Essentials of Biomolecules:
Nuclic Acids, Peptides and Carbohydrates
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Lecture No. 18
Chemistry behind DNA damage and mutation

Hello everybody and welcome again to the lecture. So, we are discussing module 4, and we have been talking about the DNA damages and DNA lesions that are formed due to exposure of our genomic DNA to the external and internal agents. So, and we have also seen how the lesions are responsible for creating the mutations in the genome which in turn is responsible for uncontrolled cell divisions. We have started with the oxidative DNA lesions.

We have seen the how under oxidative conditions saturated in the presence of free radicals in the inside the cell, how the DNA nucleobases undergo damages undergo different kinds of reactions that produces the DNA lesions such as a 8 Oxo guanine, and 8 Oxo adenine and all this the damages of the pyrimidine, nucleobases also and then we have started working with the UV induced DNA lesions, how ultraviolet radiations are actually destroying the DNA nucleobases and doing the photochemistry.

So, we have started with the thymine-thymine dimer, and we have seen how that produces different kinds of photo chemical reactions. So today let us continue with that and we will start with the cytosine-cytosine dimers.

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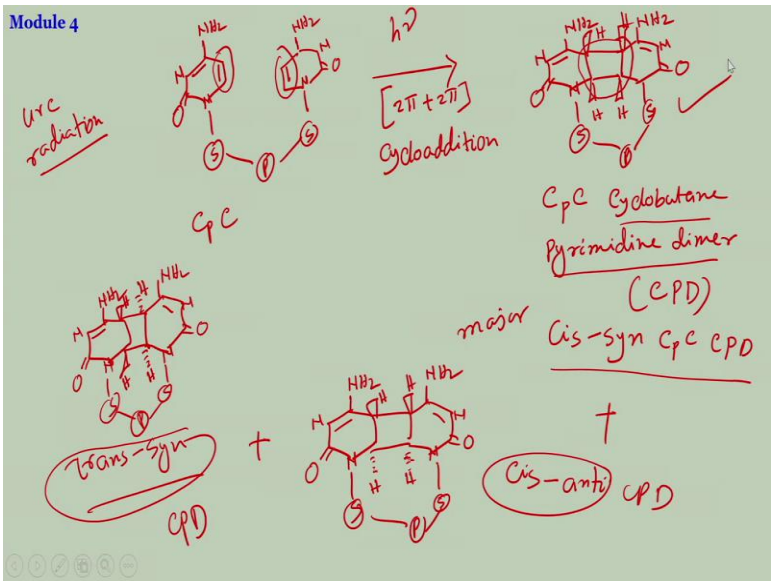

So, this is your cytosine in here is this sugar will take the dinucleotide. So you have the phosphate, sugar and this NH2. So this is our cytosine, phosphate and cytosine to see if it undergoes treatment with UV radiation then what are the photo chemistry that going to happen? h nu and of course, primarily we are talking about the UVC radiation along with UV radiations that are primarily responsible for the photochemical reactions of the DNA nucleobases.

UVC and UVB most of these cases so, first of course, you have the pi 1 here, pi 1 there so, it will undergo 2 pi plus 2 pi cyclo addition and you expect to get I mean NH2. So, this is the cyclo butane ring, this is cyclo butane ring that is formed because of reaction of 2 double bonds and we call it cytosine cytosine cyclo butane pyrimidine dimer in short we usually write as CPD. And now, this compound can have different stereoisomers the major one is of course, the Cis and Syn.

So, this is H, all H are off the plain and therefore, the relation between this and this is Cis, because they are both in the same off the plain. And listen between this and these are also the same also the Cis so, this is termed as Cis and this is Syn. Cis Syn cytosine, CPD that is the major lesion that is formed plus this is up the plain, this can be down the plain. So this is Cis anti and CPD plus this can be trans and syn.

Trans and Syn configuration would be something like this H up the plain they say to down the plain. So, these are trans this is up the plain. So, this is seen and this both have to be syn this is same, so, this is also syn, this is trans. So, this is trans syn. CPD listen of cytosine. Now let us pick up this one and move forward.

