Medicinal Chemistry
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Nucleic Acids as Drug Targets Part-1

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Welcome back. So far in the past few lectures we have seen how you know drugs can be developed against enzymes, so enzymes are the targets for new drug development and here we looked at various aspects of inhibitors, the types of inhibitors and so on and we also understood the mechanisms of how enzymes act and what kind of binding forces are present and how we could mimic the active site in the enzyme and for us to or we need to make molecules that can go and bind to be appropriate binding site in the correct manner, so that it can inhibit the enzyme.

So similarly we have also looked at how receptors function and how certain receptors I mean most receptors are present on the cell surface and they act as very important signalling hubs of signalling and how we could develop new drugs against receptors. So we also saw that you know we could have very high level of signalling so in which case we have to inhibit the particular receptor or we could have very low level of signalling in which case we will have to enhance the receptors function or we will have to increase the number of ligands.

So both of these approaches will require you know slightly different strategies and so now we will look at the third major drug target which is nucleic acids. So we have already looked at

how you know how the major nucleic acids inside the cell are basically DNA and RNA. We have looked at the various types of RNA for example and how it can function inside the cell and of course DNA by enlarge is the primary storage of genetic information inside the cell and so DNA becomes very attractive target for drug development.

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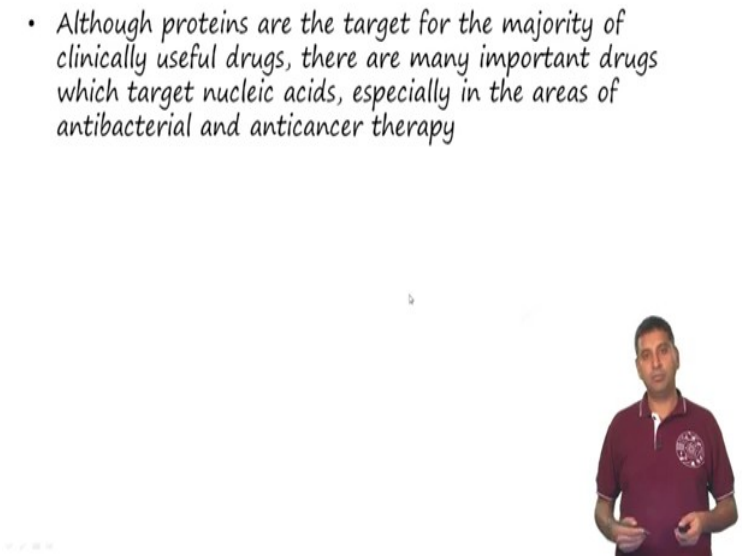

So although proteins are the targets for the majority of clinically useful drugs, like we looked at enzymes inhibition of enzymes or enhancement of receptor signalling or inhibition of it all of these are proteins and so proteins are the major targets, but nucleic acids are especially important when we are looking at antibacterial and anticancer therapy. So in today's lecture we will look at certain examples of how nucleic acids can be targets for drugs which are already in existence and how we could understand (this) how these drugs act in order for us to develop new drugs.

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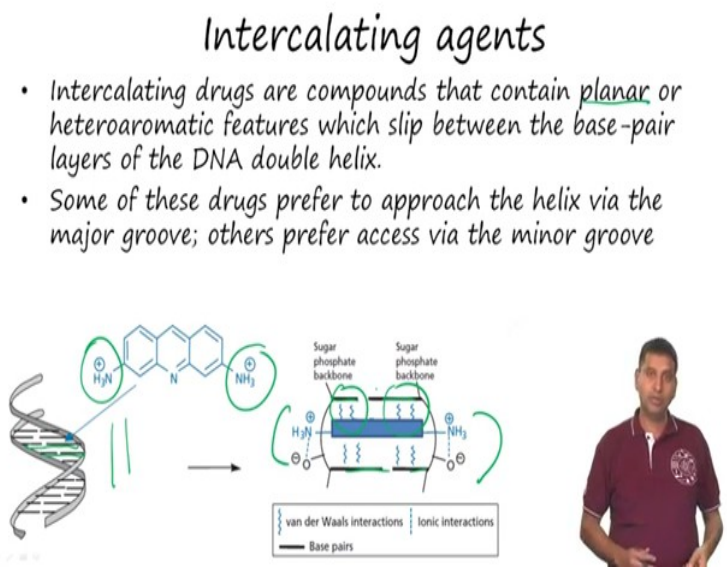

The first major class of nucleic acid interacting agents are what are known as intercalating agents, okay intercalation is basically a situation where a drug goes and slips between base-pairs in a double helix. So here is the Watson Crick double secondary structure that is shown here and there are as we looked at there are very important interactions that happen and the nucleic acid bases are interacting with each other through pi stacking and so that creates a situation where or a place where this drug can go and bind, okay.

So these drugs as you can imagine would be typically planar, okay with either aromatic or heteroaromatic features. So once this gets in then what happens is that it can then bind to the two different base pairs as shown here and some of these intercalating agents also have charged species on the periphery, okay so these charged species which are positively charged ammonium salts for example can then have ionic interactions with the phosphate backbone and lastly we could also have Van der Waals interactions as shown here with the bases, okay.

Now some of these drugs prefer to approach the helix via the major groove and others prefer to access via the minor groove, we have already looked at the major and minor groove in the in one or the previous lectures.

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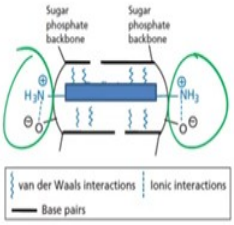
- Once they are inserted between the nucleic acid base pairs, the aromatic/ heteroaromatic rings are held there by van der Waals interactions with the base pairs above and below.
- Several intercalating drugs also contain ionized groups which can interact with the charged phosphate groups of the DNA backbone, thus strengthening the interaction.



Once they are inserted between the nucleic acid base pairs, the aromatic or heteroaromatic rings are held together by Van der Waals interactions (and) with the base pairs above and below. Several intercalating drugs also contain ionized groups as we discussed earlier and these can interact with the charged species. So what in this process happens is that we have a very strong interaction that is through Van der Waals interaction as well as ionic interactions and (these strengthen these are) these can go inside the DNA and therefore they can form pretty stable species.

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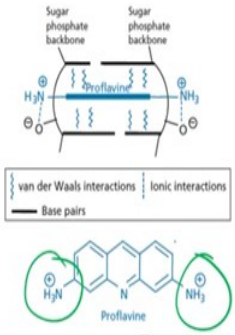
- Proflavine is an example of a group of antibacterial compounds called the aminoacridines, which were used during World Wars I and II to treat deep surface wounds.
- These drugs proved highly effective in preventing infection and reduced the number of fatalities resulting from wound infections.
- Proflavine is completely ionized at pH 7 and interacts directly with bacterial DNA...



So the example that we are going to look at is Proflavine. Proflavine is one of the antibacterial compounds and under the major class of compounds known as aminoacridines

and these were used during World Wars 1 and 2 to treat deep surface wounds infections. And the drugs proved highly effective in preventing infection and reduce the number of fatalities. So Proflavine as you can see is completely ionized in pH 7, okay so it has an amine and so the amine can pick up a proton and form an ammonium salt and therefore it is completely ionized.

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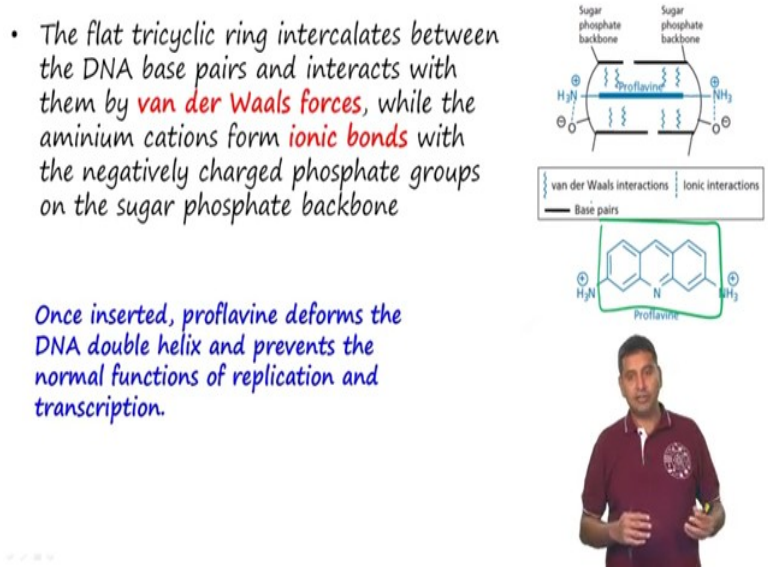

And because it has a flat tricyclic ring as shown here, it can go inside the between the two base pairs and once it inserts again as we looked at earlier it will form stable Van der Waals interactions as well as ionic interactions and after this insertion happens the double helix becomes deformed. Now after the double helix is deformed what perhaps happens is or what we could imagine that could happen is that it would prevent the normal functions of replication and transcription.

