Essentials of Biomolecules:
Nuclic Acids, Peptides and Carbohydrates
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Lecture No. 19
DNA Repair

Hello, everybody and welcome back. So, we are working on module 4, where we are discussing DNA damages and they are mutations in the last lecture, we have seen how the UV induced DNA lesions are formed, how the photochemistry works and how they are at the nuclear bases are undergoing structural variations or structural orientations that are responsible for inducing the mutations in the genomic DNA.

So, we have seen that if you irritate the nucleic acids that may primarily I have talked about the nuclear basis that undergo different kinds of lesion formations. So, I will just quickly recap and then we will move on.

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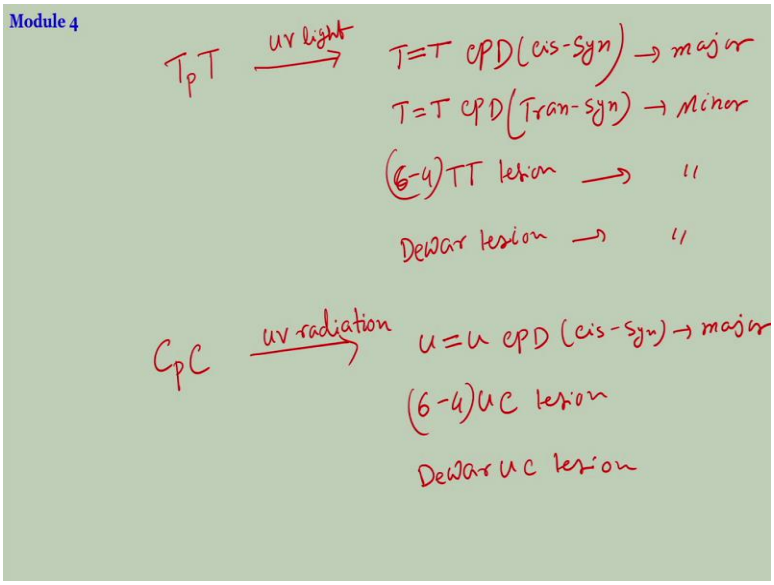

So, if you have a dinucleotide with 2 thymine and thymine with the for split here, and if you studied against the UV light, primarily the UVC and if we have radiation, then the products that are generated are majorly the TpT double bond T is CPD lesion cyclo butane pyrimidine dimer

and mainly in the form of Cis Syn that is your major content along with T = T CPD with Tran Syn geometry minor.

The other one is also minor, the sys anti and then you have 6 -4 TT or lesion. TPT that is also a minor product and then this 1 was converting into the Dewar lesion isobar or Dewar lesion that is also the minor product. Similarly, if you start with CPC both sides since the neighboring nucleobases aside to since UV radiation then what to form is you first get the CC, CP deletion which is deaminated into the eurosil, UUCPD and Cis Syn that was your major component along with 6 – 4, 1 eurosil and 1 should be cytosine UC lesion.

And the Dewar UC lesion similarly, to see can give rise to a variation of products and all these compounds have enormous differences in their chemical structures. And we have seen how the minor compounds were highly mutagenic in nature. So, even if they are formed in very little quantity within the cell, they are highly mutagenic in nature because they are structural orientation is drastically different compared to the original nuclear basis.

So, they are kind of like poisons, less quantity, but highly effective, very dangerous. So, a lot of research has been carried out to understand how the lesions are formed lot of methyl have been developed to identify all these kinds of lesions. So, I have restricted myself only to the 2 kinds of lesions, oxidative lesions and the UV induced lesions. There are other kinds also so, a lot of studies have been performed to identify various structures or various kinds of lesions that are produced and their structures are elucidated.

Sophisticated techniques instrumentations like laser spectroscopy, high end spectroscopic techniques were used LCMS that is liquid chromatography coupled to the mass spectrometry LCMS. That is that gives studies the fragmentation patterns of the organic components So from all those studies, different kinds of lesions were isolated, and their structures were elucidated from those factors, we have tried to gain insight information that what could be their properties, what kind of mutations they could induce.

And in the last lecture, I had shown you table where a total gene or total cell have been studied to know what kind of religions or how many quantity of the mutations that have been actually formed due to that lesion formations if you formed a lesion inside the DNA if you damaged a particular nuclear base in a genomic DNA, how to know what kind of nutrition that lesion in is going to induce how to know it experimentally.

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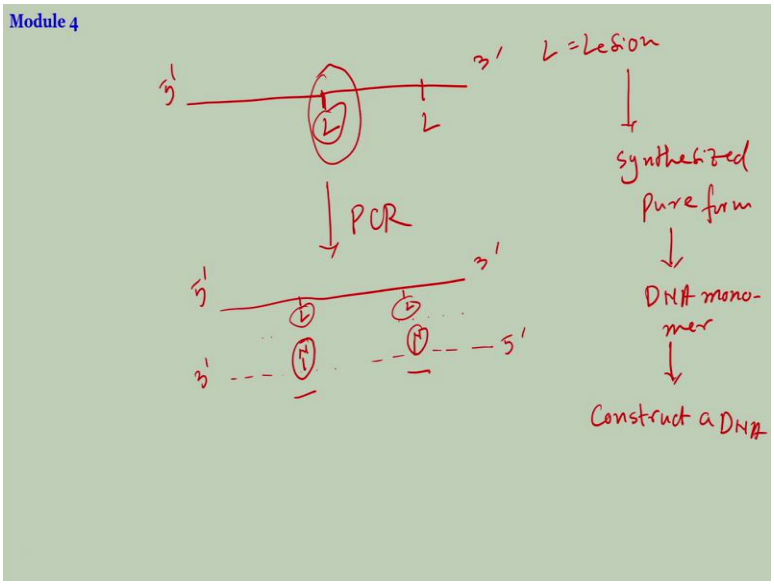