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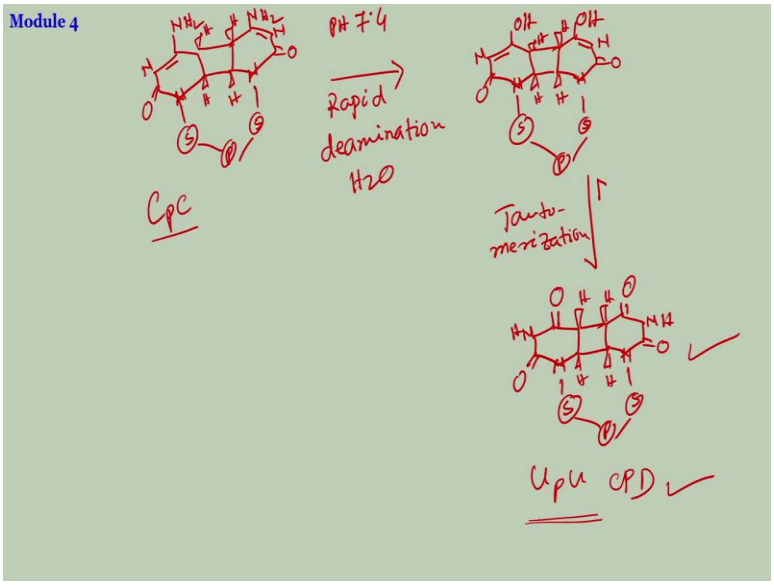

The major one there is something else that are going to happen with that. In NH2 double bond N Syn sugar, phosphate, let us take to Cis and Syn. Now, this one does not stop here because, see, you have the amine groups here and once it loses the aromaticity So, once you form the Cyclo butane pyrimidine dimer, the double bond character is lost and therefore, the compound loses and then this group is kind of isolated the double bond is not delocalized overall to the ring.

So, that makes amine group quite susceptible reaction. So, in physiological condition pH 7.4 this amine undergoes a rapid hydrolysis, we call it deamination. So it reacts basically with water and that will give you OH here, OH there this Syn of course, this process is quite fast process. It happens spontaneously in water. And then to look there, this is basically NOH form so it will undergo Tauto merization to produce ketone, this would be NH. Now is this structure familiar to you?

Can you recognize this? If you look here, so you have started with cytosine, which has thymine in without amine in with just the double bond oxygen if you had the double bond here, this

would have been eurosil. So this is now basically, eurosil, CPD so you have basically come down from a cytosine, cytosine dinucleotide. After the photo reactions, you will finally have come down to listen cyclo butane pyrimidine dimer, not for cytosine, but for eurosil. And in a short while we will see how this is responsible for induction of mutations in the genome.

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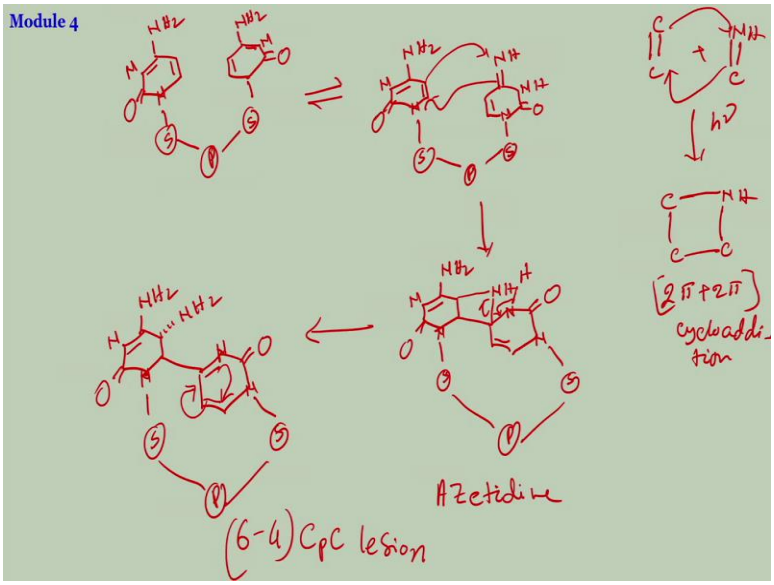

And now cytosine - cytosine, the other photo products. What other kind of photochemistry that might happen? So, this is now this compound you can write in innolate form base amine form this is another tautomer of this form double bond here and now, just like thymine - thymine, this can also undergo Paterno Buchi reaction between if you have a double bond carbon and carbon, in this case, this is carbon heteroatom in this case this is NH under radiation, you can have the cyclo addition reaction, which is known as Paterno Buchi reaction we have talked in the previous lecture.

