Essentials of Biomolecules:
Nuclic Acids, Peptides and Carbohydrates
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Lecture No. 17
Chemistry behind DNA damage and mutation

Hello, everybody, and welcome back. So today we will start the module 4, which is about DNA damage, mutations and cancer. So, it is now very clear here that the cancer cells or the growth of the tumor cells are because of the induction of genomic mutations which means that injection of mutations in the genome. And those mutations are mostly responsible for developing tumors or cancers. Now, the question is, why mutations, why the mutations will occur at all in DNA.

So here, we will see how DNA damage occurs, and how the damaged DNA plays roles in developing mutations in the genome. So the cause of mutation is actually the alterations of the structures of the DNA. More specifically, most of the time is the nucleobases, DNA nucleobases undergoes structural changes that induces the mutations in the genome, which in turn is the reason for cancer.

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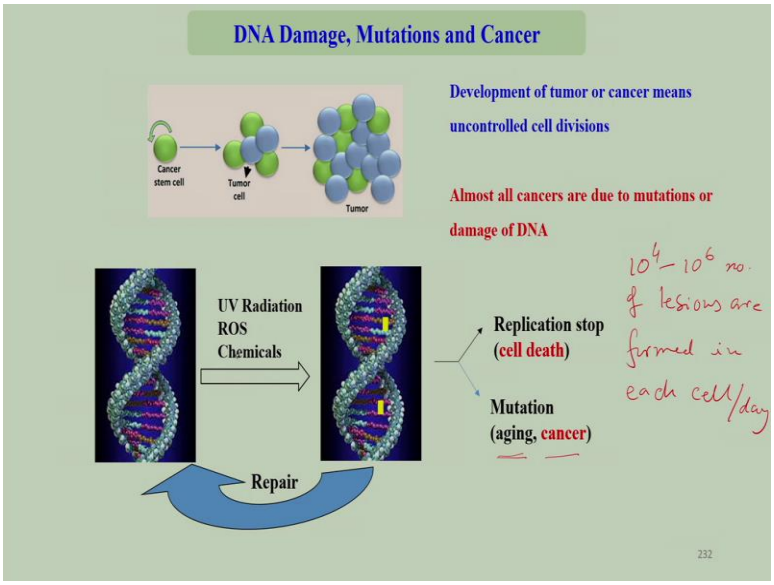

Here this is what is the excessive when we talk about cancer or tumor that means the uncontrolled cell divisions, this slide I have shown before already. So, tumors or cancer means the uncontrolled cell divisions, almost all cancers are due to mutations or damage of DNA. So, if this is a DNA and inside the cellular system inside the cell DNA, it interacts with many chemicals with it is exposed to several factors, such as the UV radiation, ROS it means the reactive oxygen species that are generated inside the cells, other chemicals, all those things, they lead to the lesions are called the damages in the nucleic acid structures to the nucleobases.

And therefore, the structure of the nucleobases or the chemical nature of the nucleobases has got changed. Now, what happens force we have very good repair system repair mechanism is really sound those lesions are those damages are usually repaired back to the original DNA by our repair system, but sometimes, or many times it does not happen. So, if the lesions are not repaired back to the original DNA, then it can lead to the several problematic consequences.

One is, of course, it very often stops the replication process, which is called the cell death, the cell will be death up to some time, because there will be no further replication or it can induce mutation in the genome that causes either aging or that is also the primary cause of cancer. So one statistics is that around into the ten to power of 4 to the ten to the power of 6 number of lesions are formed in each cell per day, every day, around a million of such kind of damages all the lesions are formed in the DNA in every cell every day.

So, you can imagine the total number of lesions that are forming that we are experiencing every now and then. But we are still alive, which means most of these damages are actually repaired back immediately to the original DNA and that functions properly, but many times inside tumor cells inside cancer cells that does not happen. So prime factors that lead to the damages are UV radiation, reactive oxygen species in short we call it ROS or the chemicals.

Now, the question is why the mutations will happen if even if there is damage? Why would mutations happen? So, this is the question will unsure in this whole module actually. So, I start with oxidative DNA damages or oxidative lesions. Oxidative lesions means when the DNA is undergoing chemical reactions under oxidative conditions.

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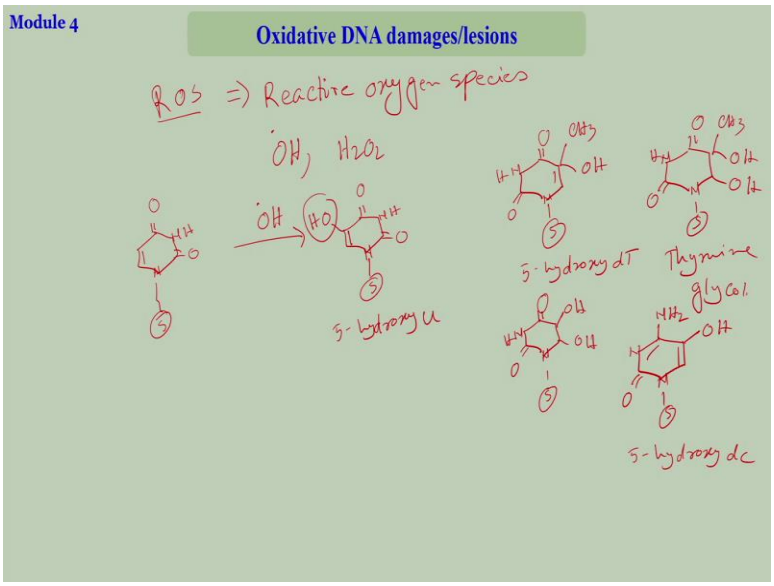

So, here from comes mostly the ROS are always reactive oxygen species. So, what happens inside ourselves lot of radicals, lot of peroxides our form every now and then because during the metabolic process and all this stuffs a lot of radicals are generated they are called the reactive oxygen species such as hydroxyl radicals, it can peroxides are the radicals of the peroxides like that. And of course, you can understand when the radicals are formed they are highly reactive, that is why it is called the reactive oxygen species the highly reactive.

