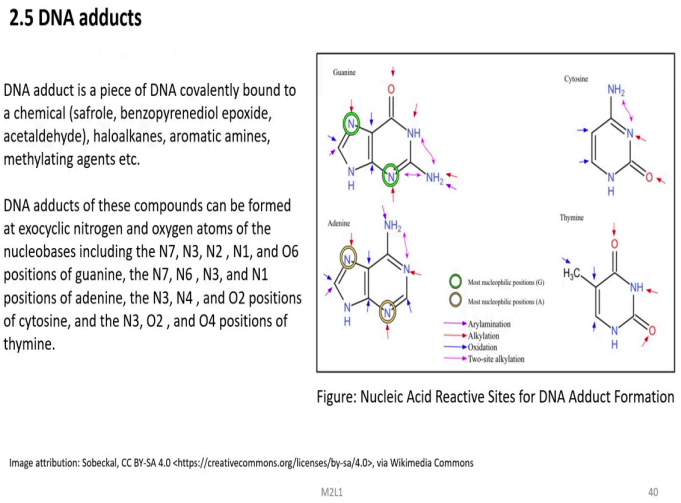

DNA adducts: What are DNA adducts? DNA adduct is a piece of DNA covalently bound to a chemical which may be safrole, benzopyrenediol, acetaldehyde etcetera. Haloalkenes, alkanes, aromatic amines, methylating agents, they may all be bound to the DNA molecule covalently.

The DNA adducts of these compounds can be formed at exocyclic nitrogen and oxygen atoms of the nuclear bases including the N7, N3, N2, N1 and O6 positions of guanine the N7, N6, N3 and N1 position of adenine the N3, N4 and O2 positions of cytosine and the N3, O2 and O4 positions of thymine and you can see the various positions in the guanine adenine cytosine and thymine molecules.

So, you can see the those with the green colour had a most nucleophilic positions in G and here with the yellow colour are the most nucleophilic positions in adenine and some of the arrow indicates the propensity for the various reaction as shown over here in this diagram.

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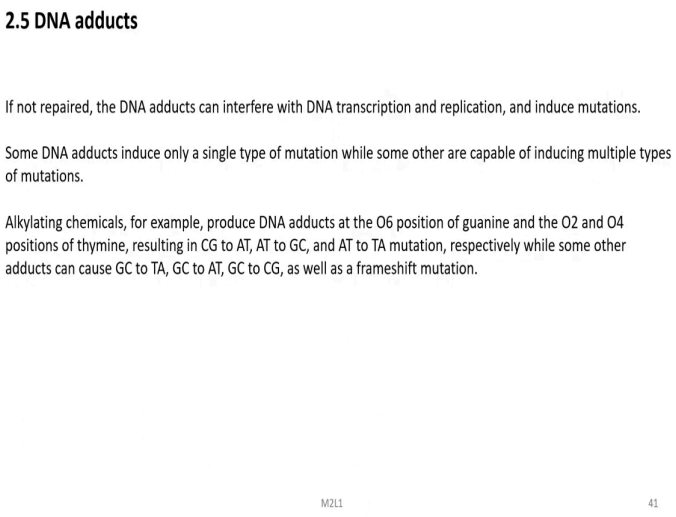

So, we know that DNA adducts are getting covalently bound to the DNA molecule through the various bases. And if it is not repaired left unattended the DNA adduct will interfere with DNA transcription and replication and this will induce mutation. Some DNA adducts induce only a single type of mutation whiles others are capable of inducing multiple types of mutation.

Alkylating chemicals for example, produce DNA adducts at the O6 position of guanine and O2 and O4 position of thymine resulting in GC to AT, AT to GC and AT to TA mutation while some other adducts can cause GC to TA, GC to AT, GC to CG as well as a frame shift mutation.

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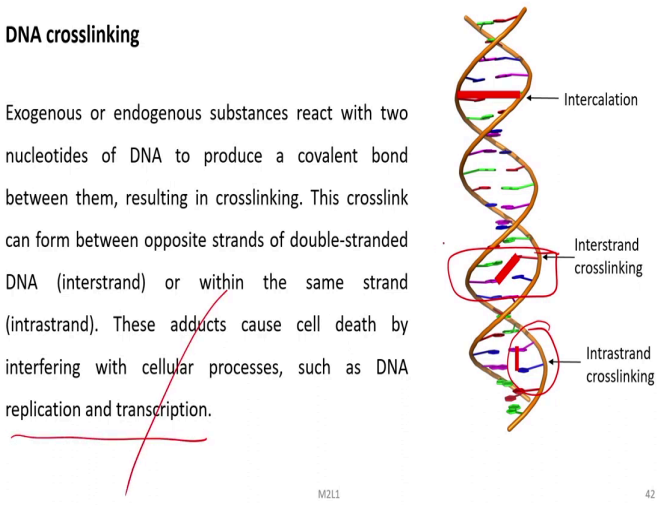

We have also already told that another kind of damage is DNA crosslinking. So, it may happen due to exogenous or endogenous substances which react with two nucleotides of DNA to produce a covalent bond between the two. And this results in the crosslinking. This crosslink can form between opposite strands of the double strand DNA double strand which is the called the interstrand linkage or within the same strand and this is called intrastrand linkage. So, you can see the linkage is happening within the single strand and here the linkage is happening between both the strand.