So, this kind of geometry that will be forming between the carbon and the heterodyned that is why this has a different name. So, this is also 2 plus 2 cyclo addition reaction 2 pi plus 2 pi cyclo addition. So, the same thing can happen here between the these 2 and that will generate in here this I should write the oxetane intermediate first this this is formed and you have the double bond here and this is your double bond oxygen and in this would be N here 1234, 1234.

This would be NH so, this intermediate for the thymine thymine dimer, we have seen that this one was called the oxetane intermediate with the oxygen here, and with the nitrogen here, this is called as azetidine intermediate. And of course, this is not stable. So, this rapidly will break down in this fashion, here you have the hydrogen. So, this will come, this should be out and what you will find this so, this ultimately comes down the plain here is your phosphate 1, 2.

Now, this has become double bond over here that will want you had another double bond here this is so, this is your 6 - 4 cytosine - cytosine lesion or 6 - 4 photo product. And this can further undergo isomerization in presence of radiation, not in the same light but with a little bit of higher wavelength 320 nanometer around. This undergoes isomerization to give you the Dewar relance isomer these double bond come here through the nitrogen of course, and then this was there just isomerization.

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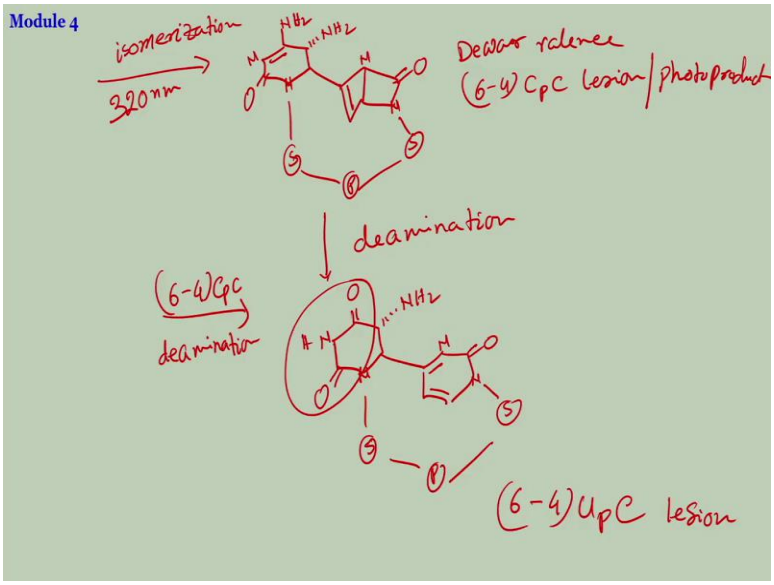

So, this happened under isomerization under 300 around 320 nanometer light NH2 here are producing amine then if I write the deaisomer so this is the deaisomer here you go Dewar ralence 6 – 4 cytosine lesion or you can also call it photo product, the 6 - 4 photo product or these both normal 6 - 4 in this position can undergo rapid deamination as we have seen before and that will produce ultimately 1 eurosil kind of thing.

Of course, this can also undergo, but this is to some extent stable because there is no double bond attached to it I am writing the plain version, it can be the deaisomer also here and here or the dewar and you can get eurosil so then this would be now we can call it if it is 6 - 4, I have written the 6 - 4 eurosil p cytosine basically lesion so, you see how the structure looks very, very good different compared to when you had started with the original dinucleotide. Now, the structure is very much different this spot.

And therefore, it can pair up or when it forms the base pairs it does not always forms the base pairs with then we have started with the cytosine so, initially it was a having the base pairs with guanine. Now, after attending the structure, so much after alteration of the geometry, then and the property of this molecule, then it no longer appears with the guanine it appear some other nucleobase that will see therefore, it will change your genomic sequence.