For example, I take a single strand DNA here, 5 prime, 3 prime. And here there is a lesion I am writing l for a lesion. This can be any kind of lesion from this lesion has been formed, how to know what kind of mutation, this lesion is going to induce are going to induct into the genome. So, a way to know is this and here comes to organic chemistry again, chemistry can play a major role an identifying or in knowing or in studying the formation of the mutations.

So, what you can do is, you can synthesize a whole length of the DNA in which chemically incorporate there is pure lesion. So, you can see synthesizer lesion in the laboratory in pure form and then convert it into a DNA monomer and then construct this DNA incorporating lesion at 1 point of space here or maybe again here you can synthesize a DNA in which you know that you are a pure lesion has been incorporated into it and you will also know its location.

So, next what are you are going to do is obviously you cannot put this gene into the living cell and study the replication. Because in that case, there is a very good chance that the lesion will be

immediately repaired and you cannot find a trace of it. So, living cell study may not be very helpful here that is what people actually do is study this in vitro replication means the PCR so you have this DNA, use these DNA with the lesion in it as a template.

And try to do the PCR with other ingredients of course, polymerase and DNTPS and all then you can know what kind of the complimentary strand this is going to synthesize. So, polymerase will pick up a suitable this is the compliment to the strand which was synthesizing or which you have synthesized. It was in the PCR here and then followed by sequencing of this complimentary strand that will tell you what nucleobase has been taken up by the polymer is opposite to this laser and now, if you do the sequencing, then you can know the identity of these two nucleobases.

And from there, you can have the accurate idea of what mutation this lesion would induce because the mutation would be the complimentary of this nucleobase. So that is how people find out how individual lesions, what kind of mutations that will be bringing in into the genomic DNA. So now, let us move on to the other part of it, that is, how to repair the damages. How the cells adopt methodologist or how the cells adopt processes to repair various lesions that are formed or repair of DNA lesions or damages.

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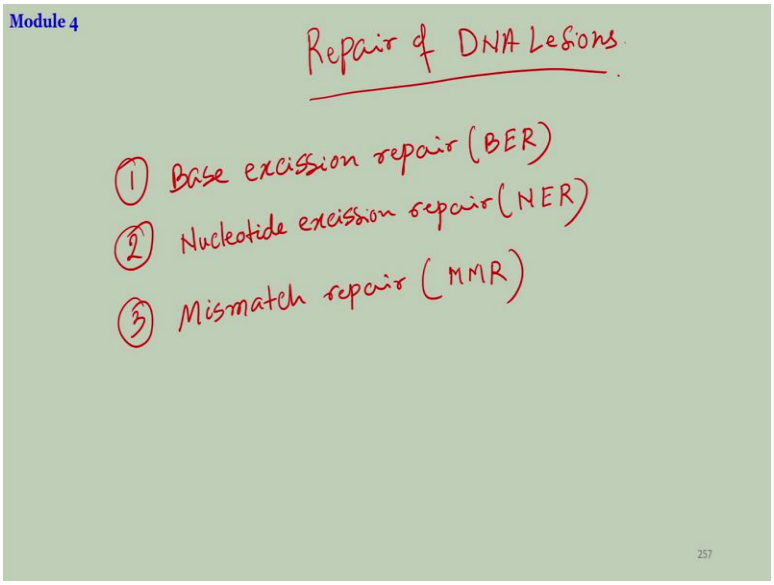

And I have talked about 3 different methods that the cell adopt to repair that damage sites of the in the genome, one is the Base Excission Repair. This is called BER and the second one is nucleotide excision repair that is your NER. And the third one is mismatch repair. Show short form is in our MMR. So depending upon the kind of reasons one of this procedure would be adopted by the cells.

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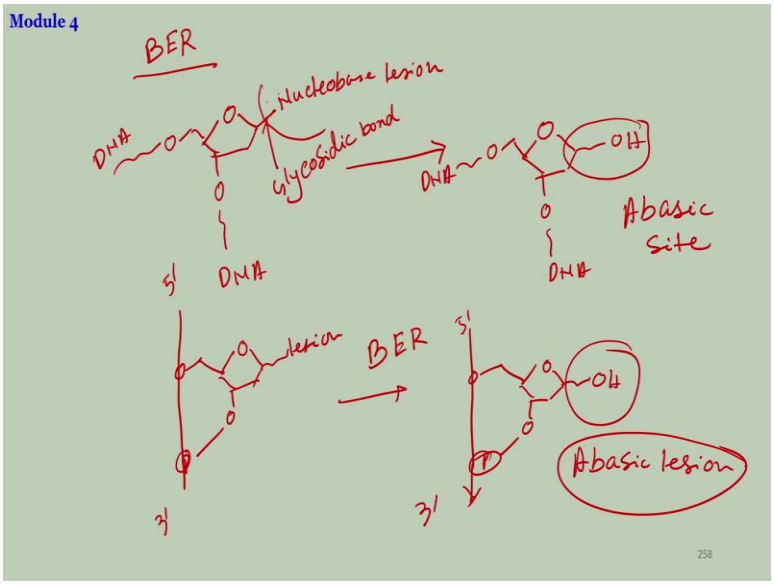