So, they will interact with something and DNA is very susceptible to reactions because it has multiple double bonds, it has functional groups. So, it undergoes reactions with these reactive oxygen species. I will draw some of the structures that are actually formed in DNA upon the oxidations. So, if you have here is the sugar. If you have eurosil, not under oxidative condition, they perform multiple number of damages actually.

So we call them either damages or lesions. If I write a single sugar just as for sugar, un hydroxyl group is appearing here, this is called 5 hydroxy. If this is deoxyribose sugar, or if this is eurosil is in our DNA. So this is 5 hydroxy eurosil. Similarly, thymine forms this is stage 3 which it forms 5 hydroxy thymine dt fall or it also forms double bond is out all this is known as thymine glycol. Because this is CH2 unit glycol unit, that is why this is called thymine glycol.

Similarly, eurosil glycol is there this is eurosil glycol, cytosine, I am drawing for the pyrimidine basis first then I will go to the purine this is 5 hydroxy cytosine. So these are some of the structures of the purine, pyrimidine nucleobases that are formed due to reaction with the ROS species. They are actually been isolated quite well study they are formed in the body.

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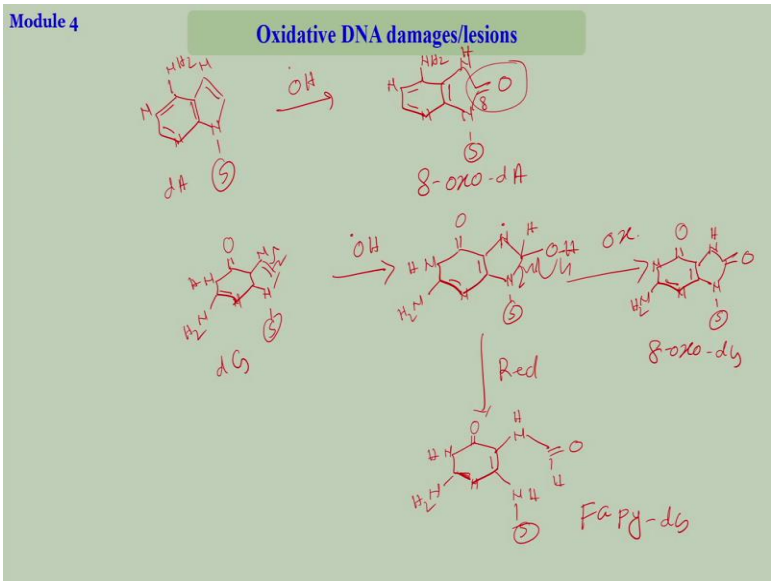

Similarly, if you look at the purine nucleobases this is your adenine. If it is deoxyribose, then it reacts with hydroxyl radicals and produces here this produces our double bonded oxygen over here and this is eighth position. If you do the numbering you will find that this is the eighth position. This is known as 8 oxo because it is oxo group deoxy adenine. Similarly, one of the highly notorious oxidative damage for warning is I will start with the warning.

So, this is deoxyribose guanine radical in so, if this is cleaved and this is a radical which will react basically and this is electron deficient center. So, you can, it will provely have this is the most susceptible carbon this these upon one electron oxidation will produce this double bond here would be NH and this is called Oxo guanine. I think you have already seen the structure of this one we have we have been doing the synthesis of the nucleobases.

And this is one of the most notorious that is formed in the genome. So, this is upon the oxidations of the di-deoxy guanine. From here, if you do one electron reduction, then it is basically has NH this will be reduced. So, you can think of kind of cleaving this homiletically

and living this homiletically also. So, this one gets clipped homiletically so, 1 NH dot would be eliminated that H dot will be here so, it will be NH.

And since this is going to be the double bond, something else has to cleave it will be this. So, what you will have NH and here you will have the NH with the sugar this this component. This is called this is a name these kinds of lesions are usually called fable lesions this is fable deoxy guanosine. So, these are the some of the oxidative DNA damages that happen because of the exposure of the genomic DNA with the ROS with the reactive oxygen species that are formed in good quantity during metabolic process. So, we are seeing that the basis will react it will produce the change in the structure. So, question is how come it is related they are related to the mutations. So, here it is.