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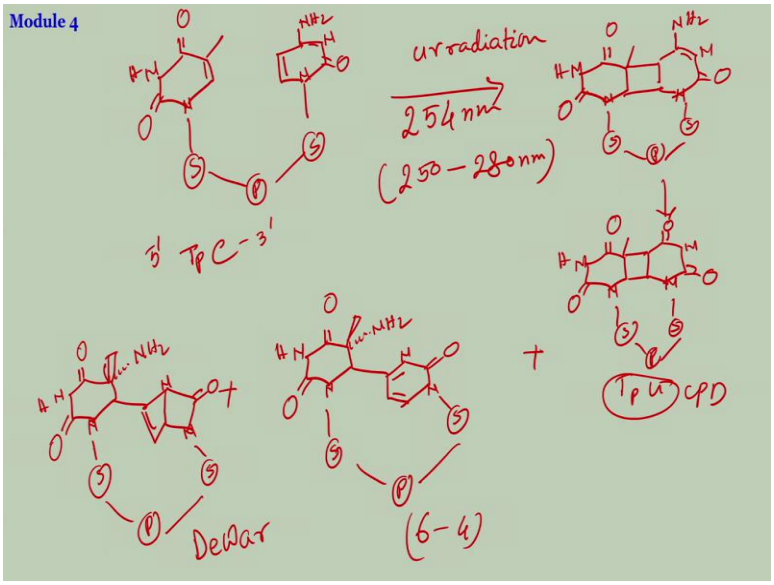

Now, quickly if you take the thymine - cytosine, so, we have said that primarily when you are exposing the DNA to the UV radiation primordial pyrimidine nuclear bases are susceptible for reactions to do the photochemistry that includes thymine - cytosine, and if it is RNA, then it is eurosil. So, it can be thymine - thymine, it can be cytosine - cytosine, or it can be thymine - cytosine or cytosine - thymine.

So if you have the thymine - cytosine then what are the products that will be getting? This is your time in this is your cytosine. So, TPC I am always doing from this or whenever writing this fashion, this means the 5 prime and this is 3 prime. It can be other way around also and you can write the relevant photochemistry or photo products. So UV radiation so when we are doing the UV radiation, if we see radiation, as I have said is the most intense or have the highest impact on the nuclear bases.

And that usually we do it at 254 nanometer it can range of course, from 250 to 280 nanometer. If you had any light in between this, you will see this photochemistry. So let us write only the products so you can have this followed by of course, without do write here. This will be formed initially followed by hydrolysis or the deamination that will eventually give you here there is a thymine. So you get thymine P eurosil TPD, I am not writing the stereochemistry it majorly of course is the Cis Syn.

And then additionally you can have the transit and assist synchrotrons syn anti configurations. So here again from the cytosine you are coming down to eurosil plus 6 - 4 here you will have the methyl group and NH2 this, sugar phosphate and here is this is 6 - 4 there is no question of hydrolysis here and the corresponding Dewar, phosphate and double bond here, this is the Dewar. So these are the photo lesions or the photo products that you can expect if you read irradiate a thymine cytosine dinucleotide for our simplicity.

We have only taken the dinucleotide they are actually part of these are actually part of the DNA. Now let us see how these structures can induce mutations in the genome.

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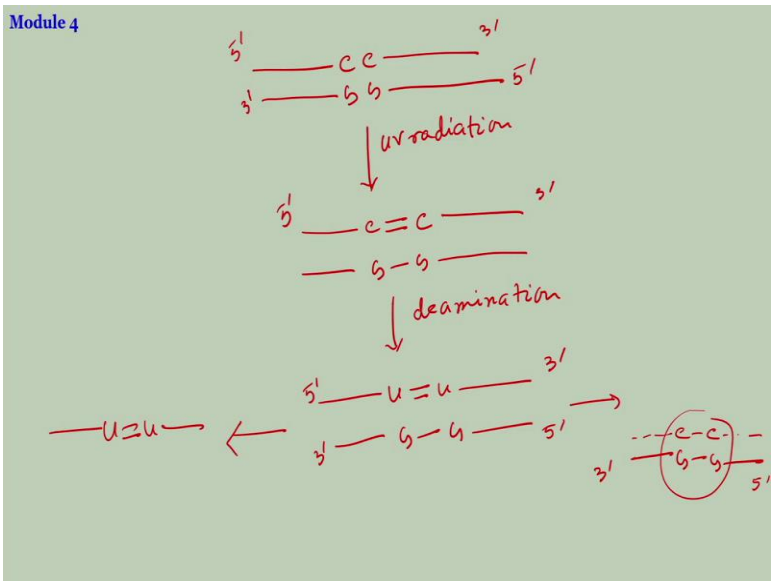

So for example, let us take 5 prime 3 prime, CC sequence, opposite to it has GG that is the normal gene double helix, 3 prime to 5 prime. Now you have irradiated with UV radiation and let us say you are formed so a dimer I am writing is as double bond it forms 2 bonds. 1 is for the single and then you have this is cyclo butane basically you have GG here now; this head undergone deamination and it will give you eurosil that was produced.