So let us start with base excision repair. As the name suggests, this means execution of our nucleus base. So, if you have the DNA is continuing. Here is your base right. So if this base is damaged, I read this as lesion nucleobase lesion. So, this is the damaged nuclear base that has been formed in the DNA base excision means that it drops off the nuclear base part out of the DNA. So, this is your bond is your glycosidic bond.

So, the repair mechanism, once it finds out that there is a problem here in the nuclear waste, then it cleaves of the glycosidic bond and then removes the lesion base out so, you have a way to know here That is your DNA basis out and you are left with the hydroxyl group here. Now, this DNA which devoid of any nuclear waste in the sugar is known as a basic site or a basic DNA or a basic sugar meaning that it is devoid of the nuclear waste.

So, only the sugar monitory is present here. So, that is the process of base excision repair. Obviously, it is done with the set of enzymes that we will see now. So, basically if you have a

DNA here 5 prime to 3 prime this is your skeleton phosphate is there and let us say here is the wage 5 prime to this is your 5 prime are not lesion here O and then for phosphate and it is going out so your DNA after the base excision repair we look like at the beginning wage.

And this will go on single stranded DNA without a base a basically is not so this is actually also a kind of lesion that I have not done talked about I have talked about only the basis. So, sugar lesions a basic lesions are actually formed in large quantity in cells also so, now, how the process looks like.

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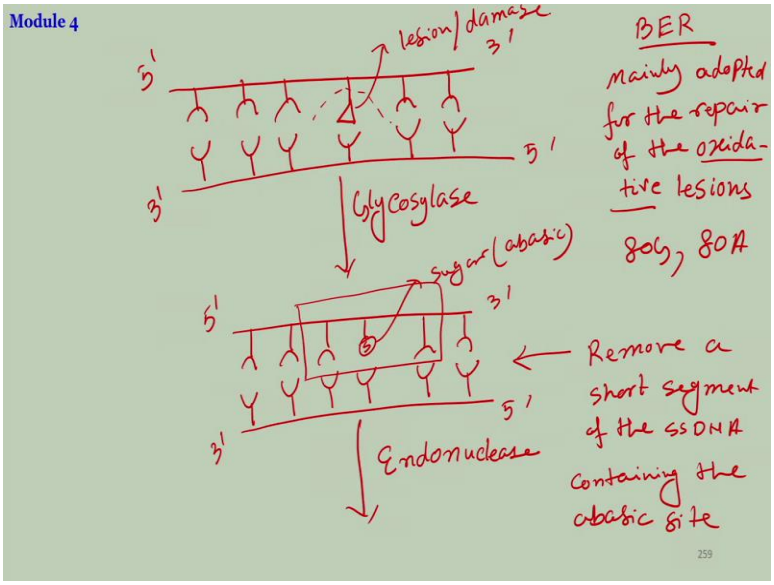

5 prime to 3 prime all the nucleobases and here there is lesion, let us say nucleobases, nucleobases complimentary strand, 3 prime, 5 prime base pairs. Here there is something nobody original one that was there. Against this the rest period let us say this is your lesion or damage, base excision repair mainly occurs of the DNA lesions, which are oxidative lesions mainly adopted for the repair of oxidative lesions such as 8 Oxo guanine 8OG, 8 Oxo adenine 8OA and so on.

So, the first is removing the nuclear waste out of the DNA and this is done by the enzyme. How does it remove by cleaving the Glycosidic bond? So, therefore, the enzyme is called Glycosylase is the enzyme that cleaves the nucleus out or that leaves that like acidic mode and he will lead with sugar. So, this is your sugar and a phosphate 5 prime to 3 prime this now, what you have to

do, there should be something incorporated the correct nucleobase has to be incorporated in this position, the knowledge will be repaired.

So, what the repair mechanism does, it does not take any chance. It does not take any risk. What it does, it will chop Once this a basic site is formed, it will chop off a portion of the single stranded DNA containing the ABC lesion. So, this will remove or exceeds short segment of the single stranded DNA containing the abasic site in and around a little bit left little bit right. So this is just like surgery if we have an wound when doctors go for a surgery, they always remove a little bit in and around the only area show that the infection does not spread. So, same thing happens here so the repair system has to be very precise.