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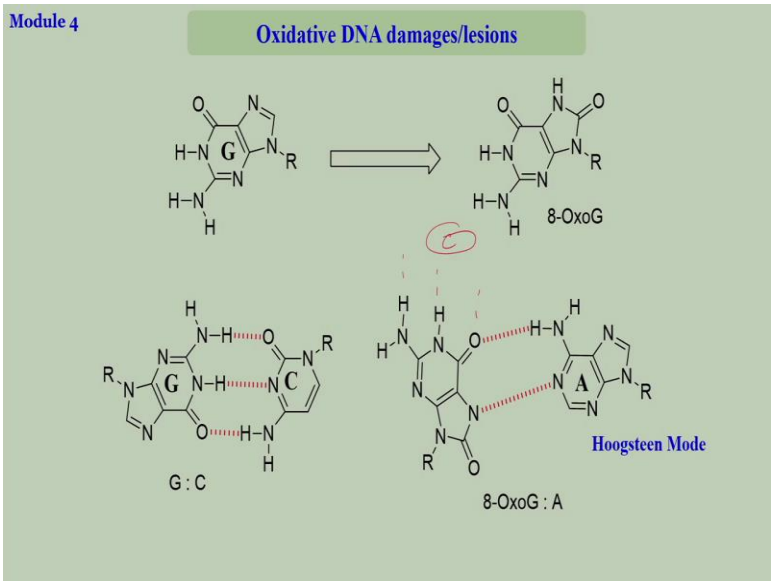

So, from guanine you get the 8 Oxo guanine this is the structure. Now, when you have the G that we are with cytosine we know there are 3 hydrogen bonding and all hydrogen bonds are off the creek nature. Now, when a 8 Oxo bond is forming, this is the structure of the 8 Oxo bonding because, so what you are forming 1 oxo group here, additional double bonded and now what happens it changes the orientation of the nucleobase 2 things can happen.

One is 8 OxoG still pairs of OL with the cytosine counterpart just like this 123 to this end as usual with cytosine that is fair, then there is no problem or this actually can form an alternative

based with adenine, because of this structural change, sometimes even this can participate. So, if it were with adenine, then you have 2 hydrogen bonds in this fashion. And you see this is hosting base because both 6 membered ring and the 5 membered ring are involved. So, this is Oxetine base spearing on behalf of the 8 Oxo guanine and now if the 1 in 8 OXoG and adenine spear of, then we have the problem how?

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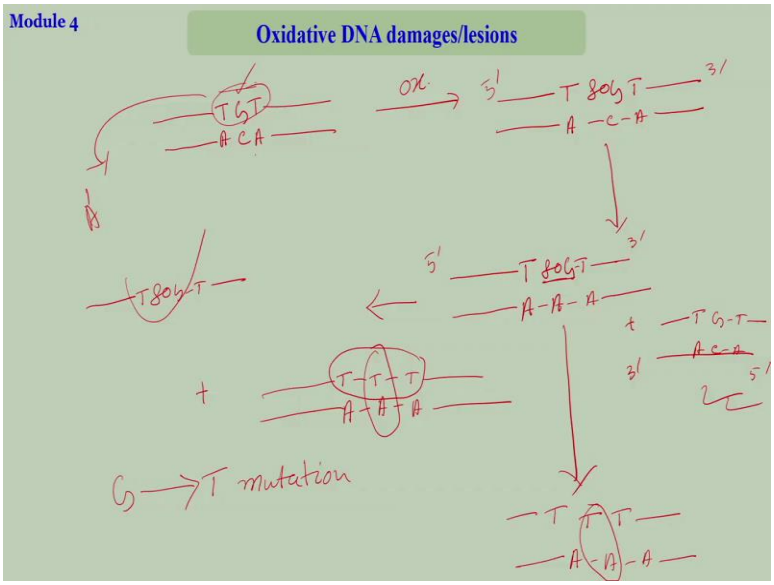

Let us say start with 3 membered maybe T here let say this is your DNA with the G here double stranded DNA in the gene. Now it undergoes oxidation and this forms T now I call AOG instead of G and other T, this you still have your ACA in a counter strand. Now what happens of course, as I have said most of the cases, our repair system will find out that there is some abnormality over this DNA strand, and it will repair it.

Now, if not, let us say it does not get repaired, then what are the consequences? So when for the solution happens, then this would be used as 1 of the primary strand 1 of the templates strand, if this is let us say this is 5 prime to 3 prime. So this would be used as 1 template and the 3 prime to 5 prime with ACA would be used as the other template. During replication or during cell division, so, now, if this is acting as the template, the synthesis of the new DNA strand will be GT this and you get the original gene back, no problem there.

Now here if this is a template, when this is going to synthesize the counter strand, this will be a now since there is 8 Oxo warning, if it pairs up with adenine, it will pick up another adenine over there. And then this so, here is all right. Now we will see when this goes further for the cell division T8OGT plus this is AAA. So, when this strand, this is a normal strand without any damage sites over there. So, the repair system would not be able to see, it will take this as a perfect strand perfect template and it will synthesize the counter strand on it TTT.

Now, you see, this word has got changed now, your original DNA was GC sequence here it has become TA sequence and this will continue repair mechanism will not be able to find out because this is very normal. This is all known to the cells all the nuclear bases are known to the cells. Now over time whatever this at some of the other time this should be detected by the repressed system and will to be chopped off and it will take the correct stand opposite to it.

So, this will be repair at some point even if the repair happens from here, let us say it is the same thing it got clipped off, it will make a space here T and in that case, it will act this as the template and oppose it to A, another T will be picked up. So, even when this is gone, chances is high that 1 T you will be picked up in place of a toxic warning. So, in both cases, you are actually getting a gene which is different from the original. In other words, you are getting a mutation from G to T.