And now comes the important point that when you go for the replication process with this so in 1 case this will be divided into 2 strand to the replication of course, but it will be divided into 2 individual single strands even in this should be acting as 1 template and on top of it the new DNA will be synthesized by the set up those enzymes replication. So it will be fine. So, this is fine. You have your original DNA that is being replicated.

Other case you have now eurosil - eurosil as the template. Now using this; your new strand would be synthesized. I will show you later little later, that of course, that this thing would be repaired immediately. But many times they are overlooked by the repair systems and stays like that then what is the problem that is going to happen?

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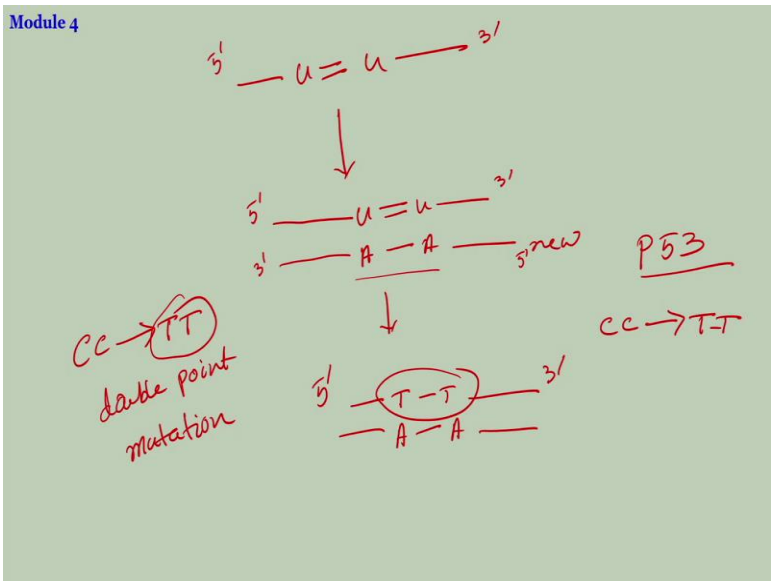

So you have now the eurosil - eurosil and this was in 5 prime to 3 prime orientations. Now eurosil does not exist in DNA in DNA what exist is thymine. So the polymerize systems when this is using this as a template and synthesizing the compliment to the strand. They will initially read these as thymine because it is a pyramidine and they will take off adenine negative state. So the new strand this is the new strand will take adenine in here and then eventually, when this was for further replication, eventually they should be taken as template and they should be replaced adenine-adenine and you have thymine-thymine.

So, eventually this damage will be spotted and will be relieved or it will be executed will be repaired. So, but in the meantime, you have synthesized a template strand, which is a pure DNA strand without any damage here so, that will be taken as a proper template and it will take up time in time in against it and it will go on. So, what do you get here? 5 prime to 3 prime strand thymine - thymine. What did you have originally? Originally you have 5 prime to 3 prime strand you had cytosine-cytosine.

So you are getting a double point mutation. So, cytosine-cytosine into thymine-thymine in double mutation or double point mutation, single mutation is called point mutation. If there is only 1 base that is mutated that is called point mutation, or also known as single nucleotide polymorphism. And this is called double point mutation or simply double mutation. So that is how the DNA lesions dimer, particularly this thymine and cytosine-cytosine and thymine is

responsible for inducing a double mutations, which is a big reason for creating the genomic malfunctions.

So in fact, this has been observed the tumor suppressor gene. There is a name for it. I am one particular gene called P 53 a tumor suppressor gene and in p 53. And this has been observed cytosine - cytosine sequence has been seen to be mutated to thymine - thymine sequence. And that is, of course, is one of the reason for the malfunction of the P 53 gene. And now, let us look at the other lesion 6 – 4 lesions.