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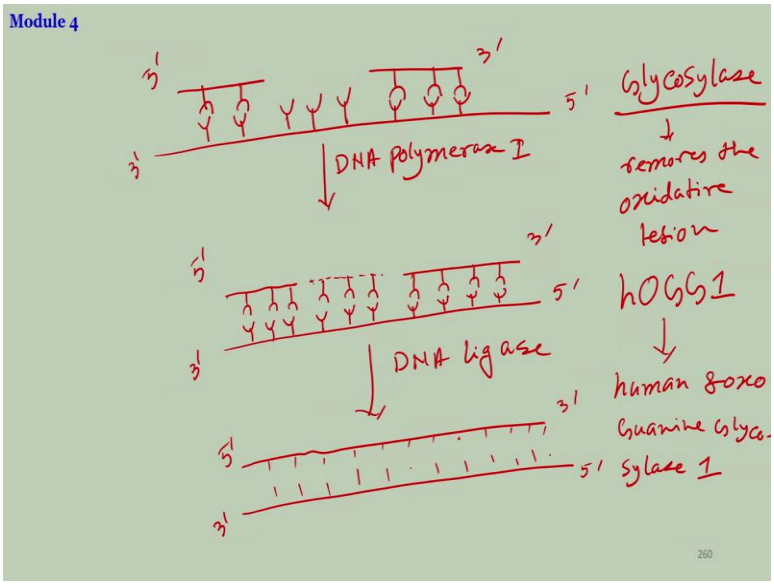

So, after cleaning this thing up, what do you have is this 5 prime, 3 prime and your whole DNA this enzyme I can write here this enzyme which actually cleaves the DNA section out is known as endonuclease. There are different types of endonucleases this 1 kind of endonuclease would be used to remove the section of the single stranded DNA that contains the abasic site. Now, when you were talking about the oxidative lesions, most of the times or a major portion of the oxidative lesions are the purine are coming from the pure in nuclear bases guanine and adenine.

So, this side what the abasic site that we have been calling is also known as Apurinic site or Apurinic base because it is now devoid of the pure in nuclear base and the endonuclease is

known as a Apurinic endonuclease, if the lesion is coming from the pure in nucleobases, then this endonuclease would be used. Now, we have an empty space here, which you need to reconstruct. And suddenly you have to synthesize this portion using the complimentary strand as the template.

And obviously, here comes your polymer is so, DNA polymerase is 1 is it was here that will fill up this gap taking the 3 prime to 5 prime mint or the strength as the template. So now it will incorporate the correct nucleobases is here. Now, there is still the gap here because it was synthesized discretely and now you need to stitch them together as usual, you need DNA ligase and now you have a completely repaired strand with the correct nucleus in place of where there was that damage originally.

So, this is 1 of the processes one of the 3 kinds of process that I have talked about. Base excission repair excision means cleaving it off or removing and this is mostly applicable for the lesions which are oxidative in nature. Key enzyme is glycosylase that actually removes the lesion oxidative limit it removes the oxidative lesion, 1 kind of glycosylase that we have the human glycosylase is known as that has a hOGG1.

There are many glycosylase. So, this is human 8 Oxo, O is for Oxo and that inevitably means a talk show for glycosylase 1 or hOGG1. What is the enzyme that actually repairs all the oxidative lesions, most of the oxidative lesions that are formed inside our cells, it correct itself and this particularly is the human so that is about the rough sketch of the basic session or appear.

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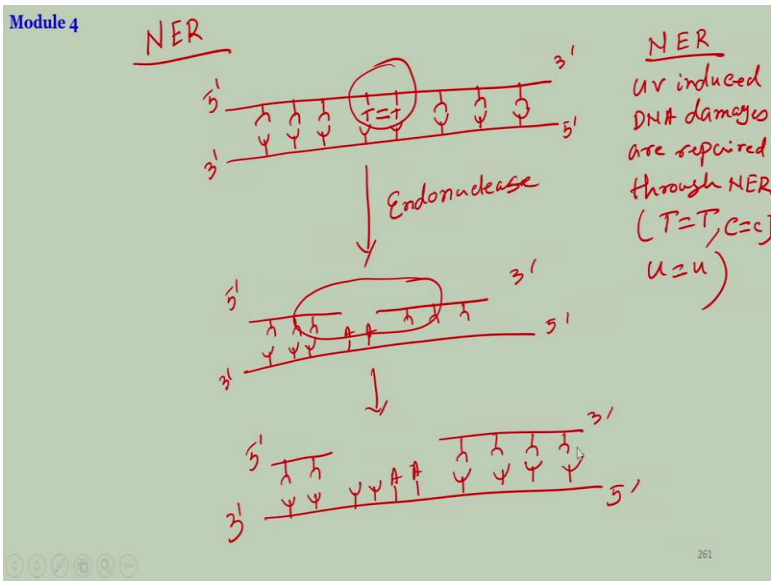

Now let us move on to the nucleotide excision repair in our in this case, basis not removed, but the whole nucleotide is removed. Let us start with a particular example maybe we can use the TT here d and T double bond. So here it would be a here would be a originally, nucleotide excision repair is adopted for the lesions, which are induced lesions, there are other kinds also in our domain whatever we have covered is the induced lesions are repaired through this procedure or through this process UV induced DNA damage are repaired through MER.

For example, TT, CC or CC becomes UU eurosil and so on CP diligence for example. So, what it does is, as the name says that it clips off the nucleotides nucleotide means the base, the sugar, the phosphate whole chunk of it because what happens, the CPD kind of lesions are not very well recognized, they look like because you have seen the structures there is only a cycle of mutant pyramid ending in between them. Other than that they are a whole other framework is almost the same as the original nucleobases.