And there are also certain other phenomena where there is no any linkage, but certain molecules get intercalated between the stack to bases. So, whenever some kind of intra or interstrand cross linking takes place it causes cell death because it halts certain processes like replication and transcription.

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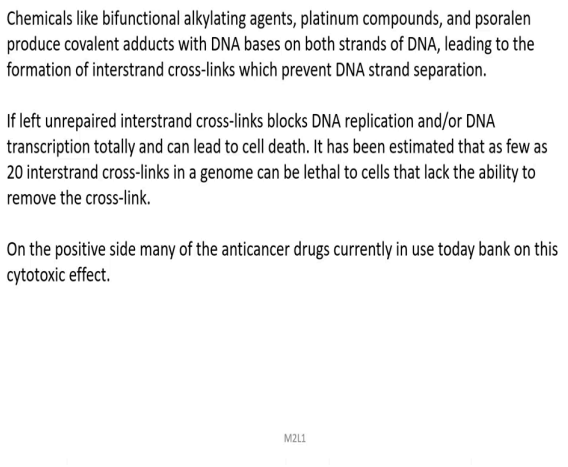

Chemicals like bifunctional alkylating agents, platinum compounds and psoralen produce covalent adducts with DNA bases on both strands of DNA, leading to the formation of interstrand cross links which prevent DNA strand separation. In the earlier figure you can see here this interstrand cross linking taking place where both the strands are covalently bound to one another. So, this will prevent the separation of the two strands; however, in intrastrand crossing this does not occur because there is no any cross linking of the two strands.

Now, if this cross linking is left unrepaired the inter strand cross links blocks the DNA replication and or DNA transcription totally which leads to cell death. It has been estimated that as few as twenty intrastrand cross links in a genome can be lethal to cells both in bacteria and humans that lack the ability to remove the cross link. On the positive side many of the anticancer drugs currently in use today rely on these cytotoxic effects occurring due to the intrastrand linkages.

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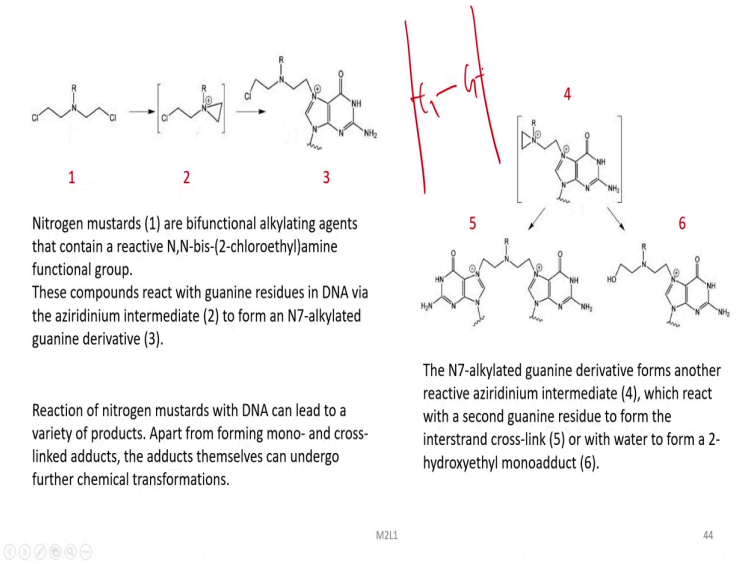

So, let us examine little bit detail, how these intrastrand cross linking takes place due to certain agents. Here we are taking the example of nitrogen mustards which are bifunctional alkylating agents and they contain reactive N,N-bis-(2-chloroethyl)amine functional group as shown in number 1. These particular compounds react with guanine residues in DNA via the aziridinium intermediate as shown in number 2 to form an N7- alkylated guanine derivative as shown in number 3.

One this once these N alkylated guanine derivative is formed this forms another reactive aziridinium intermediate as shown in number 3. So, there are two steps in which these aziridinium intermediates are formed once in the first step and another one is in the second step from 3 to 4.

This aziridinium the second aziridinium number 4 reacts with second guanine residue to form the interstrand cross link number 5 or with water to form 2-hydroxyethyl monoadduct. So, this cross linking happens in two steps; first one guanidine gets covalently bound to a adduct and in the second step this guanine with the adduct binds with the second guanine.

So, thereby it forms a covalent bond between the two guanines located in the two strands of the DNA and thereby cross linking the two DNA strands.