So this is called originally you had the G in place of the G now we have the G here. So this is usually this is how we write G to D mutation, it is a single point mutations, single base mutation. So your gene is now changed and that is a problem. In the next module, we will see, of course, that how the proteins are synthesized using the gene as its coding unit. So, maybe you all know that a 3 letter alphabet or 3 alphabet nucleotide sequence, codes for 1 amino acid.

So when protein synthesis happens, these nuclear bases in a pair of 3, they actually code for a single amino acid and that is how our protein is synthesized, predetermined sequence. Now, if see, let us say TGT codes for 1 amino acid, which is maybe A prime talking about A prime is 1 kind of abundance it the gene has been transformed into TTT. Now, this sequence may not code

for the same amino acid, it may code for a different amino acid, if it does, then your protein got changed and it may not function.

That is where the problem is that is why the mutations are so notorious, sometimes not all the times of course, there are so many mutations going on. So, if the mutations that happen in the important region of the gene, they are susceptible to alter cellular functions and many times induces uncontrolled cell divisions. So, that is how the DNA lesions are related to mutations. So, specifically, we have to know about the oxidative DNA lesions. Now I will come back to another kind of problem that leads to mutations and that leads to huge lesions formation, he was damaged of the DNA, this is that UV radiation effect.

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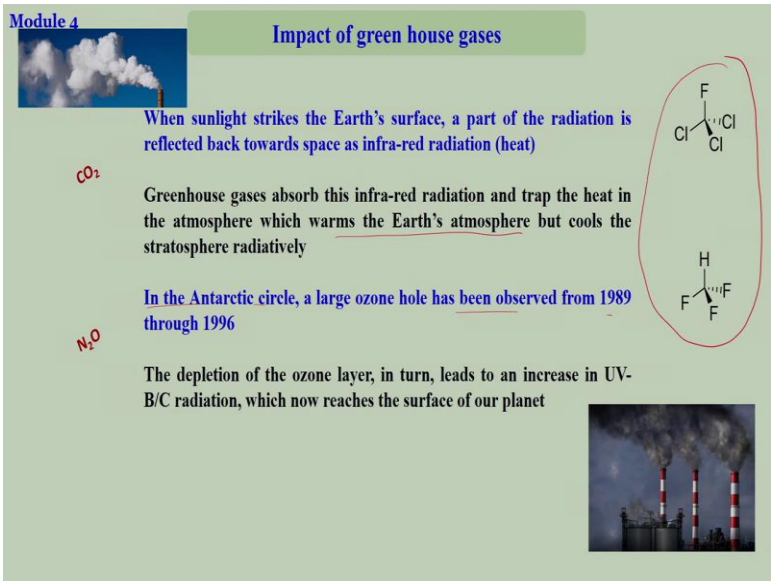

So, impact of greenhouse gases, this is everybody knows nowadays that what the greenhouse gases what the pollutions are doing to the earth or to our body as well. So, the greenhouse gases means the gases like carbon dioxide, nitrogen dioxide, and most notoriously and in large quantities are called the chlorofluorocarbons. They are used in huge quantities in various sectors of life. So, these are called greenhouse gases because they are not natural, they are actually effluent or they are emitted from our daily used materials.

So what are their problems? When sunlight strikes the Earth's surface, a part of the radiation is reflected back towards space as infrared radiation or as heat. So most of the sunlight that reaches

the Earth's surface is actually radiated back up as a heat. So it is a reflection and that keeps the Earth's temperature proper, it maintains the temperature of the earth. Now what happens this greenhouse gases, they actually shield the back radiation.

They absorb the radiation or they absorb the reflected light from the Earth's surface mostly. And they trap the heat because of the fine particles because of their huge quantity operations in the air. So, they trapped most of the radio since that was supposed to go back to the upper atmosphere, which we call stratosphere. So, as a real result, what happens it increases the temperature of the earth, because the radiation is not moving out of the earth.

So, it is accurate getting accurate accumulated and it increases the temperature of the earth. So, which was the Earth's atmosphere and since the radiation is not reaching back to the stratosphere, in the stratosphere, it is getting cool the temperature is dropping down. So, earth is getting hotter as we know today as the global warming and the stratosphere to some extent is getting colder. So, this is a fact that in the Antarctic Circle a large ozone hole has been observed from 1989 to 1986.

So this whole thing is related to the depletion or to the destruction of the ozone layer. The depletion of the ozone layer leads to an increase in the UV radiation, which now reaches the surface of our planet I will show you.

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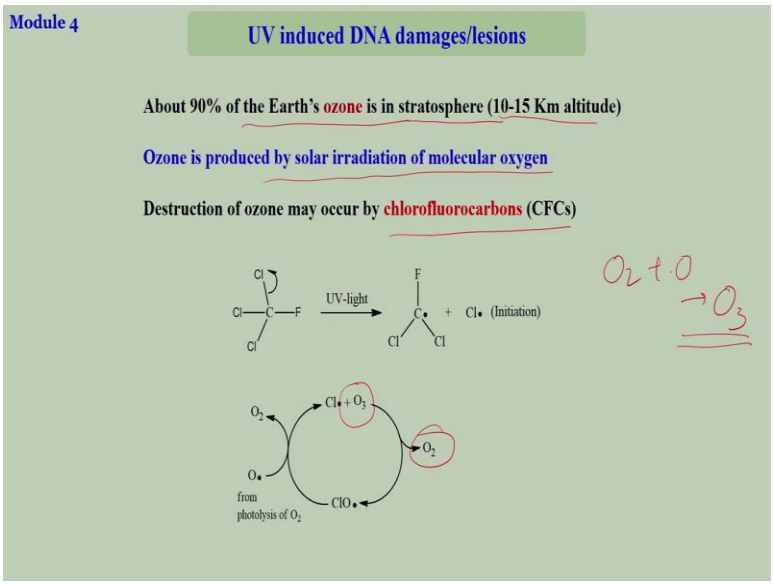