So, sometimes the repair system can cannot understand whether this is correct or this is wrong, so, it does not take any risk. It simply cuts off a portion. First it drops off the part of the where there is the least in part, that whole nucleotide part is cut off 3 prime keeping a here by the name of course is endonuclease. And then again, just like the other surgery we have seen, it does not take a risk, it removes a certain part of the sequence just to make sure nothing else has been 5 prime so you have a gap here that is created by 5 prime, 3 prime.

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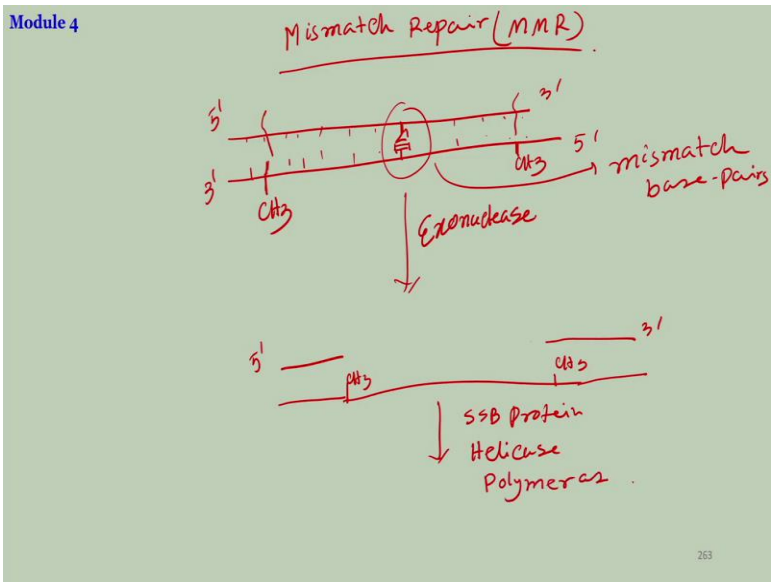

Here this followed by the action of DNA polymerase 1 and number 2 is DNA like is that will make your original strand back that so this is the early periods rent repaired DNA. So that is how the nucleotide excision repair works. Some of the examples of the nucleotide excission repair enzymes are I will give you some names. If we are you do not have to remember the names be if we are CVPR basically, see these are some of the enzymes that are involved in that nucleotide excision repair.

The third category is mismatch repair MMR our mismatch repair is it may not be from our lesion you have a double stranded DNA. But somewhere there is a mismatch of nucleus waste mismatch a base pairs. So if this is I am writing this as triangle this as a rectangle so that they do not match each other they are not complimentary to each other. So, this is mismatch portion, mismatch base pairs not necessarily they came from any particular kind of lesion and it is simply has been awkward because of the mistakes during the replication process. Of course they can happen anywhere.

So, if there is such kind of mistake that against a time in cytosine has been incorporated wrongly by the polymer is during the replication process. Then what happens is after a DNA synthesized in the cell after a replication process happens, then the cell has proofreading system just like our own writings. Once you write a certain document, then you always do a proofreading will tweet

again and again whether there is any mistake with spelling mistakes or anything other kinds of mistakes there similarly, cell also has that.

So once the replication is done, the proofreading system will scan through the entire gene. And we will try to find out if there is any mistake in its work. And here comes the mismatch. If there is any mismatch, if it is located by the proofreading system, then it requires to be repaired. And this is what the system is mismatch repair. It is little bit different than the basic session and the nucleotide excision.

One thing, what I did not tell you is that our DNA undergoes methylation inside the cell. And that is a very natural process actually, DNA methylation is a kind of natural process that happens to DNA, normal our DNA as well as micro organ, the DNA of the prokaryote as well. This is sometimes to protect the DNA so that they cannot be recognized by the enemies. So the methylated sites are the target here are the recognition sites, in this case in the mismatch repair.

So, let us say there is a DNA methylation on this base here and there is another Over here in this mismatch repair, what the repair system does or what the enzyme does, it will recognize the 2 closest methylated points closest to the mismatch and then it will cleave off a shortened part closer to this methylated sites and it will give you here. So, it will recognize this and it will chop off in this part 5 prime here is your methylated site so, here it will cleave off.

On the other hand you have another methylation here. So, it will cleave from in this site 3 prime and you get empty space, it will make 1 cut here to make another cut here and the whole chunk is out. So, you have something like this it created and then it will be filled up. Of course, now the enzyme because there are 2 cuts here, the enzyme is exonuclease for this cut actually, here, this enzyme is exonuclease. So, you need SSB protein of course, that you had required the SSB proteins and all sorts in the previous ones also helicase to stabilize and then followed by polymerase.