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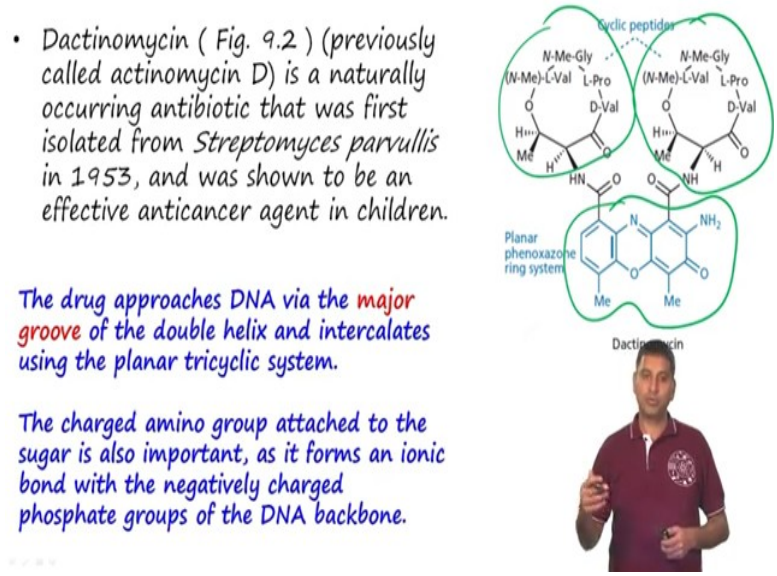

Another example of an intercalating agent is Dactinomycin (so) it is previously called as actinomycin D. It is a naturally occurring antibiotic that was first isolated from Streptomyces parvullis in 1953, it has been shown to have an (effect) as an effective anticancer agent in childhood cancers. If you look at the structure of Dactinomycin it has the planar phenoxazone system and it also has a peptide over here which is a cyclic peptide.

And the drug approaches DNA via the major groove of the double helix and after it intercalates, the planar tricyclic system gets between the two base pairs as we have seen previously. The charged amino group attached to the sugar is also important and it forms an ionic bond with the negatively charged phosphate groups of the DNA backbone.

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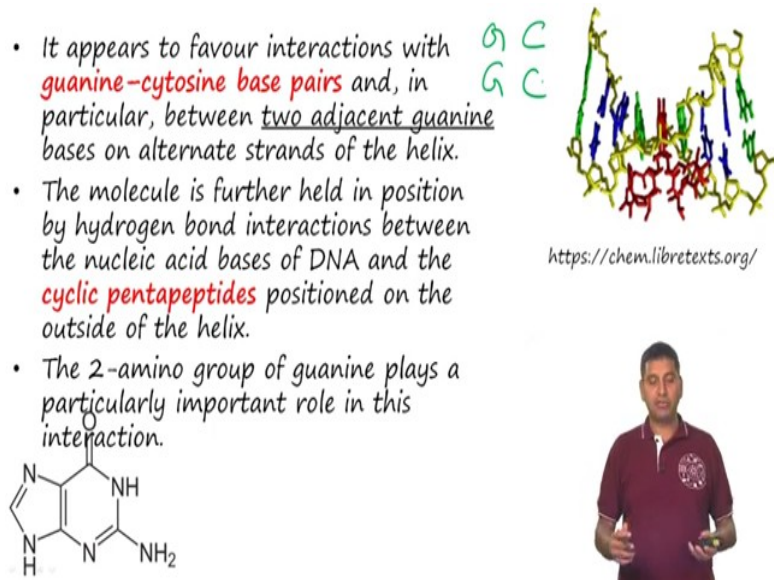

It appears to favour interactions with the guanine-cytosine base pairs that is GC base pairs and in particular between two adjacent guanine bases on alternate strands of a helix. So you have GC interactions and if you have two G's then it prefers to bind to these types of DNA base-pairs. The molecule is further held in position by hydrogen bond interactions between the nucleic acid bases and the cyclic pentapeptids as we looked at earlier.

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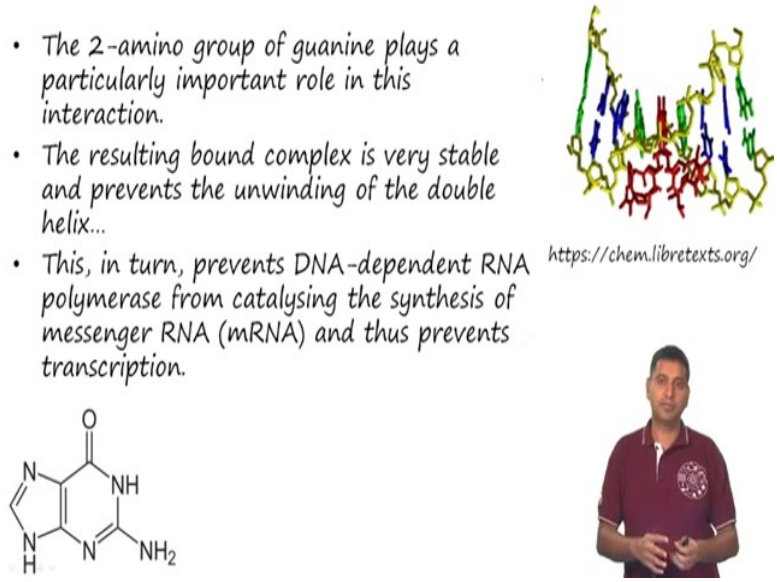

The 2-amino group of guanine plays a particularly important role in this interaction. The resulting bound complex is very stable and it prevents the unwinding of the double helix. So this in turn, prevents DNA-dependent RNA polymerase from catalysing the synthesis of mRNA and thus it prevents transcription.

So the effect of Dactinomycin in actively dividing cell is that once it goes in it goes inside and does intercalates, after it intercalates it forms an extremely stable bond with DNA and after this complex is formed because this complex is so stable it prevents the unwinding of the double helix and if unwinding of double helix does not happen as we know from previous lectures that the single strand DNA is not produced which is going to transfer the information to mRNA and so therefore transcription is inhibited and as a consequence if transcription is inhibited then (you would) the cell would stop replicating or stop dividing.

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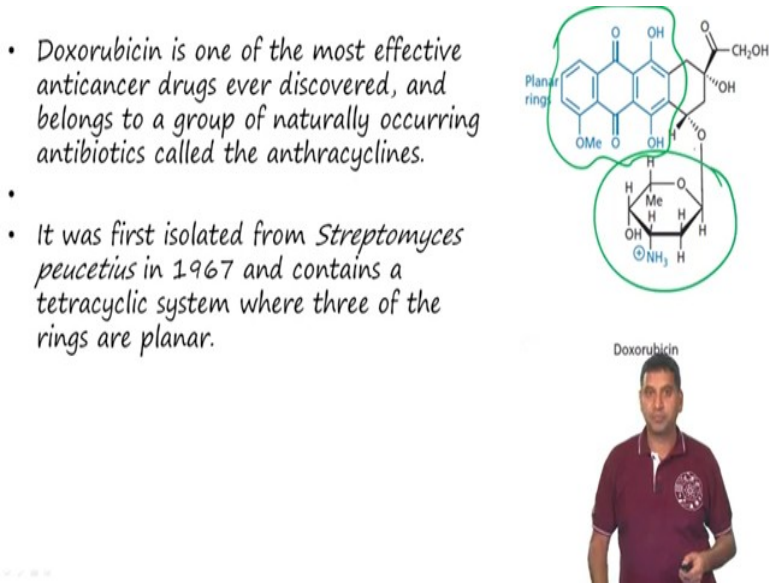

The next example or we will look at is Doxorubicin, it is among the most effective anticancer drugs ever discovered and it belongs to a group of naturally occurring antibiotics known as anthracyclines. It was first isolated from Streptomyces peucetius in 1967 and contains a tetracyclic system where three of the rings are planar, okay. So again the common group that we see here is that it should have three rings and they are all planar. Doxorubicin also has an amino sugar, okay.

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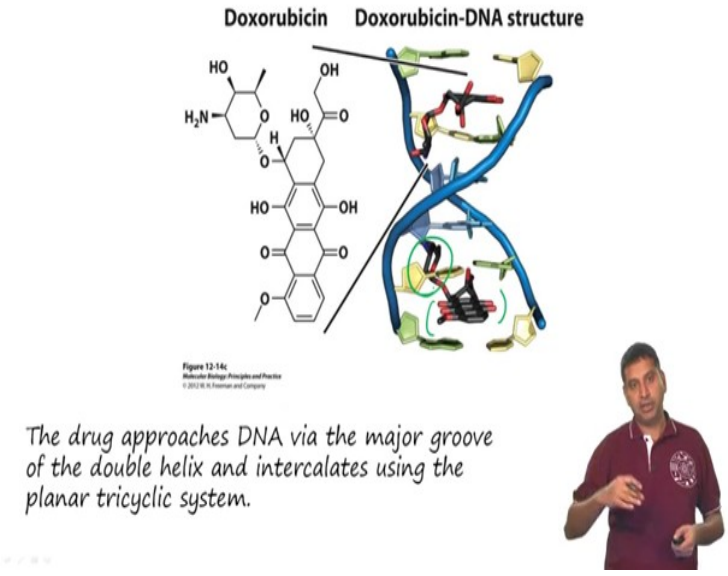

Here is the structure of Doxorubicin approaching DNA and you can see here that this is the structure of Doxorubicin you can see the planar rings over here and this is the sugar over here and it intercalates using the planar tricyclic system.