So ozone, or ozone layer, it stays back in the stratosphere, which is around 10 to 15 kilometer altitude off the surface of the earth. Now, ozone is usually produced by the solar irradiation of molecular oxygen. So when the production of ozone is through molecular oxygen, oxygen reacts with another radical of oxygen. 1 O2 molecule reacts with another molecule of radical subtypes of oxygen that produces the ozone that is how the cycle goes on in the stratosphere, it keeps the density of ozone intact there.

And the ozone layer what it does is it shields the UV radiation from reaching the Earth's surface. So the UV ray or UV radiation cannot reach our earth because of the ocean shield. Now, due to this chlorofluorocarbons, this is the scheme or this is the cycle that is given the chemical reactions that goes on the chlorofluorocarbons, they undergoes radical productions. And those radicals actually clips the ozone into the molecular oxygen back. So ultimately, or do you get is the destruction of the ozone.

And as I said, that the temperature in the stratosphere was getting cooler that further accelerates the radical reactions. So, it helps the cleavage or it helps the destruction of the ozone layer. And since the ozone layer is depleting, now, the UV radiation is reaching the earth and that is the real problem.

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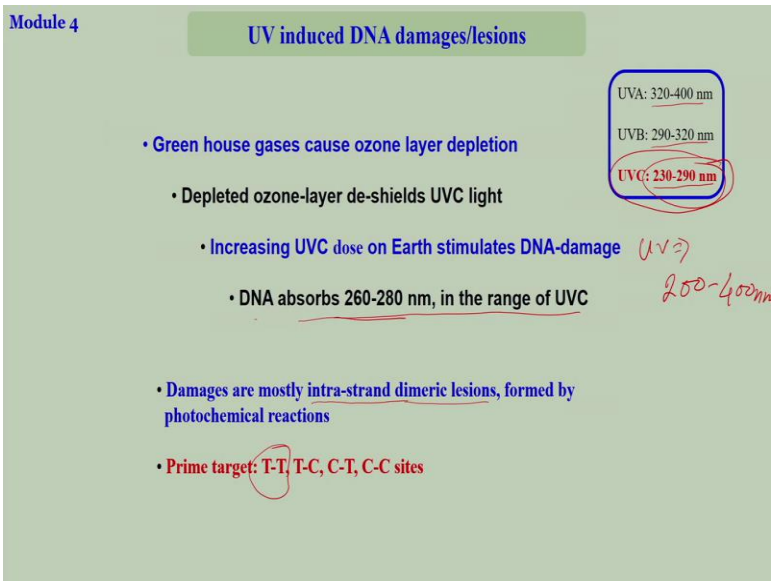

So, here if you look at the table this table given here is the range of the light if we radiations is in the range of 200 to 400 nanometer and it is divided into 3 different categories UVA, UVB and UVC. If UV has a range around 320 to 400 nanometer UVB is 290 to 320 nanometer if UVC is to 230 to 290 nanometer. Now, what this radiation is going to do to our health. So, if you see out of all these categories, this guy, if we see radiation it falls in the range of 230 to 290 nanometer.

Now, if you remember in the biomolecule so the question is, is it going to affect the biomolecules is it going to affect our help? So, if you see the DNA or the protein, we have mentioned, that DNA mostly absorbs UV radiation at around 260 nanometers. That is the absorbance maxima. So, which means is that it is in the range of that maybe 230 to 270 nanometer or 280 nanometer and proteins mostly absorbed in 280 nanometer.

That is how you measure the protein concentration by measuring the absorbance at 280 nanometer, but in for protein not all amino acids are active for absorbing if we radiation only a very few of them like tryptophan and so on they absorb the UV radiation. So, for DNA it absorbs between 260 to 280 nanometer and protein absorbs between maybe 270 to 290 nanometer something like that so, all of that range is basically within this frame UV radiation which means, if we see light falls on our body, the DNA or certain proteins, certain amino acids can absorb that radiation.

And therefore, they can do chemistry photochemistry because if the molecule absorbs or radiation, it goes to the excited state and from excited state can look different reactions. So, that is where the problem mainly the UVC radiation which used to be protected because of the ozone layer is now reaching the earth and that actually damages the DNA nuclear bases. So, greenhouse gases cause ozone layer depletion depleted ozone layer de-shields UVC, light increasing UVC dose on earth stimulates DNA damage.

And the reason is DNA absorbs within that range of the UV radiation. So, primarily there are many kinds of DNA damages happens because of the UV primarily, they are interest strand dimeric lesions, those are formed within the same strand to nucleobase to each other. They form a dimeric lesions and the prime targets are the, most amount of induced DNA damages occurs on the primary nuclear basis.

Thymine - thymine, thymine - cytosine, cytosine - thymine, cytosine - cytosine sights are more susceptible to undergo DNA damages or DNA lesions. So now I will show you the chemistry that goes on and what are the products that are formed. And later we will see how these structural changes lead to mutations.