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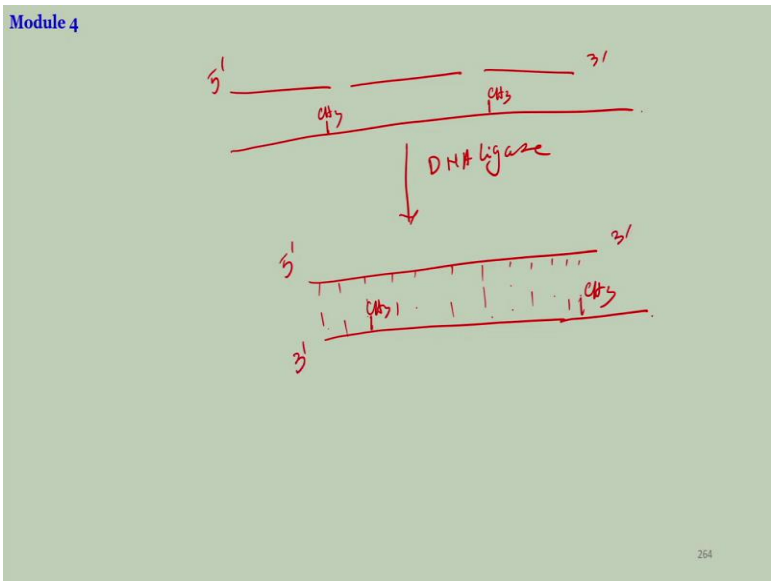

And then it will be synthesized descriptively it was caught in the methylated site here is CH3 and here is your CH3 followed by DNA ligase. CH3 you will get your mismatch repaired. So that is; what is the mismatch repair system? So, these are the prime processes primarily these are the 3 methods that are widely occurs inside the cellular system to rectify the lesions or to rectify a mismatch.

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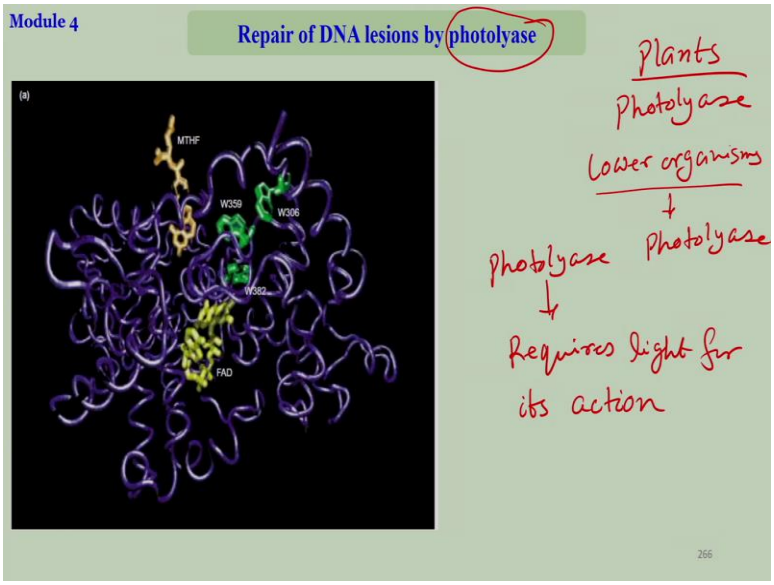

Now, there is another kind of repair and that is done by a certain enzyme called photolyase. So, this happens in the plants system all plants are most of the plants have this enzyme called photolyase. Most of the plants have lower organisms also have lower organisms also have these enzyme photolyase. Of course you can understand the leaves in a plant they undergo or they

experience or they are exposed to radiation much more and also they harvest light for the photosynthesis.

So, they are also the photochemistry occurs in the DNA that forms the CPD dimers in 6 – 4 photo products and so on also happens to the lower organisms as well. And since the plant can harvest the light, it can use the radiation for the repair also. So, the photolyase enzymes there are many classes of photolyasers, CPD photolyaser there that is cyclo butane pyrimidine dimer photolyase, they are a 6 - 4 quarter product photolyase that had been isolated.

So, we have done some work on that field also. So, photolyase is the enzyme or one of the few enzymes actually that request light for it action So there are a few enzymes that uses light as a reagent for its function like our eyes have crypto chrome, is a photosensitive enzyme. The photolyaser is on such kind. So, this is 1 of the structures of photolyase, which shows that it binds to certain co enzymes it requires certain co enzymes these is empty. So, it requires certain coenzymes or co factors for its function.

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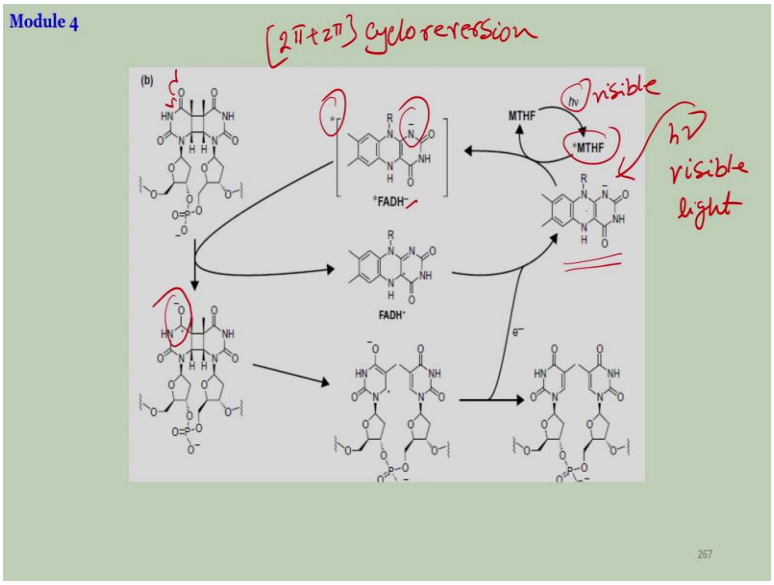

What photolyase does is actually you have formed the CPD dimer cyclo butane pyrimidine dimer to 2 pi plus 2 pi cyclo addition reaction what the enzyme does is does the reverse of it cyclo reversion. So, 2 pi plus 2 pi cyclo reversion that is the job of the photolyase since it is doing a reversible process of course, the radicals are involved because, when the photochemistry

happened, the photochemistry occurs through which means to the radical formations which means after the CPD form.

