Biochemistry
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Lecture - 18
Nucleic Acids III

Our third lecture on nucleic acids is going to speak about the stability of DNA okay and what are the certain factors that are going to lead to the denaturation of DNA or the renaturation of DNA and how that is extremely important for designing of drugs for the cleaving of DNA as I mentioned in the last class, where we want to consider that the protein synthesis is inhibited or stopped okay.

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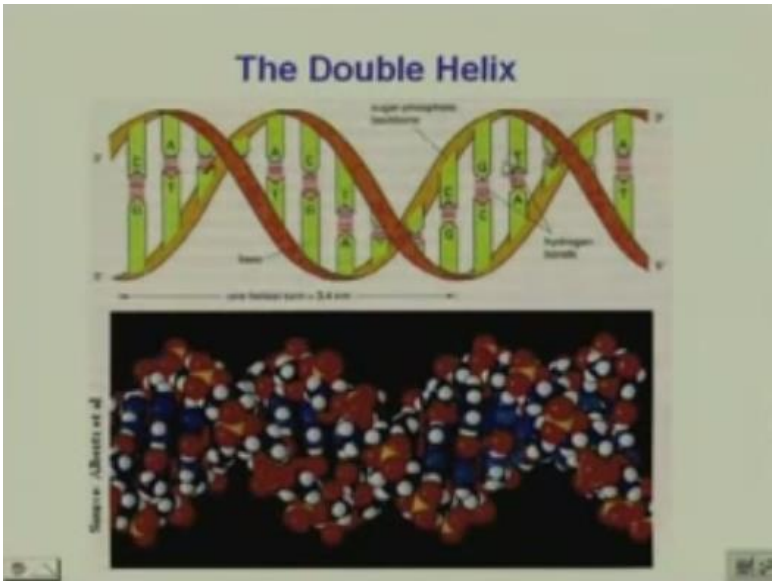

If we go back to look at the double helical structure of DNA, we have our sugar-phosphate backbone and we have the specific bases linked together. Now apart from the hydrogen bonding present here, we are going to have certain other interactions that are going to be responsible for the double helix to maintain its structure in solution.

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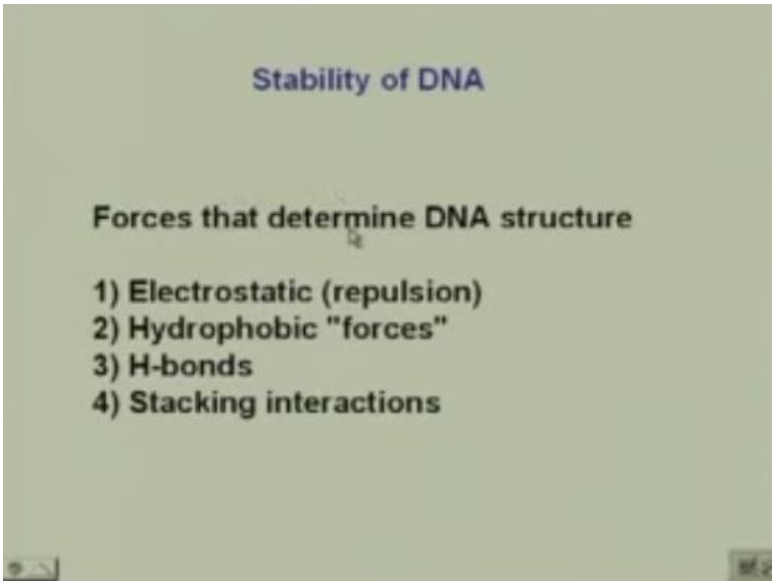

Now the forces that actually result in the DNA structure are electrostatic forces that are mainly repulsion forces. Now can you guess why we would call them repulsive forces, why were the electrostatic forces in the case of DNA or the structure of DNA that I showed on the previous slide, we call repulsive. What do we have in the backbone, we have sugar-phosphate.

What is the charge on these phosphates? they are all negative. So the strands were tend to be as far away as possible right, which was going to result in an electrostatic force that is going to be basically a repulsion. We have hydrophobic forces. We have hydrogen bonds obviously between the bases and also possible between the other hetero atoms present in the bases with the water that is around and we have stacking interactions between the bases, between the bases at different levels.

Now considering the properties of the Watson Crick Base pairing, the base pairings are planer okay. So in the resulting planarity that we have between the G-C and the A-T pairs, we are going to see a stacking interaction that again can be disrupted with any agent that penetrates this region between the bases. So we have these four types of forces that actually determine the DNA structure.