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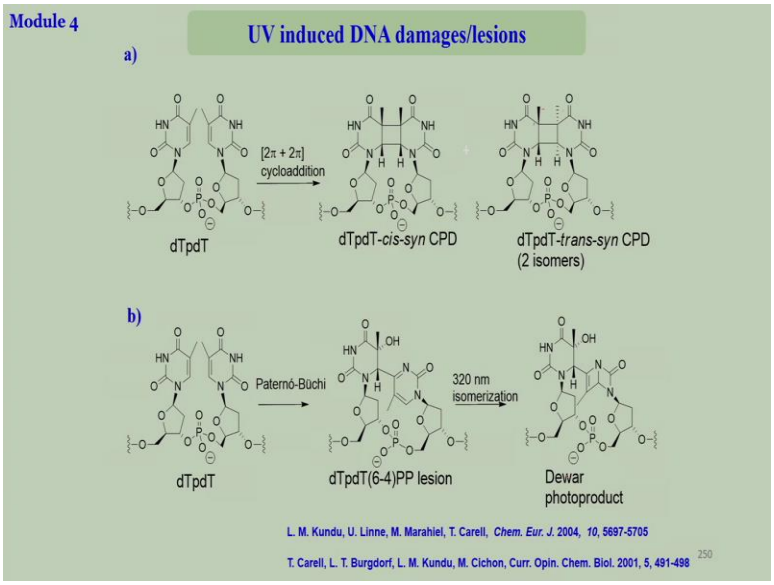

So, this is how the dimers are formed or the photochemistry goes on 2 plus 2 cyclo addition, this is thymine dT, I have written pdT also because to specify the deoxyribose and then 2 plus 2 cyclo addition reaction, it undergoes since CPD you can see here and then trans syn this is trans syn it can have 2 isomers then, you can have the better nuclear reactions between this double bond. That is producing a 6 - 4 PP lesion photo product.

6 - 4 PP lesion here that we have already seen and upon further isomerization under 320 nanometer radiation, it will produce the Dewra ralence product or Dewar photo product.

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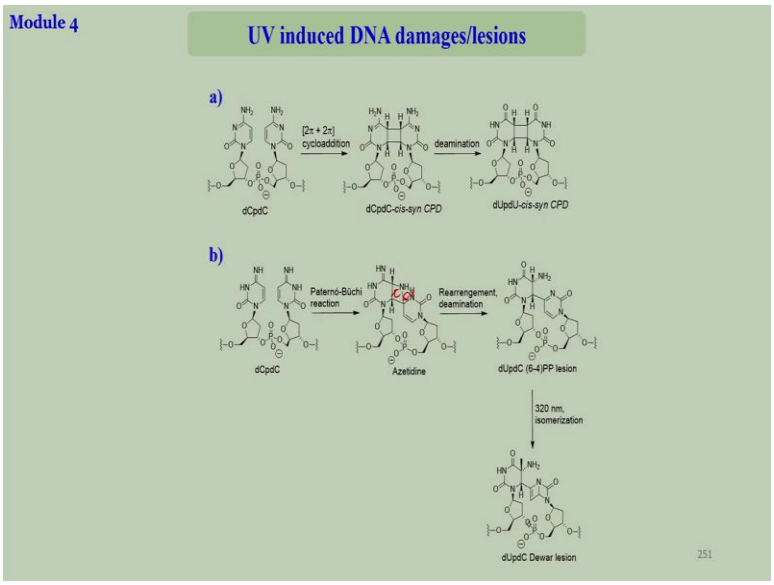

Similarly, if you have the Cis - Cis dinucleotide it will produce them majorly Cis Syn and then the deamination will occur to produce the Cis Syn DPD reaction as it intermediate this rearrangement followed by the deamination the rearrangement is this as I have shown this will come here it will form the double bond and then it will break this carbon nitrogen bond. You will get the NH2 here. 6 – 4, equalization and the deamination over here. So you have now isomerization that will give you the de isomerization.