If you want to break the CPD, you need to inject electrons here to create radicals and that will do the subsequent chemical reactions. So, you need to inject an electron there and that electron come from the coenzyme flavin adenine nucleotide FAD and the electron to the FAD actually comes from the methyl tetrahydrofolate the second coenzyme I am just show you the mechanism of action how it works. So, photolyase binds with this as well as this and it is radiation from sunlight.

Photolyase absorbs the radiation from sunlight. And this radiation is not the UV. This is the normal radiation visible light chlorophyll also absorbs the visible light during photosynthesis. So, photolyase, harvest or photolyase absorbs the visible light through this MTHF because empty a HF absorbs that range of the spectrum. Then MTHF with radiation this is visible it goes to the excited state MTHF it forms with the negative charge here.

The funnels the energy to FAD, because if FAD has to transfer an electron it needs to be reduced to minus FAD negative charge has to be created here, then only it can inject the electron and it has to go to the excited state. That is why you need the light radiation makes the electron jumps to the excited state. So, visible light is absorbed by MTHF and then funnels to that FADH goes to the excited state and then gets reduced to minus FADH minus why not directly light is absorbed by FAD because FAD usually absorbs the UV range.

But you do not require UV range light, because the plant is constantly harvesting the natural visible light. So it channels the energy here. And now, if transfers on electron to the dimer, therefore, you get this if you inject 1 electron, this is the key to growth, obviously, it will be open. It will be 1 here, and then the electron extra electron is there extra electron. Of course, will go to oxygen because it is more electronegative.

So, we will get all my O- here at a radical here. Now, what is this radical is formed, it can trigger the opening of it here to here. So, unwinding basically reversible process, it forms this ultimately

form generates this radical and that radical again goes back the electron again goes back to the MTHF, and you have your repaired DNA or the CPD gets repaired. So, that is how the photolyase acts for the CPD lesion.

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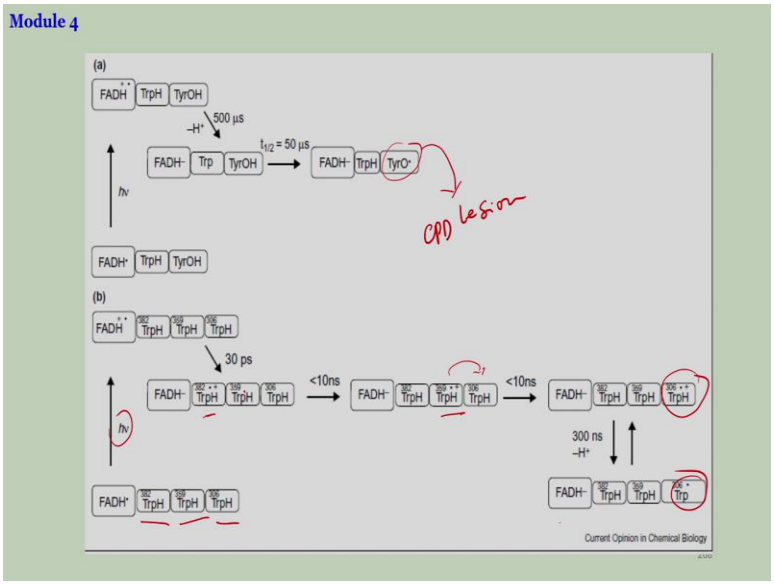

So, here comes how the study was, how did you know all these processes is happening that MTHF is transferring to FADH is now doing the job and all sorts of things, these were studied in 2 spectroscopic techniques as well as through EPR. Because if you can detect the signal electrons so, here you do not have to remember this, this is just for your information. That if you have initially you have the active site of the protein contents at tryptophan and tyrosine next to FADH.

So, FADH goes to excited state then tryptophan and tyrosine is there, look at the time scale is only 500 microseconds it takes to reduce the FAD into the FADH minus then tryptophan and tyrosine then the radical is transferred to the tyrosine here from FADH minus in 50 microsecond that is 1 kind of mechanism and from tyrosine this will be transferred to the lesion CPD. Lesion that is what the function of the enzyme itself apart from the cofactors a second mechanism is FADH in another kind of photolyase.

So, there are 2 kinds of photolyasers for CPD itself photolyase 1 and photolyase 2. So, other photolyase has tryptophan - tryptophan - tryptophan, 3 tryptophan in the active site of the protein active site of photolyase and that is close to the FAD. If I can go back here is the FAD and here

is the active side of the protein the amino acids so, FAD goes to the excited state to the light goes to radical formation, then it gets reduced to the tryptophan, then tryptophan injects pumps electron to here, it gets there, again pumps electron to here is kind of like electron hopping or tunneling of electrons, you get this and you get the, the radical on the tryptophan finally.