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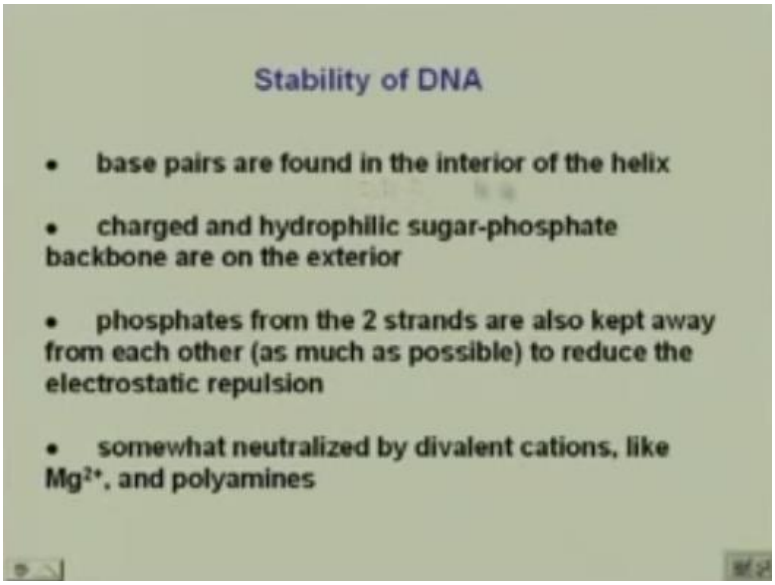

Now the base pair are usually found in the interior of the helix okay, which is going to result in the stacking interactions between the bases. The charged and hydrophilic sugar-phosphate backbone is on the exterior, which results in the electrostatic repulsion of the strands. The phosphates from the two strands would obviously be as far away as possible why because they are negatively charged and they would reduce the electrostatic repulsion if they would be as far apart as possible.

Some of this is neutralised by polyamines, why would they neutralised by polyamines or magnesium because there would be positively charged, they would counteract the negative charge of the phosphate and result in stabilisation. Usually, we have magnesium and polyamines that would result in the stability of DNA. So there are certain forces that keep the DNA structure together, keep it stable and we are going to learn now of what agents can actually disrupt the structure of DNA.

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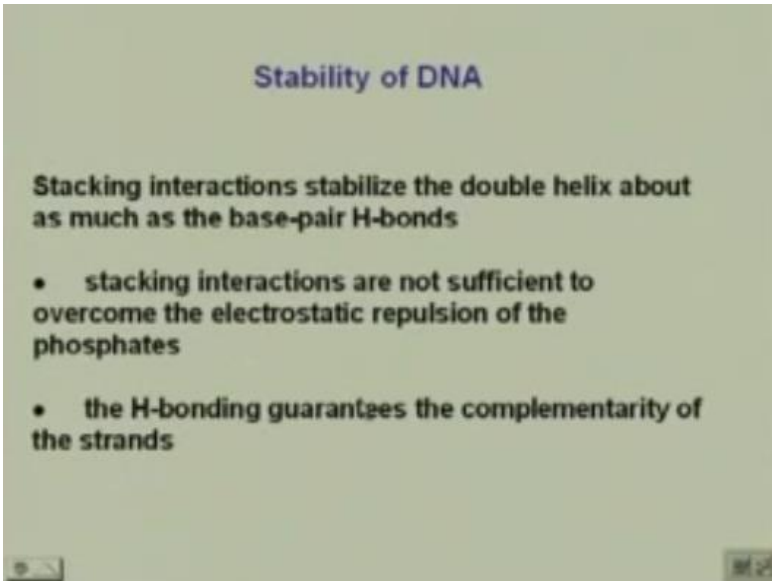

The stacking interactions stabilise the double helix as much as probably close to the base pair hydrogen bonds okay, because what do we have in those cases, we have a pi-pi interaction okay and we have a series of such pi-pi interactions right. So the stacking interactions actually stabilise the double helix as much as the base pair hydrogen bonds. The stacking interactions are sufficient to overcome the electrostatic repulsion of the phosphates okay.

So the phosphates will remain as far apart as possible that would be stabilised by some positively charged divalent ions or polyamines and the hydrogen bond between the purines and the pyrimidines guarantees that there is going to be complementarity of the strands. This is extremely important in DNA because you understand when we go from a double helical DNA to forming the daughter DNA, then what happen, you have to have perfect complementarity.

It is not like RNA, where you can have a single strand and you can have complementarity for some region of the RNA, but not for the whole region. So we have these stacking interactions, we have the electrostatic interactions and we have the hydrogen bonding interactions and this is what wholes are DNA together.