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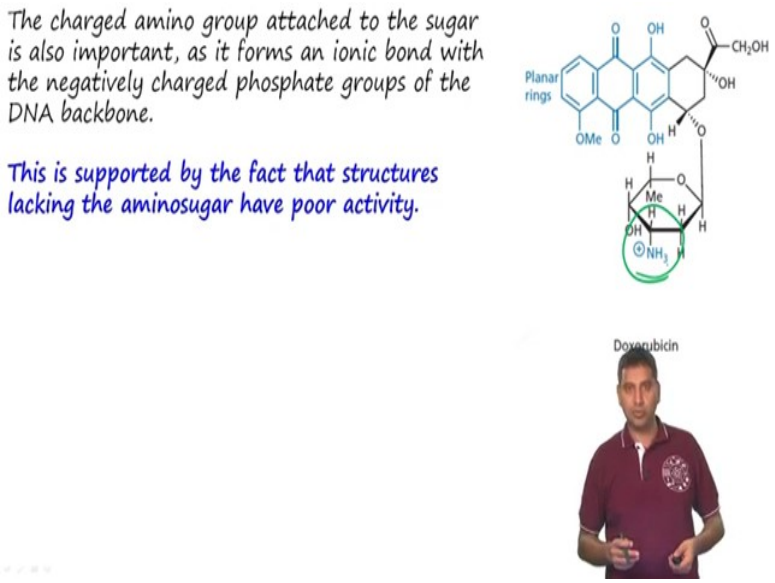

The charged amino group attached to the sugar is also important as it forms an ionic bond with the negatively charged phosphate groups (so) such as shown here. A number of experiments have been conducted to understand the intercalation by Doxorubicin and the data from these experiments support that once you remove the amino sugar the activity of Doxorubicin goes down or the activity of the Doxorubicin analogue goes down. So therefore the amino sugar plays a key role in the activity of Doxorubicin.

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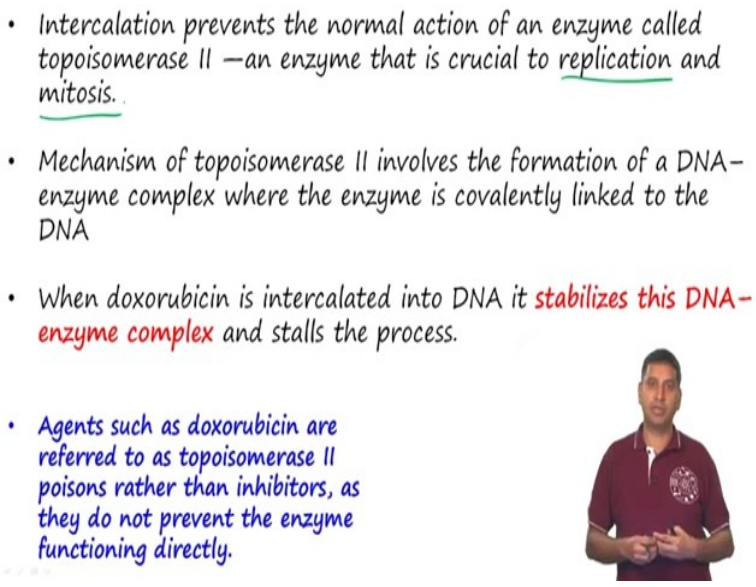

Again intercalation prevents normal action of an enzyme called as topoisomerase 2. This enzyme again as we looked at earlier is an enzyme that is crucial to replication and mitosis. So the mechanism of topoisomerase 2 involves the formation of a DNA-enzyme complex

where the enzyme is covalently linked to DNA. Again recall we have looked at this in one of the previous lectures about how topoisomerase 2 acts.

So when Doxorubicin is intercalated into DNA, it stabilizes this DNA enzyme complex and stalls the process. Agents such as Doxorubicin are referred to as topoisomerase 2 poisons rather than inhibitors, as they do not prevent the enzyme functioning directly, but what they do is that they prevent the by making a stable complex they prevent transcription from occurring.

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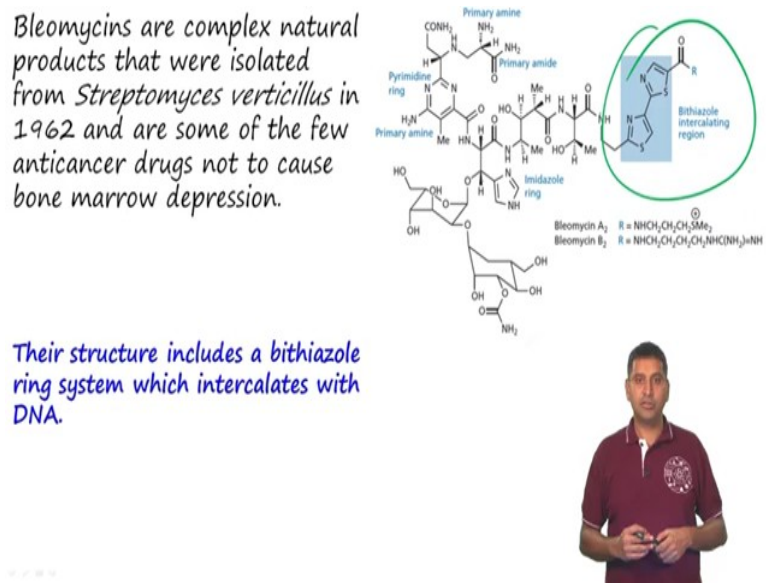

The next class of DNA interacting agents are Bleomycins. Bleomycins as you can see are complex natural products and they were again isolated from Streptomyces species in 1962 and (they are one of the few) they are among the few anticancer drugs that do not cause bone marrow depression. So in many cases because anticancer drugs target actively dividing cells, they cause nonspecific action on the bone marrow and so any drug that prevents or inhibits the growth of cancers but does not affect the bone marrow is very useful. One of the key elements of the structure of Bleomycins is this bisthiazole ring as shown here and this bisthiazole ring is believed to be the major intercalating species.

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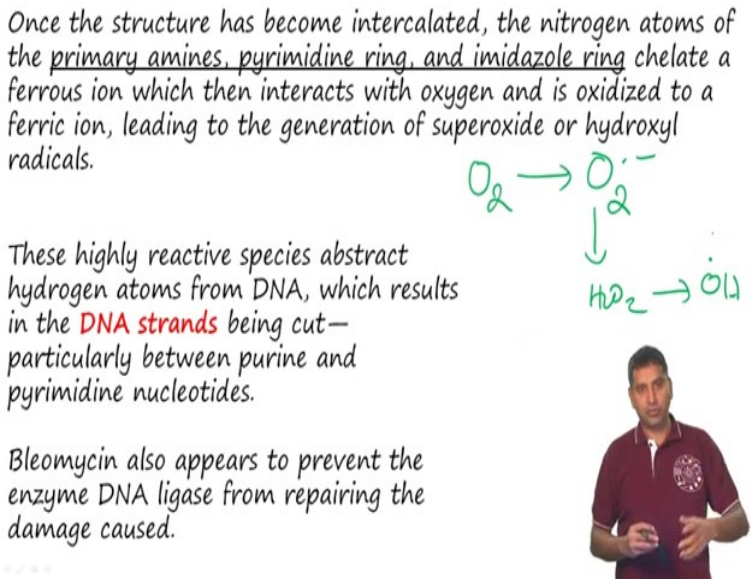

Once the structure becomes intercalated, the nitrogen atoms of the primary amines, the pyrimidine ring, and the imidazole ring chelate an ion that is ferrous ion which then interacts with oxygen and is oxidized to ferric ion. Now during this process oxygen forms, picks up one electron and forms super oxide and super oxide can subsequently get converted to hydrogen peroxide which then eventually forms hydroxyl radical. These together are known as reactive oxygen species and the cell is very efficient at getting rid of this some of this reactive oxygen species.

However, since the generation of reactive oxygen species occurs near the DNA strands, this can result in the DNA strands being cut particular between purine and pyrimidine nucleotides. Bleomycin also appears to prevent the enzyme DNA ligase from repairing the damage caused and whenever there is a damage that occurs on DNA there are number of enzymes that come and help in repairing the damage that is caused.