So, studies have also been done using only tryptophan if we can make a radical on tryptophan, it can also do the repair process. So, a lot of molecules had been synthesized that covalently outside the enzyme, just without using the enzyme FADH conjucated to tryptophan conjucated to tyrosine and the repair were also were studied and through them also, this kind of mechanism were developed, or so this is other kind of the repair system to the photolyase.

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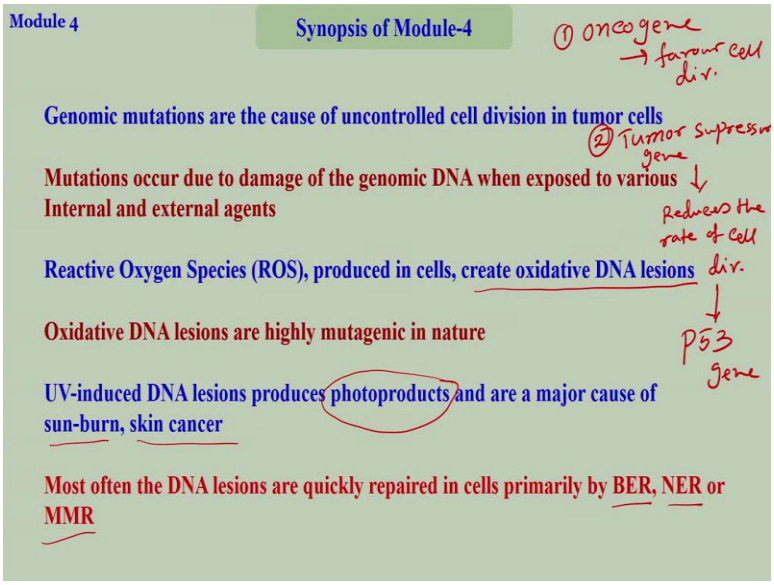

So with this, I think I will conclude model for that in this whole model. We have talked about the genomic mutations, how are the formed? What are the chemical basis behind the formation of mutations in the genomic DNA, and how the chemical structures or alteration of chemical structures can govern the biological processes again, so, first means, genomic mutations are the cause of uncontrolled cell division tumor cells. Nearly all tumor cells have the mutation

In the genome, and that is the primary cause of their uncontrolled cell divisions. For example, in our cell when cell division happens, there are two genes that control the cell divisions. One is called oncogene. The role of oncogene is actually it accelerates cell division. So it will favor the

cell division. Favour cell division means favor, the divisions of favor the replication itself and there is a second gene known as tumor supressor gene.

That actually reduces the rate of cell division rate or the speed of cell division. So one gene is accelerating cell division, the other gene is decelerating the cell division. Now when these two genes act properly, then we have a controlled cell division that happens all the time in our body. Now, if there is mutation happens in either of these 2 genes, for example, if the tumor suppressor gene is mutated, then it cannot function again anymore.

I had given 1 example that P 53 gene is a kind of tumor supressor gene. So, if the tumor suppressor gene is mutated they cannot function it cannot stop the cell division or it cannot control the speed of the cell division. In that case what will happen, oncogene will function only and your cell division will be more and more. That is where the uncontrolled cell division happens. That is what happens in the tumor cells. So, genomic mutations are the cause of uncontrolled cell division in tumor cells.

Mutations occur due to damage of the genomic DNA, when exposed to various internal as well as external agents, such as the radicals. So, reactive oxygen species or ROS produced in cells, they create oxidative DNA lesions. And all these are those chemicals are produced during metabolic process or also the other processes reactive oxygen species for example, the peroxide radicals superoxide radicals, hydroxyl radicals, they are highly reactive in nature.

So, they are responsible for oxidative cell divisions, oxidative DNA lesions oxidative DNA lesions are highly mutagenic in nature. You have seen how a talk show go on in because of its structure property, it has totally changed, it is basically adding ability and therefore, the capacity of inducing mutations is high induced DNA agents to do says photo products. So, we have seen the photochemistry or photochemical lesions that are generated due to radiation of the DNA and are a major cause of sun burn and skin cancer.

So, when you are exposed for a prolonged time to sunlight if you especially go to a high altitude area, trekking for example, then you will most often see that you have to use a heavy amount of

cream on your surface of the skin to prevent it from sunburn is basically is because of the formation of that in these 2 reasons different kinds of the intelligence and skin cancers are most of the cases are because of the DNA damages that are formed due to UV light.

Most often, the DNA lesions are quickly repaired as we have seen in cells, primarily by base excision repair, nucleotide excision repair or the mismatch repair process. Along with of course, the one, I have added one, additional one that is then photolyase that is mostly for plants as well as the some of the lower organisms. They have this kind of repair systems which require visible light to do the reversible cyclo reversion process.

So yeah, that is all for this model that how the mutations happened and what are the cause of the mutations? And what is the effect of the mutation that is not controlled cell divisions. So with this, I will conclude this model. And then next we will go to module 5. Thank you.