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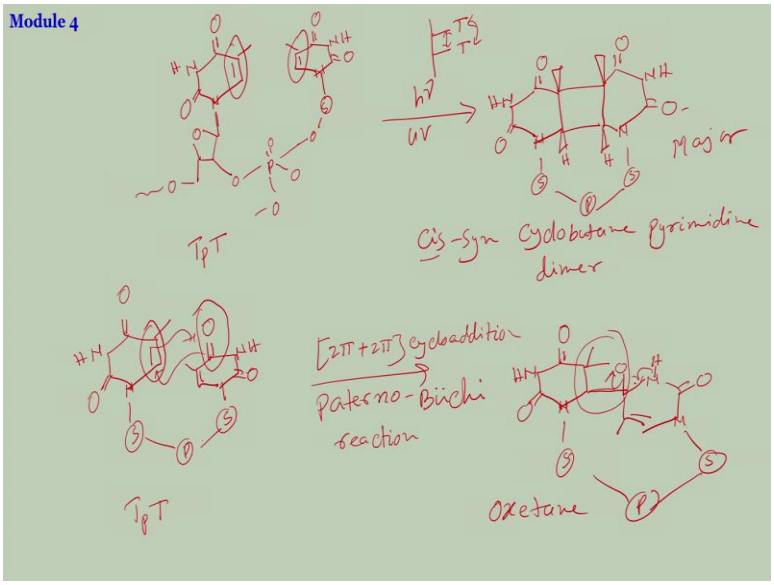

So if this is your thymine this is the sugar that is how it goes and here you will have O minus another thymine in, sugar O, and the phosphate I am not drying the sugar. So it is basically

within the same strand, if you have a thymine and if you have another thymine in next to each other then there can be reactions under the UV light. Now if you have H nu, UV basically UV. What is going to happen? So, they sometimes change the backbone also, today, we are not going to discuss that with the backbone, but mostly they interfere with the nucleobases part as well.

So, in short, this you can write T P; P for phosphate T, if you remember your chemistry, the basic photochemistry if you have 1 alkene plus another alkene. If you sign light on it, if we expose it to radiation, then it will undergo 2 pi, electrons are there plus 2 pi Cyclo addition reaction and it will form it is like this of course, when you are talking about the photochemistry they are all homiletically bond single arrow right.

So, it will form this is called the cycle addition reaction. And there are 2 pi wants involved. So 2 pi plus 2 pi cycle addition. Same thing might happen here and same thing does happen here this is the double bond. This is a double bond carbon double bond. So, what it forms is it forms the cycle addition I am not writing that I am writing sugar here phosphate here and if you look at the stereochemistry there can be 2 different kinds.

One is this is CST up the plain, 1 is CH3 and 1 is H, H is up the plain also for this is up the plane as well and this is the major quantity that is formed this is called cyclo butane pyrimidine dimer because your formula cyclo butane pyrimidine due to the nuclear base pure pyrimidine nuclear base cyclo butane pyrimidine dimer is happening between 2 of them so, it is forming a dimer and because of the stereochemistry.

This is particularly called Cis Syn geometry so, these 2 are cis orientation that is why cis and this 2 are also in cis orientation, which is if you are 2 of them, then you call it syn instead of calling both cis, the usual notation is, it was cis syn or trans something like that so it is a cis syn cyclo butane pyrimidine dimer, which is the major lesion of DNA when this is exposed when DNA is exposed to UV radiations especially the UV radiation.

So this is 1 kind of treatment or 1 kind of reaction that happens second is this maybe this here this the same TBT now, apart from this 2 plus 2 cyclo addition reaction there can be another type

of cyclo addition reaction that is also 2 plus 2 cyclo addition. So, this is 2 pi plus 2 pi cyclo addition same is this, but it has also a name see, this is the double bond that we have selected and on the other nucleobase we have done with this carbon double bond.

So, there is another double bond exists that is this if know if these 2 double bonds therefore, cyclize product that is known as that has a name it is known as Paterno Buchi reaction actually, this is a German name, it is moving reaction Paterno Buchi reaction. So here your cycle addition would be this way goes here and goes here and the product would be something like this. This would be your NH. Here is your super double bond this is thymine.

Here is the thymine and the rest of it sugar P sugar this with the formation of this 4 membered ring and this ring is known as Oxetane intermediate basically it does not stay along that way. It is called the Oxetane intermediate. Now, what this will happen immediately of course, oxygen intermediates are not at stable. So, it will immediately be cleaved like this so, there will be 1 OH coming in and there will be 1 double bond over here.

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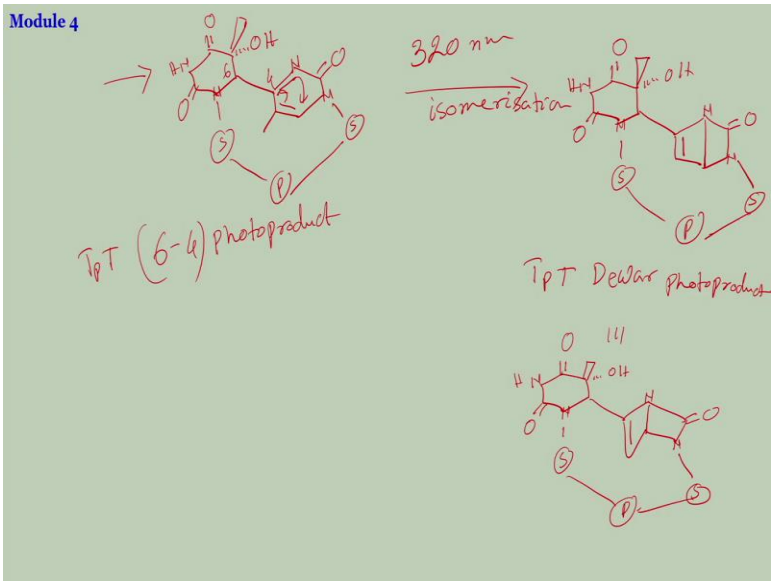