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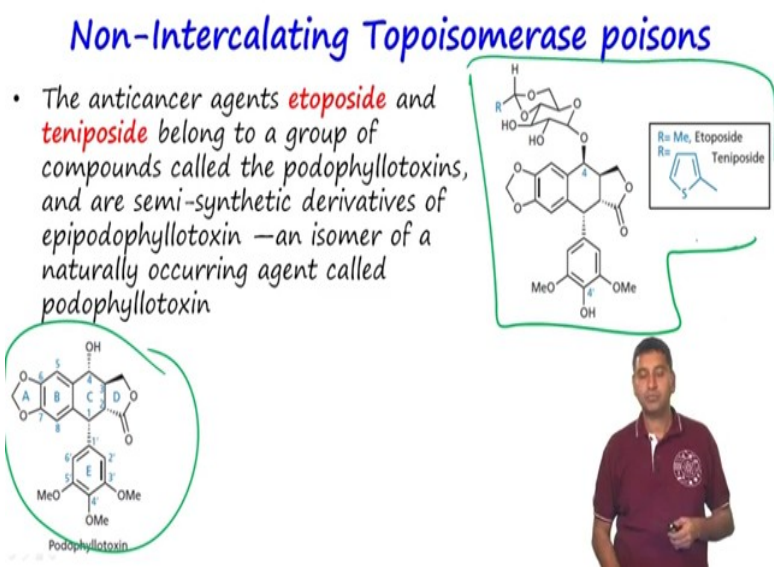

Let us now look at some Non-Intercalating Topoisomerase poisons. The anticancer agent etoposide and teniposide they belong to a class of compounds known as podophyllotoxins. These are semi-synthetic derivatives of epipodophyllotoxin an isomer of the naturally occurring agent called as podophyllotoxin. So here is the structure of the natural isomer and here is the structure of etoposide and teniposide.

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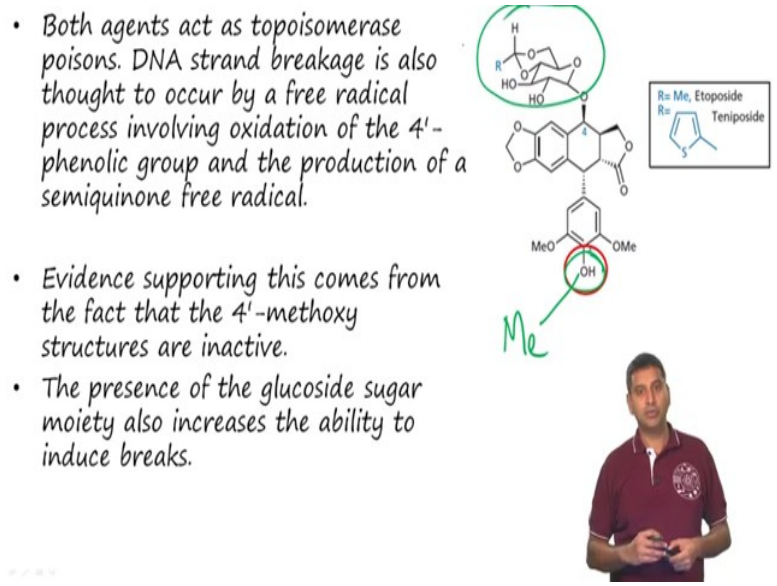

Now both these agents act as topoisomerase poisons. DNA strand breakage by this class of molecules is thought to occur by a free radical process that involves 4 prime phenolic group and what happens or what is proposed to happen is that this 4 prime phenolic group forms a semiquinone radical which then generates again reactive oxygen species. Evidence for this

comes when if you remove this or if you remove the if you are able to derivatize this oxygen into a methyl then the compound becomes inactive. The presence of the glucoside sugar moiety also increases the ability of this molecule to induce breaks.

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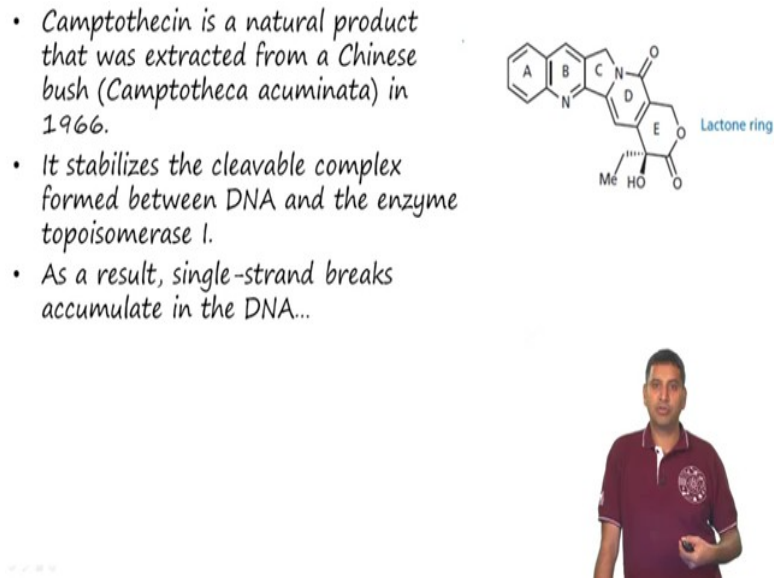

The next class next important molecule that we would look at is Camptothecin. Camptothecin is a natural product that was extracted from the Chinese bush Camptotheca acuminata in 1966. Camptothecin stabilizes the cleavable complex that is formed between DNA and the enzyme topoisomerase 1. So by doing this what happens is that it results in single-strand breaks that accumulate in DNA.

You may remember the topoisomerase 1 and topoisomerase 2 both nick DNA for their function. The difference between topoisomerase 1 and topoisomerase 2 is that topoisomerase 2 cleaves only one strand of DNA, whereas topoisomerase 2 cleaves both the strands and since the complex between DNA and enzyme topoisomerase 1 is stabilized the single-strand break persist and it does not and therefore this accumulates in the DNA resulting in inhibition of proliferation.

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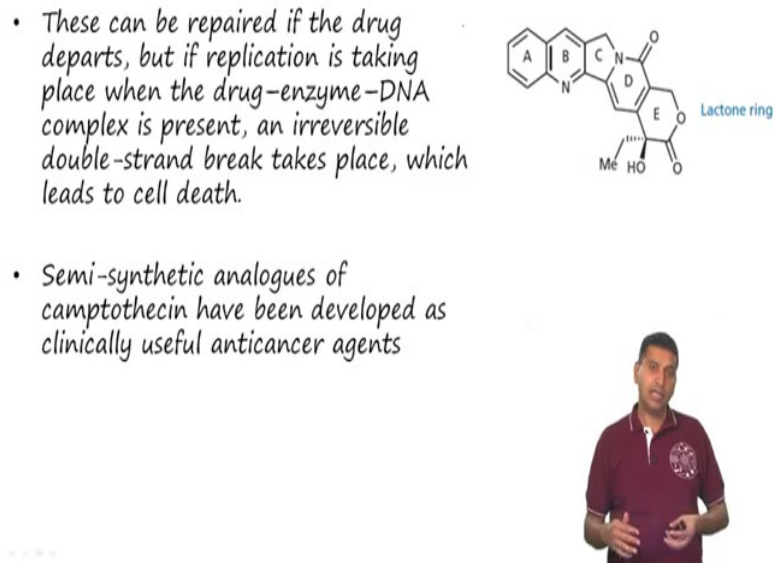

These single-strand breaks can be repaired if the drug departs, but if replication is taking place when the drug enzyme DNA complex is present, an irreversible double-strand break takes place, which then leads to cell death. Semi-synthetic analogues of camptothecin have been developed as clinically useful anticancer agents.

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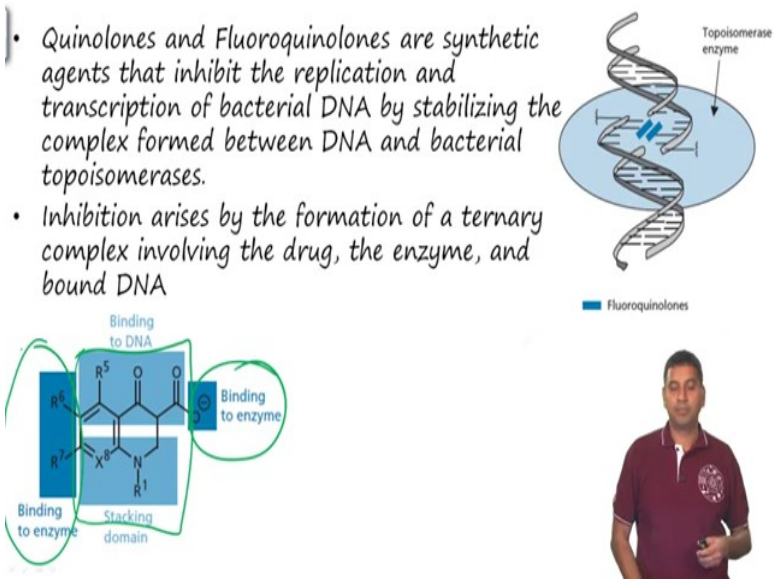

The next class of drugs that interact with DNA are Quinolones and their fluorinated derivatives Fluoroquinolones. They both are synthetic agents and they both inhibit the replication and transcription and they are antibacterial compounds and so they inhibit replication and transcription of bacterial DNA. So what happens is that they stabilize the complex formed between DNA and bacterial topoisomerase.