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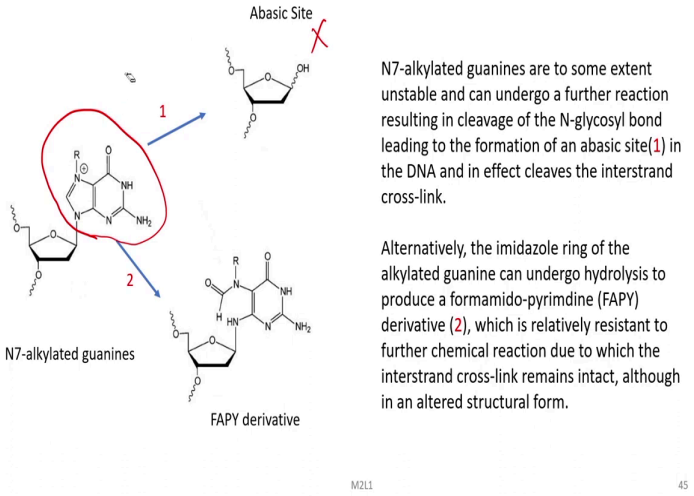

Now in certain cases there may be formation of abasic sites as well. Here for example, one single N-alkylated guanine is being shown here. Now, these N-alkylated guanine to some extent is unstable and this can undergo a further reaction resulting in cleavage of the N-glycosyl bond leading to the formation of an abasic site. So, this particular guanine is removed from here.

So, the base is removed in this case. So, a abasic site is created. Alternatively, in the second pathway you can see the imidazole ring of the alkylated guanine can undergo hydrolysis to produce a formamido-pyrimidine or FAPY derivative which is relatively resistant. So, he is opening up over here you can see here this is relatively resistant to further chemical reaction due to which the interstrand cross link remains intact although in an altered structural form.

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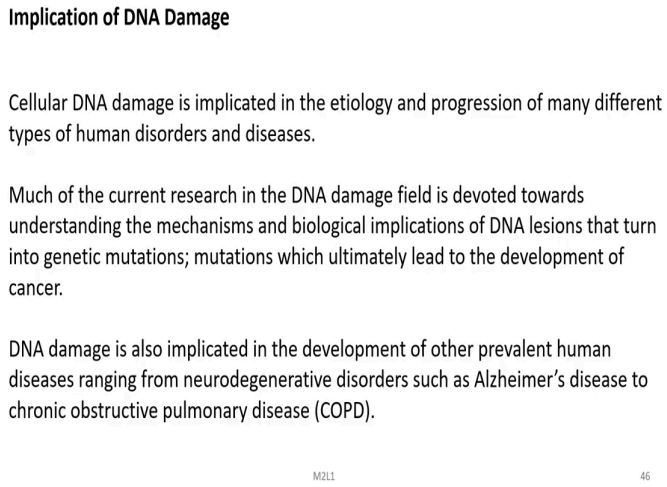

So, we have seen that by various mechanisms due to various factors, endogenous and exogenous DNA molecules get damaged because it is a comparatively very active molecule or susceptible molecule. Now, what happens if DNA damage occurs? What are the implications of this? Cellular DNA damage is implicated in the etiology and progression of many different types of human disorders and diseases.

If these are not repaired by the cellular systems in place. Much of the current research in the DNA damage field is devoted towards understanding the mechanism and biological implications of DNA lesions that turn into genetic mutations, which ultimately lead to the development of various diseases mostly cancer. DNA damage is also implicated in the development of other prevalent human diseases ranging from neurodegenerative disorders such as Alzheimer's to chronic obstructive pulmonary diseases or COPD.

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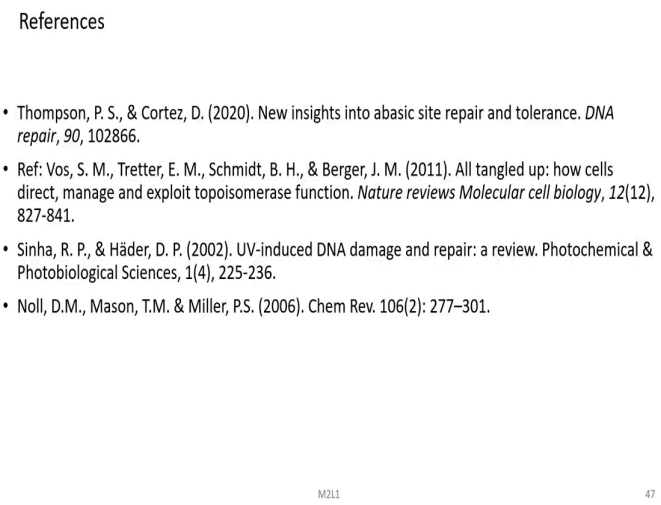

So, these are some of the references which we took for preparation of this lecture.

Thank you.