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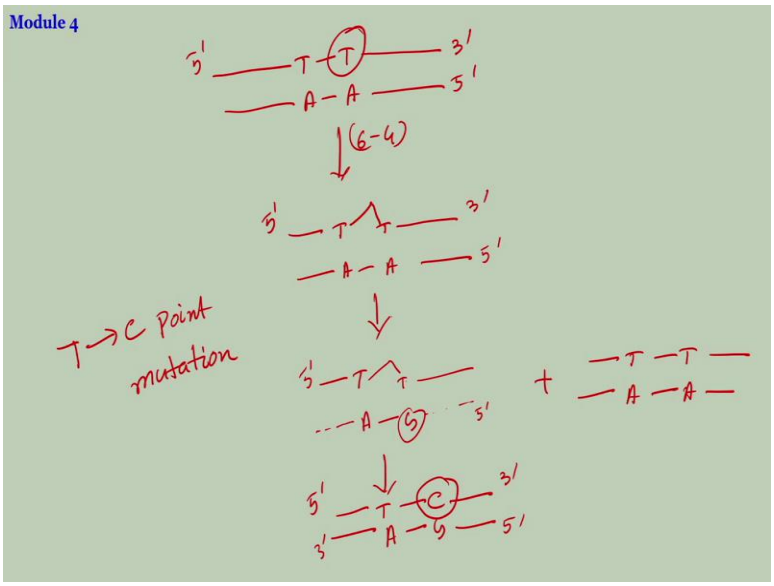

So let us continue with the mutation, 5 prime, 3 prime, this is your 5 prime. Now 6 - 4 photo product. I am writing this fashion this is this is 6 - 4 PP lesion. You have a now it has been observed that once you form the 6 - 4 photo product, if you look back at the structures here, this

is the thymine-thymine and 6 - 4 photo product. You see that this is this new structure that has been generated and this can pair up with a guanine.

So, basically when the prime strand is undergoing replication, then they should take this as a new strand plus, when this strand is acting as the replication, it will give you the original DNA. So, you get your gene intact if this is your template. On the other hand, if this is your template, then you were having a G of was T to the time and then as I said that, over time this should be gone away, but this will remain so, you have the G and here d here ultimately a C will appear up.

So, you had the time in originally, now you are getting cytosine in here. So, its thymine to cytosine point mutation that happens and of course, majority of them will repeat are back. So, these are the process, how the chemistry is involved or how you can understand the cause of mutations in the genomic DNA, when they are exposed to either oxidative condition or the UV radiation.

So, the structural parameters are the structural features play a major role in determining the mutations in the genome. Now let us talk about the repaired how the cell is repairing or what are the processes involved inside the cell that repairs the lesions or that repairs the DNA damages.

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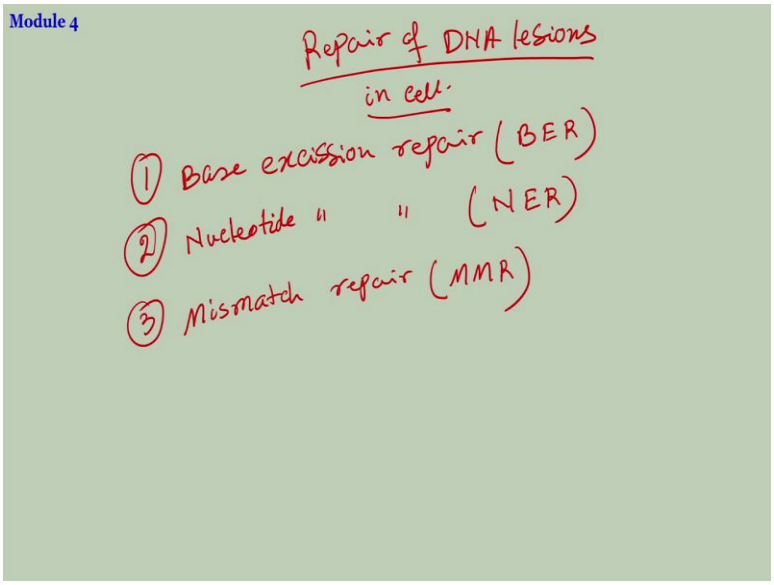

Repair of DNA lesions in cell so as I am mentioning again and again our cellular system is very well protected and we have really good mechanism that will find out immediately if there is a damage that is formed. And that we called there is a system called proofreading system. And then of course for operating systems will give the signals that there is some problem over here and then the repair mechanism will go there and do the repair.

So, there are three mainly 3 kinds of repair processes that go on in the cell. One is called base excision repair. So I am talking about both eukaryotes and prokaryotes. Base excision repair in short, this is known as BER. Second process is nucleotide excision repair. In short, this is called NER and the third one is mismatch repair. In short mismatch repair MMR. So we will briefly see how these processes act.