So once you stabilize this complex as you can recall from example of camptothecin it results in accumulation of breaks. Inhibition arises by the formation of a ternary complex involving the drug, the enzyme and the bound DNA. So here is the structure of the quinolone and what this is the area that binds to DNA and it also stacks it is a stacking domain, this is the functional group that binds to the enzyme and this also binds to the enzyme.

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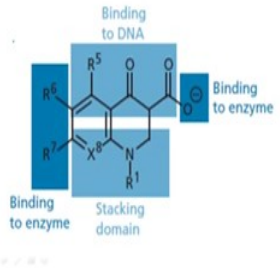
- The binding site for the fluoroquinolones only appears once the enzyme has 'nicked' the DNA strands, and the strands are ready to be crossed over.
- At that point, four fluoroquinolone molecules are bound in a stacking arrangement such that their aromatic rings are coplanar.



The binding site for the fluoroquinolones appears once the enzyme has nicked the DNA strand and the strands are ready to be crossed over. At this point, four fluoroquinolone molecules are bound in a stacking arrangement such that aromatic rings are coplanar.

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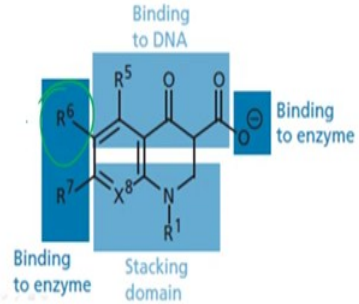
- The carbonyl and carboxylate groups of the fluoroquinolones interact with DNA by hydrogen bonding, while the fluoro-substituent at position-6, the substituent at C-7, and the carboxylate ion are involved in binding interactions with the enzyme...



The carbonyl and the carboxylate groups of fluoroquinolones interact with DNA by hydrogen bonding, while the fluoro substituent over here at position-6 and the substituent at C-7 and the carboxylate are involved in binding interactions with the enzyme together it prevents the enzyme DNA complex.

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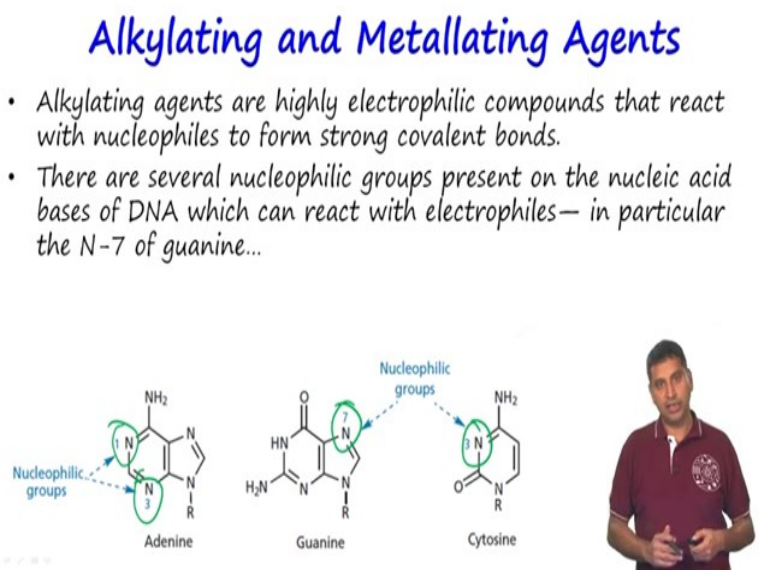

Next class of molecules that interact with nucleic acids are Alkylating and Metallating agents. Alkylating agents as the name suggests are highly electrophilic compounds that react with nuclear files to form strong covalent bonds, we have already studied in lot of details nucleophilic reactions in introductory organic chemistry course, where you have majorly S_N1 and S_N2 type of reactions.

Now (these) there are several nucleophilic groups present on nucleic acid bases. So here are some examples so you have adenine which can it has two of these which are nucleophilic, guanine has this molecule which is quite nucleophilic and cytosine again has this nitrogen which is nucleophilic and now N-7 of guanine for example is highly nucleophilic and it acts as an excellent nucleophile with certain alkylating agents.

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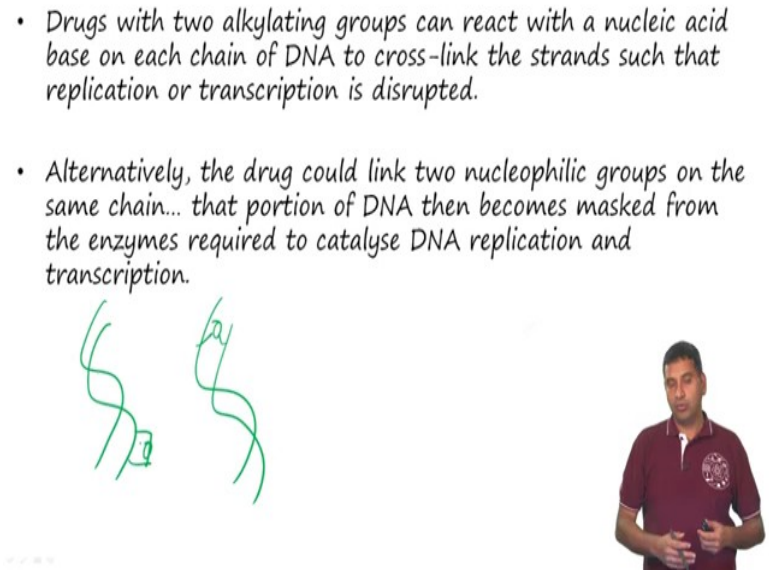

So drugs with two alkylating agents can react with the nucleic acid base on each chain of DNA and what it might do is to actually do what is do to get a cross-link. So if reacts here and then if it reacts here, here is your electrophile and then actually what it does it makes a cross-link. Alternatively the drug could link to two nucleophilic groups of the same chain that the portion of DNA then becomes masked from the enzymes required to catalyse the DNA replication.

So what could happen is you have a DNA strand here, so it alkylates here and then it alkylates here and then what happens is that, that particular portion of the DNA becomes does not participate in replication or transcription.

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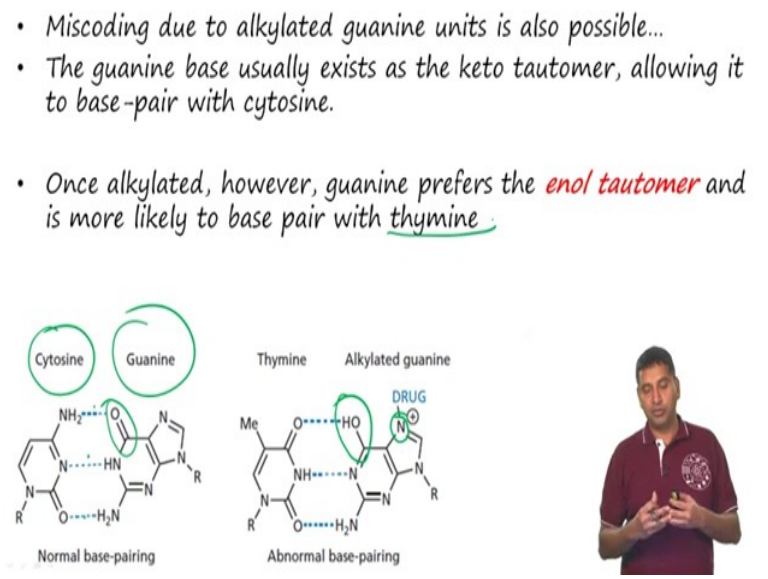

So this might result in what is known as miscoding, so consequence of miscoding is that a slightly different protein is going to be synthesized and this protein may not function the way the normal protein would function and that could again result in stress perhaps in cell death. The guanine base usually exist as a keto tautomer, allowing it to base-pair with cytosine. This we have already looked at previously so when cytosine hydrogen bonds with guanine you have three intermolecular hydrogen bonding that can occur.

But once guanine is alkylated as we saw in the 7th position, then it forms an abnormal base pairing because you have the keto isomer here getting converted to the enol isomer or enol tautomer and so the enol tautomer is actually more likely to bind not with cytosine but with thymine. So this again results in situation which causes stress.