That gives you the structure thymine up the plain, wait down the plain in is and here double bond, there was a double bond already here and there is this end with the sugar and there should be the double bond oxygen now, this position is your 123456 and this position is 1234, 4 position. So this product is known as 6, 4 photoproduct. So now you can see how the structure

got changed totally from a thymine - thymine individual upon the reaction with the UV radiations have a completely different kind of structure.

Now this is actually undergoes isomerization at around 320 nanometer here this way basically wait here if I write this fashion it will be in and straight this and here will be a double bond these can you recognize such kind of structure. These are Dewar benzene is a derivative of Dewar benzene. So, this is called the Dewar isomer it does form actually. So, this is called Dewar photoproduct all are thymine - thymine. So, this is TPT 6 - 4 photo product.

They all have been isolated they all have been characterized and in different kinds of DNA different kinds of genome had been exposed to UV radiation and they mainly form such kind of structures. So, Dewar isomers you can also write it is like OH in sugar this is ON here like this this is how we usually write that in a disconfirmation Dewar isomer. Now this is for if you have the thymine and thymine. So, these are a few of the photo products that you can get when exposed to UV radiation.

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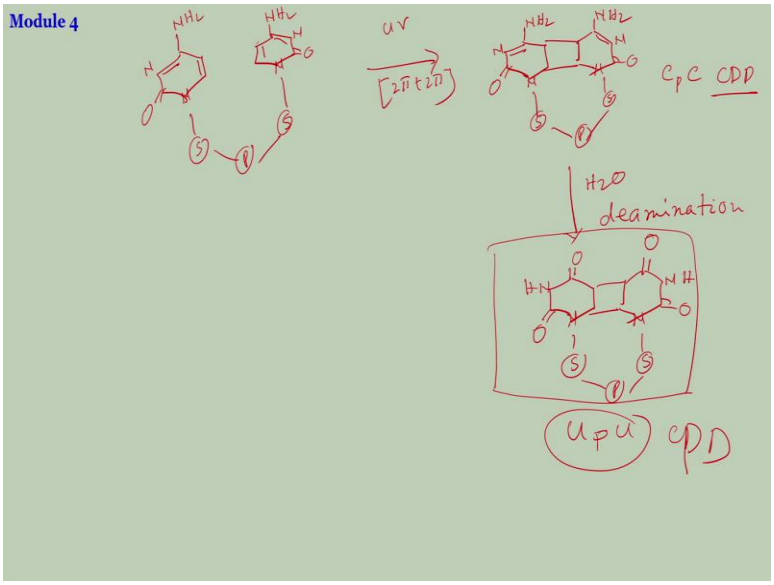

Now very interesting ones are if you have cytosine if you have to 2 cytosine, CC then what are you going to get? UV radiation and of course it is going to give you 2 plus cyclo addition products so cyclize 1 NH2 now we will move in here, no one in this this is CpC, CPD cyclo mutant pyrimidine dimer in short we call it CPD. Now, very interestingly once this is formed

from psycho since, what you have is amine group or double bonded amine group double bonded and it has obviously lost its aromaticity.

So, this makes these very susceptible for hydrolysis treatment of water deaminates the NH2 groups from here deamination quite rapid deamination actually, once you form these, if you are doing your reaction in water also form these there immediately we hydrolyzed and will not be released or NH2 will go out and it will give you this OH first and then now you can see the real reason for mutation so, this is formed.

Can you recognize what is this from where it is derived? This is basically eurosil P, CPD. So you are cytosine is converted into the eurosil. Now, if you think of DNA I will show you later if you think of DNA where you have the cytosine as a neighboring nucleobases and after the exposure to UV radiation, if they are converted it to the eurosil CPD. So, eventually they will be repaired back and it will take as U and U of course the eurosil is does not exist in in the DNA while synthesizing DNA.

So, eurosil will be over time be replaced with thymine, so cytosine will be converted originally cytosine would be converted into thymine, so very clear which sense of mutations here so, that is for the cyclo butane pyrimidine dimer.