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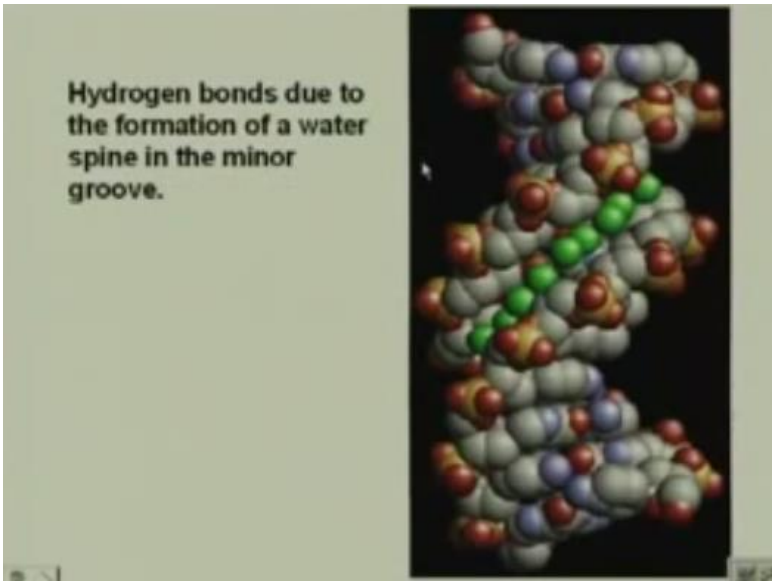

We also have this is one picture we saw previously where we also have an additional hydrogen bonding between water that goes through the minor groove of DNA right. So we have the major groove that is this large gap here and we have the minor groove and the minor groove also twists around with the double helical structure. So we have these hydrogen bonds also form in addition to the other types of bonds or the other types of interactions that are observed.

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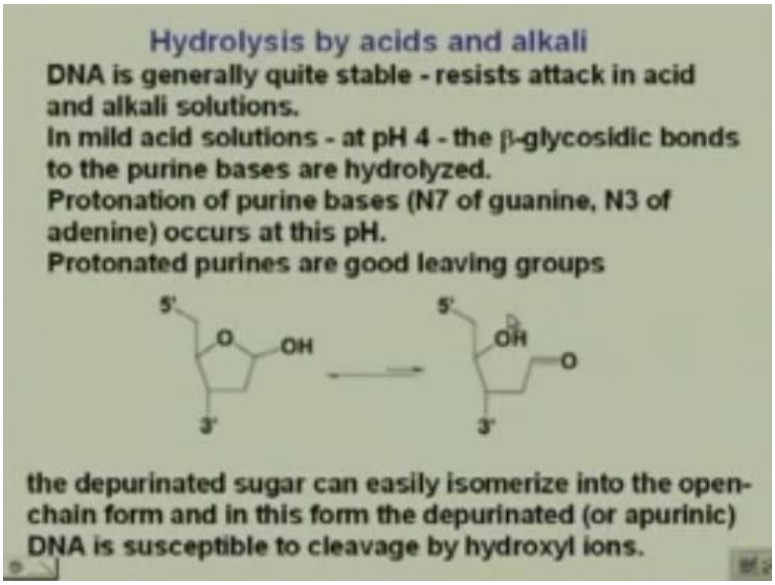

Now DNA usually is quite stable. It actually resists attack in acid and alkali solutions. Now can you tell me why it is more stable compared to RNA, why is DNA more stable compared to RNA.

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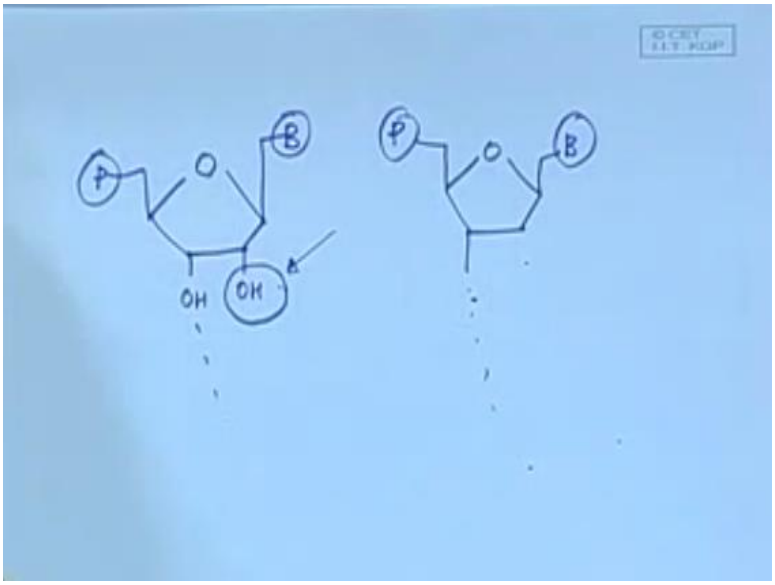

What are we differing in this right? We have our base and we have our phosphates. We have continuation here. So what do we have, in RNA we have this, in DNA you are missing that. What is linked here, you have the other strand here. You have the phosphate here then you have the phosphate again, another sugar, another base and so on and so forth.