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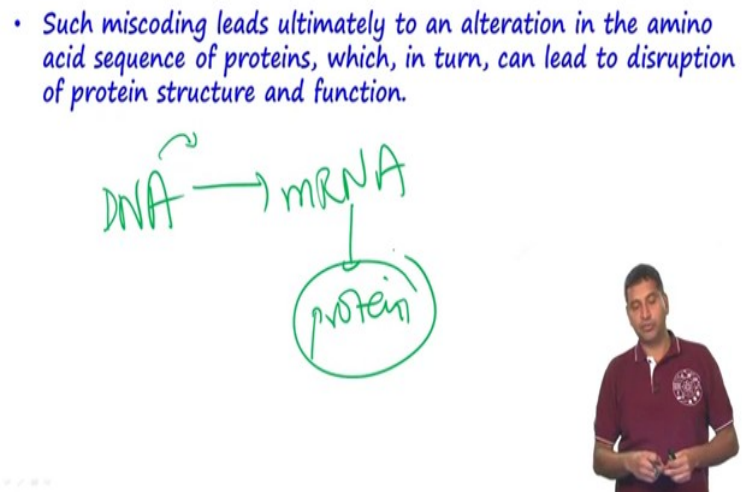

So such miscoding leads ultimately to an alteration in the amino acid sequence of the proteins because DNA is going to give rise to mRNA and mRNA is going to give rise to protein so if the sequence of DNA is normal then a normal mRNA sequence would be produced which is going to produce the normal protein. However, if we have some mismatch here then it is going to result in a protein that is going to be perhaps different in its sequence.

As we have looked at previously the primary structure of proteins are very important in how they fold and the folding is going to determine the function and ofcourse it is also going to determine the structure.

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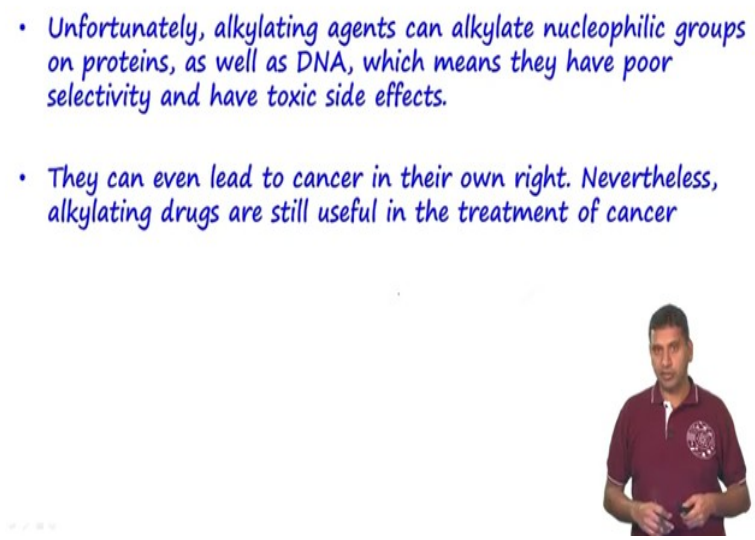

Unfortunately, alkylating agents being such reactive molecules can alkylate nucleophilic groups of proteins, as well as DNA, which means that they have fairly poor selectivity and they have very strong toxic side effects. In some cases they can even lead to cancer in their own right. Nevertheless, alkylating agents are still useful in the treatment of cancer.

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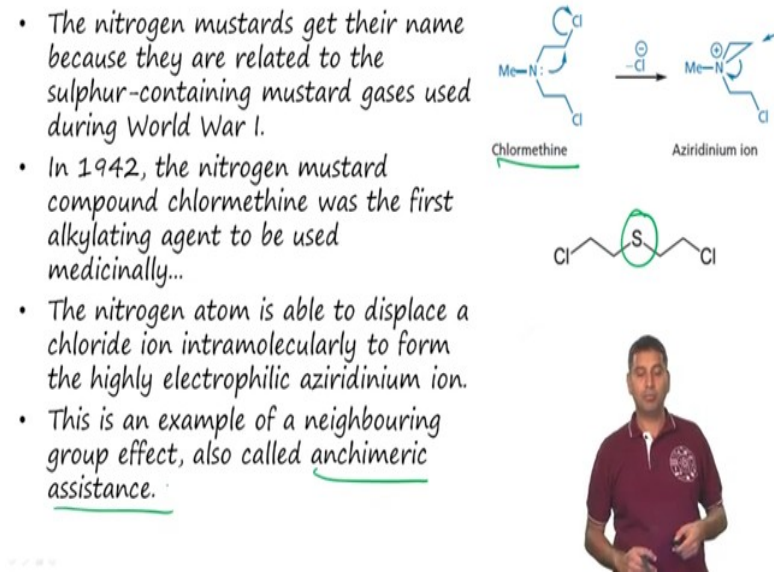

So the first class of alkylating agents that we will look at are nitrogen mustards. So these are related to the sulphur containing mustard gases that were used as weapons in World War 1. In 1942, the nitrogen mustard compound, chlormethine as shown here was first used as a medicinally and it is an alkylating agent and as you can see the nitrogen lone pair can do a intramolecular reaction, kick out chloride and form a aziridinium type of ring. This as we

have looked at previously is an example of anchimeric assistance or neighbouring group participation.

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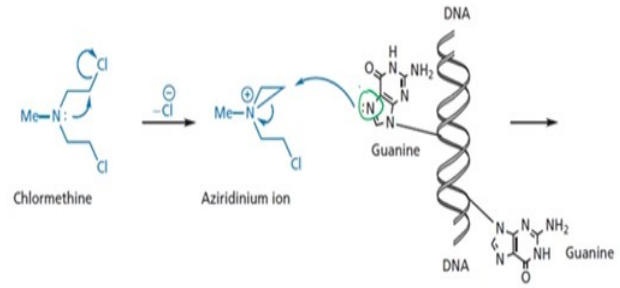

- Alkylation of DNA can then take place...
- As the process can be repeated, cross-linking between chains or within the one chain will occur

So once this highly reactive aziridinium ion is formed, then it is a substrate for attack by guanine for example and once it reacts with guanine it is going to form a covalent bond, formation of this covalent bond is an irreversible process and now the second chloride ion can be displaced from the resulting species and once it is again going to form an aziridinium ion, another molecule of guanine or another nucleophilic base can attack and it can form a cross link.

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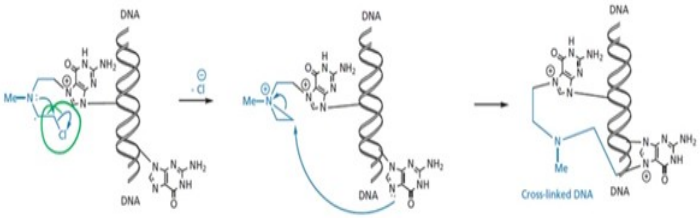

- Some improved versions of these drugs such as cyclophosphamide are now used...

So there are some improved versions of these drugs that are used such as cyclophosphamide and we will look at this later in the course.

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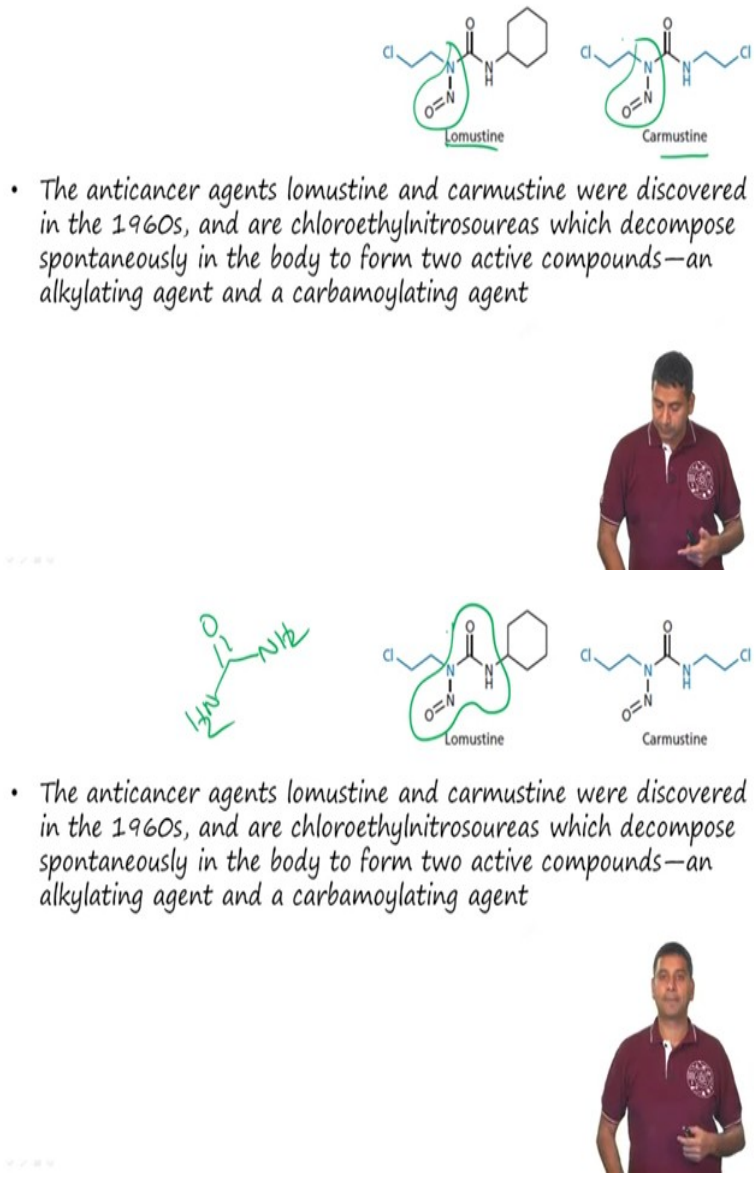

The next class of anticancer agents which interact with DNA are lomustine and carmustine, these were discovered in the 1960's and they are basically nitrosoureas which decompose spontaneously in the body to produce two active compounds, one is an alkylating agent and the other one is a carbamoylating agent. So here is the structure of these two compounds, a nitrosourea is basically this structure, okay urea is this and nitrosourea is here, okay.