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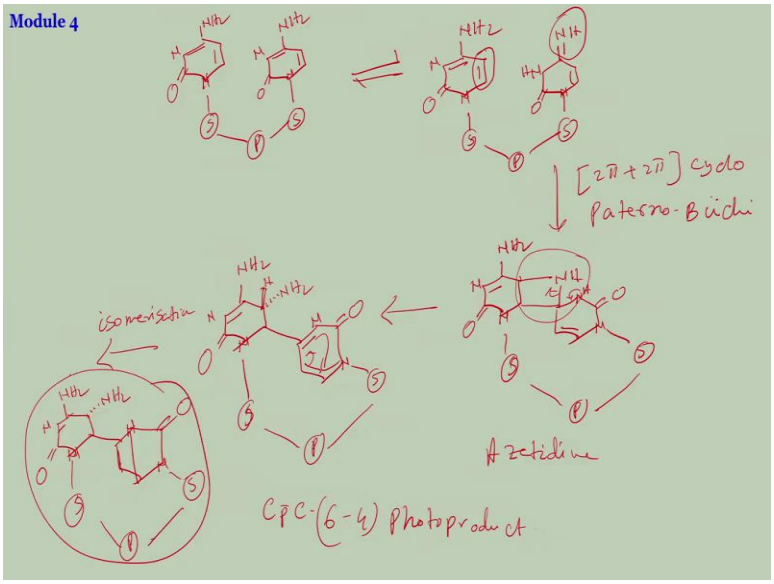

And if you do the other Paterno Buchi reaction between this write here no saying in double bond, so, these can also stay in automatic form. So, it will be in this fashion to some extent here in NH and NH this imine form now this imine is double bond here so, for thymine is a double bond oxygen that was participating in the Paterno Buchi reaction in this case this imine form with the double bond that will react with the other double bond of the other cytosine.

So, this is also the 2 plus 2 pi plus 2 pi cyclo addition and Paterno Buchi reaction and this will yield which it will yield finally the NH this will lead to Azetidine intermediate that will want so, here in that NH will be there is this double bond and here be the NH here, with other NH double bond O nitrogen with the sugar there is this as usual in NH2 in this intermediate is called Azetidine intermediate and obviously this is also not very stable.

So immediately this will be clipped off and it will give you in each NH2 here free this is hydrogen double bond sugar this and this is your 6 - 4 photo product. So, CPC 6 - 4 photo product and further isomerization will lead to the imine I mean In here straight double bond over here in sugar in NH2 sugar, phosphate sugar this is the D-Valine isomer. So, this would be the photo product primarily for the cytosine this will further deaminate here this will be deaminate here also.

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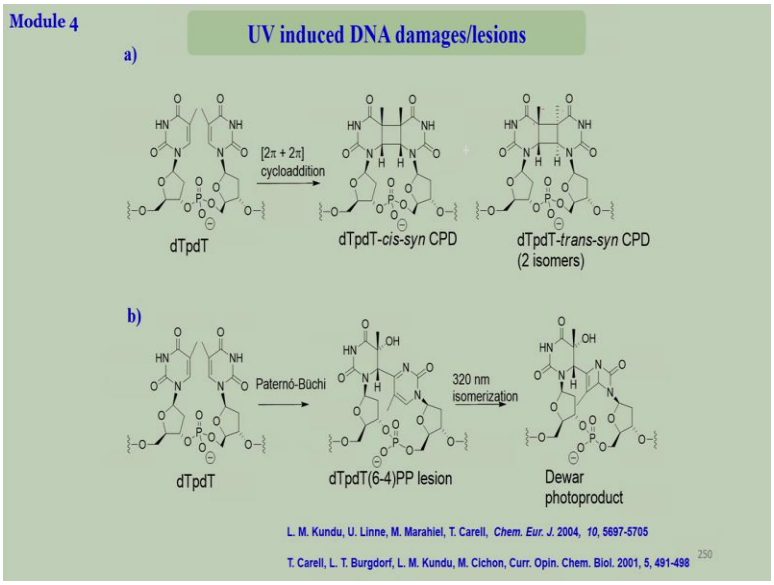

So, this is the dTpdT this is the phosphate deoxyribose 2 plus 2 cyclo addition, cis syn this is the cis syn it can also be trans syn these 2 are trans and these 2 are syn. So, this is called trans syn. This is another photo product that is formed along with this Paterno Buchi reaction will produce that 6 - 4 photo lesions followed by isomerization at 320 nanometer will produce the Dewar photo product.

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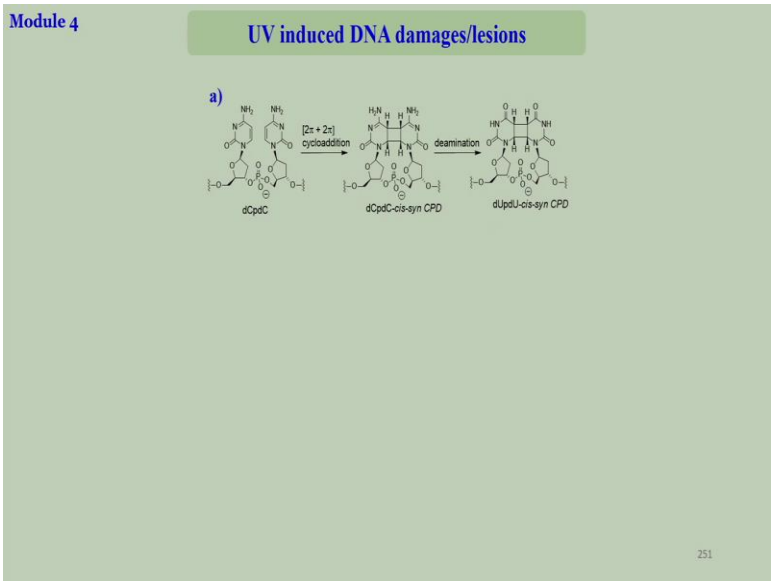

So, I have actually worked in this field. Now, if you take the example of cytosine cytosine, 2 plus 2 cyclo addition, cis syn and it will be de added to eurosil. Next lecture will continue with this. Thank you.