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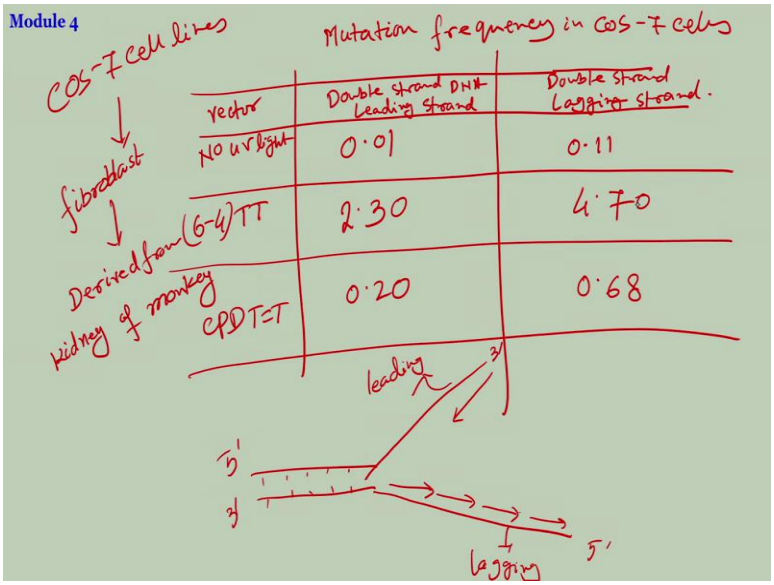

Before that I will give you a statistics that how the mutation frequencies are actually happening in cell lines. I will give you a table vector double strand DNA and this is your leading strand, double strand and lagging strand. So, the data I have is for 1 particular type of cells known as costs seven cells or we call it cell lines. This is basically a cell that has been isolated from the fibroblasts of this is kind of fibroblasts cell.

And this is been derived from kidney of Monkey that is in general at the cell lines are fibroblasts cell lines that has been obtained from the kidney of monkey 7 particularly is obtained from the

kidney of on particular kind of monkey that is in South African monkey. So, this is the cell line and with that cell line people have studied that the frequency of mutations that are occurring within the within the cell lines.

So, if you have, so, that is why I have divided into 2, 1 for the lagging strand mutation that can happen from the lagging strand using that as a template or using the leading strand as the template. So, if you have without radiation, no radiation, no UV radiation, no UV light which means the normal DNA the normal DNA. When it undergoes replication inside the cell, what is the probability of mutation so this table is for mutation frequency?

In core cell lines, see yourselves to when the when you have the normal DNA, and it undergoes replication, then also there are some mistakes that happen, but very less, the mutation frequency of the leading strand is really negligible 0.01. Frequency means how many number of mistakes or how many number of mutations happening per unit time, maybe per second. Really, really negligible, almost nothing and if the lagging strand is used as the template, then it is slightly higher 0.11 that is the mutation frequency.

Now, if you induce 6 – 4 for thymine-thymine listen in the cell line in the DNA of the of the cells, then for the leading strand your mutation frequency becomes 2.3 quite high from almost a good probability or good amount of mutations that are occurring because of the 64 TT lesion. If the leading strand is acting as the template, if the lagging strand is acting as the template then it is quite high 4.70 so, high degree of mutations observed when the lagging strand is acting as the template in which you have introduced a 6 - 4 TT lesion if it is CPD thymine-thymine.

Then is less leggings leading strand is 0.2, lagging strand is slightly high 0.6, 0.8 but still very quiet less. So, CBD lesions are not that much mutagenic although they are formed in major quantity in the DNA when you expose it to light, but they are less mutagenic. On the other hand 6 – 4 photo lesions and the Dewar relence leisons. They are formed in minute quantity, their yield of formation is quite list, but their degree of mutation is very, high.

So even a slight amount of formation of the 6 - 4 lesions can damage the DNA can cause a huge amount of mutation in the genome and that will change the function of the genome. So, this is basically if you take, what I have talked about is that is this you have formed our application for 5 prime to 3 prime, and he was synthesizing the complimentary. So this is your leading strand as the template and you are synthesizing the compliment and the strength, then these are the mistakes that might happen.

This is the frequency of the mutations in the strand that has been occurred. And similarly, this is 3 prime to 5 prime and so this is your lagging strand. And you are synthesizing in a discreet fashion. Then, what are the mutation frequencies in this newly developed strand? Are basically these here, I think we can discuss about the process of the rapier in the next lecture? Thank you.