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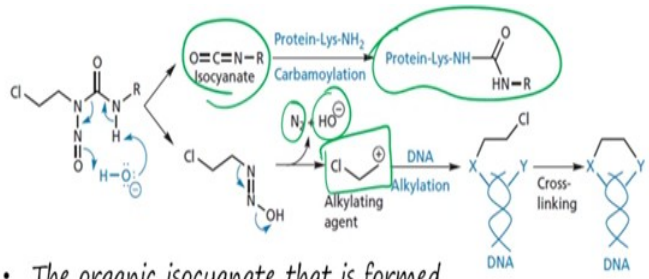

- The organic isocyanate that is formed carbamoylates lysine residues in proteins and may inactivate DNA repair enzymes.
- The alkylating agent reacts initially with a guanine moiety on one strand of DNA, then with a guanine or cytosine unit on the other strand to produce interstrand cross-linking

So let us look at the mechanism, so what happens is that this nitrosourea undergoes decomposition to produce an isocyanate RN double bond C double bond O and isocyanates are known to react with lysine residues of proteins and once it reacts with lysine residues of protein it is an irreversible process and it forms a urea derivative. The other part of the molecule produces this kind of species which can then generate nitrogen and hydroxide ion and produce a highly reactive alkylating agent and this alkylating agent can then go and interact with DNA produce a cross-link.

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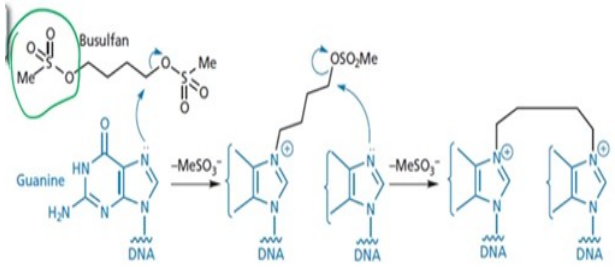
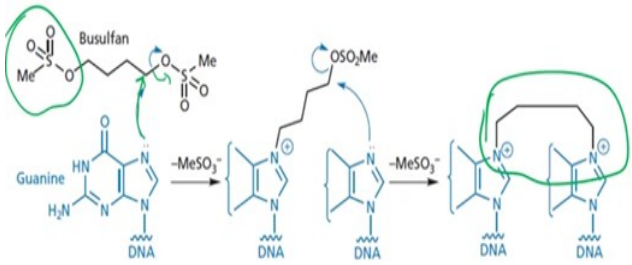


- Busulfan was synthesized in 1950 as part of a systematic search for novel alkylating agents.
- It is an anticancer agent which causes interstrand cross-linking between guanine units.



- Sulfonates are excellent leaving groups...
- However, the mechanism involves a direct S_N2 nucleophilic substitution of the sulfonate groups and does not involve any intermediates such as the aziridinium ion.

The next class or the next example of the alkylating agent is Busulfan. Busulfan was synthesized in 1950 as an effort to systematically search for novel alkylating agents. It is again an anticancer agent which causes interstrand cross-linking between guanine units. Here is the structure of this molecule and as you can see here it has a very good leaving group in the form of sulfonates and sulfonates because of their excellent leaving group ability can react with guanine and once they are kicked out they are going to alkylate guanine.

Then, subsequently another molecule or another nucleobase attacks and it produces a cross-link as shown here, okay this mechanism involves the S_N2 nucleophilic substitution and does not involve any intermediate such as the aziridinium ion.

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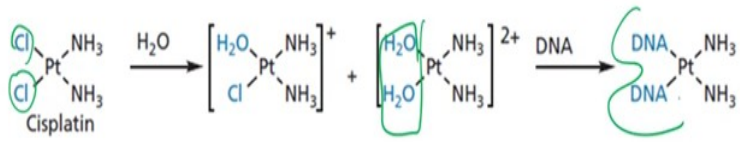

- Cisplatin is one of the most frequently used anticancer drugs in medicine.
- Its discovery was fortuitous in the extreme, arising from research carried out in the 1960s to investigate the effects of an electric current on bacterial growth.
- It is an anticancer agent which causes inter-strand cros[illegible]nking between guanine units...

The next example of an anticancer drug is cisplatin. Cisplatin is one of the most frequently used anticancer drugs in medicine and its discovery was actually accidental and it was they were doing research on investigating the effects of an electric current on bacterial growth and this happened in the 1960's and accidentally it was found to have excellent anticancer activity and again the mechanism here is that the cisplatin structure shown here and it forms the aquated cisplatin derivative which can then interact with DNA to produce a very stable complex which inhibits the growth.

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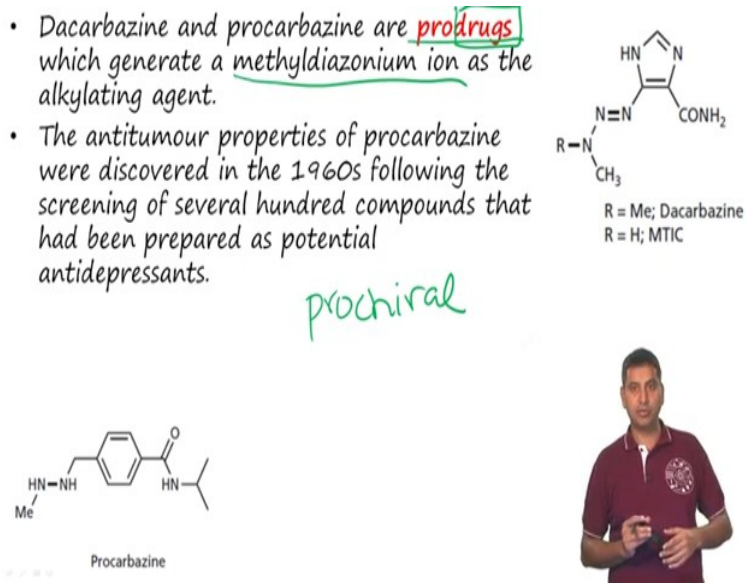

Dacarbazine and procarbazine are prodrugs, we will look at this example now and these two compounds generate a methyldiazonium ion (and) as an alkylating agent. Before we proceed let us understand this term called prodrug. So in organic chemistry we have studied the concept of chirality and any molecule that has a potential group which can become chiral is called as prochiral.

So similarly extending the same analogy we can understand prodrug as a molecule that becomes a drug. So on its own it does not have any activity, but once it gets inside the cell or inside the body it gets there are some transformation that happens and makes the active drug. So the antitumour properties of these compounds were discovered again in the 1960's after screening hundreds of compounds as potential antidepressants.

So here is an example as we looked at in the first couple of lectures where we found that when we look for some drug and we actually come across a molecule which has an entirely different activity is one of the ways in which new drug can be discovered.

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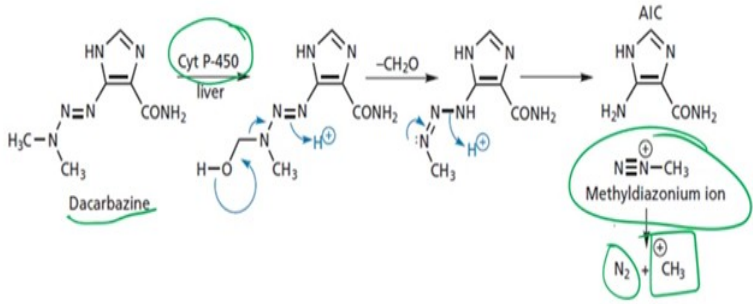

- Reaction of this ion with RNA or DNA results in methylation, mainly at the 7-position of guanine.

So now let us look at the mechanism by which this molecule acts. The major metabolism inside the body happens in the liver and the class of enzymes known as cytochromes P-450 are very crucial for metabolism. So this drug gets metabolized by cytochrome P-450 and it produces this kind of an alcohol intermediate which then rearranges and gives you a methyldiazonium ion and this methyldiazonium ion produces nitrogen and a carbocation which is methyl carbocation.

A methyl carbocation as we have seen is going to be extremely reactive and it reacts with DNA or with RNA and results in methylation and once it get methylated then the DNA is destabilized and then what happens is it inhibits eventually inhibits the proliferation.

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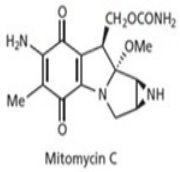
- Mitomycin C was discovered in the 1950s and is a naturally occurring compound obtained from the microorganism *Streptomyces caespitosus*.



- It is one of the most toxic anticancer drugs in clinical use and acts as a prodrug, being converted to an alkylating agent within the body

The next major class of drugs is mitomycin. Mitomycin was discovered in the 1950's and again it is being isolated from the class of microorganism known as Streptomyces. It is one of the most toxic anticancer drugs in clinical use and it is another example of a prodrug. Of course we will look at prodrugs much later in the course and this is an excellent example of a prodrug and what it does is that on its own it is really inactive but what it is converted to an alkylating agent within the body.

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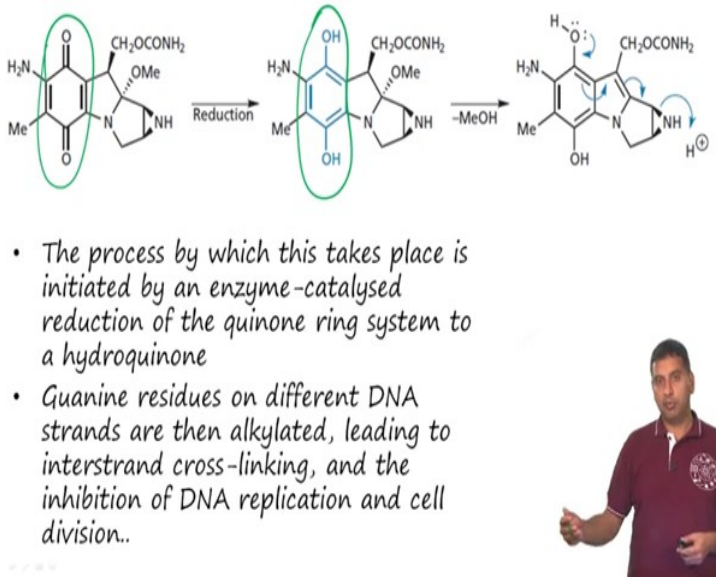

Now the way in which this happens is that there are enzymes which can carry out what is known as a bio reductive process. There are many enzymes inside the body which can do oxidation as well as reduction and these are known as oxidoreductases. So when mitomycin encounters a bio reductive enzyme this Quinone here undergoes reduction to form a Diol and now Quinone is actually an electron withdrawing group we will imagine that carbonyl groups are electron withdrawing, whereas once it forms a Diol, the Diol is fairly electron rich which can then promote this rearrangement as shown here to produce eventually pick up a proton to produce a species which then becomes the substrate for attack by DNA.

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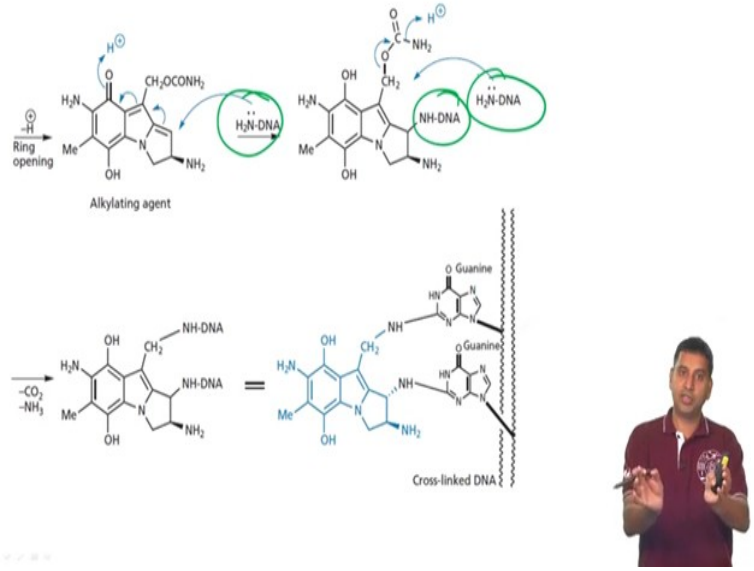

So as you can see here the amine can attack over here and this is like a Michael reaction but is not really a Michael reaction but it is like a Michael reaction and eventually forms this covalent adduct. One more round of loss of carbon di oxide and an amine generates the second site for attack by DNA and this is how mitomycin can cross-link.

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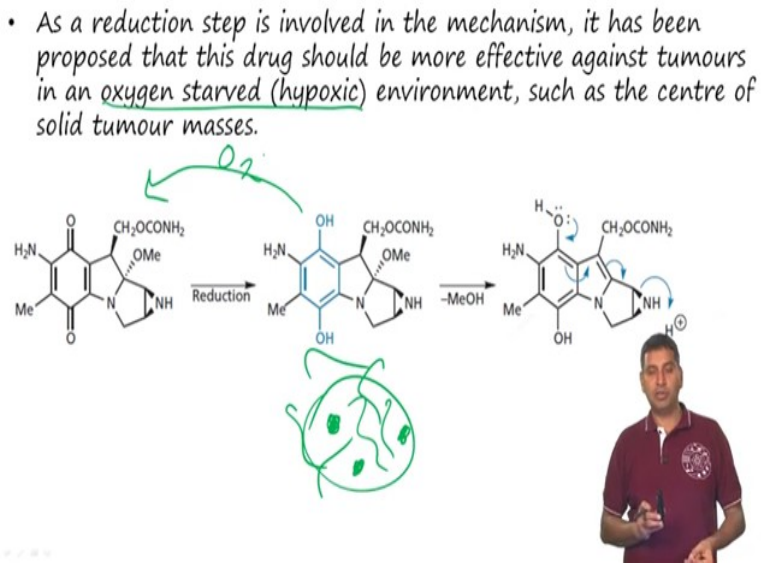

One of the important characteristics of solid tumours is that they have very little oxygen in the centre of it. So what happens is that imagine that this is a tumour and there is a large number of blood vessels that can supply the tumour, but since this is an uncontrolled growth the generation of blood vessels is not uniform. So this results in certain areas being here shown here which have less oxygen because we have less blood supply and.

So therefore when the drug gets into these kinds of hypoxic regions or oxygen starved regions, the bio reduction that happens is going to be somewhat irreversible. Otherwise if oxygen is present it is going to oxidize this back to the original drug. Now what this helps us in understanding is that mitomycin is more effective in areas where there is hypoxia and we will look at later but hypoxic tumours are some of the most difficult to cure because they have less blood supply the drug also gets in much lesser, they respond less to radiation because there is less oxygen and therefore you have a very important problem that can be solved using mitomycin.

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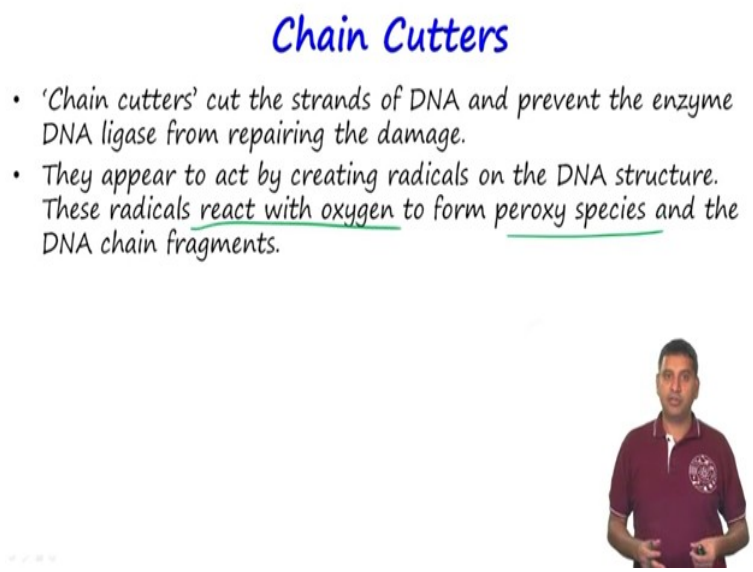

Now let us look at the next example which is chain cutters. Chain cutters as the name suggest cut that strands of DNA and prevent the enzyme DNA ligase from repairing the damage. What they appear to act by creating radicals of the DNA structure and these radicals react with oxygen to form super oxide and peroxy species which then fragments the DNA.