So what happens here is this is susceptible to hydrolysis, but DNA can resist hydrolysis, which is why ribonuclease does not work on DNA. It cleaves only RNA in the mechanism that we learnt in our enzyme mechanism classes.

So DNA actually is quite stable and in mild acid solutions at pH 4. In this case, we have a different kind of hydrolysis whereby the purine bases themselves a hydrolysed okay. Since the OH is absent that the 2 prime position unlike RNA, what happens in this case, the beta glycosidic bond to the purine bases are hydrolysed and we also have protonation of the purine bases.

Now what is going to happen if you are going to have protonation of the purine bases at the acidic solution, you are going to disrupt the hydrogen bonding right. As a result of which you are going to have this hydrolysis, which leaves your purines protonated that is going to act as a good living room and you are going to have an isomerisation of the depurinated sugar.

This is the hydrolysed sugar where you are going to have, what is this 5 prime and what is this signify, what is this 5 prime and 3 prime end signify, it signifies that this is a part of a DNA strand right. You do not have the OH here. So this is part of DNA strand and this

occurs under mild acidic conditions where there is a protonation of the purine, the purine is lost and what happens is this sugar now that is on the left hand side, which is depurinated isomerises to an open chain form.

So what have you done in mild acidic conditions, you have not only depurinated your DNA, you have also opened up the sugar right. So that where basically be a problem.

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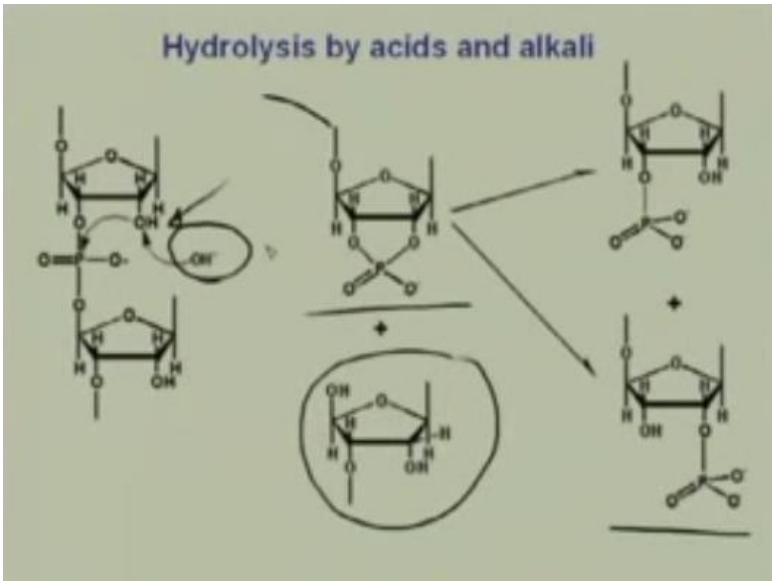

What happens in RNA, this is RNA now right, in RNA what we have here is we have the case where we have the OH at the 2 prime position. The OH at the two prime position is going to give you what this is your OH at the 2 prime position. What is going to happen in that case, you are going to have easy hydrolysis possible with an OH minus, you can have hydrolysis at the OH position.

Now happen when we consider the ribonuclease mechanism, histidine 12 and histidine 119 where important in doing what, donating a proton, so one acted as an acid and one as a base and the roles were reversed in what was called the hydrolysis step. So what do we have in a sense, we have transphosphorylation, so this is what is happening. We have, what is this, we have a cyclic phosphate intermediate which results in the phosphate being transferred.

You see other phosphate has been transferred, where was it originally here okay. So you have either go back to its original form or but it is cleaved, remember it is cleaved and so we have this part which is what, which is the rest of the chain and this part is the previous part of the chain right and we either have this phosphate remain here or go over to the 2 prime position,

is that clear.

So in this case, now this is not possible with DNA, you understand that. Why is not possible, simply because you do not have that OH there. So it is not possible, but this is easier for the case of ribonuclease.

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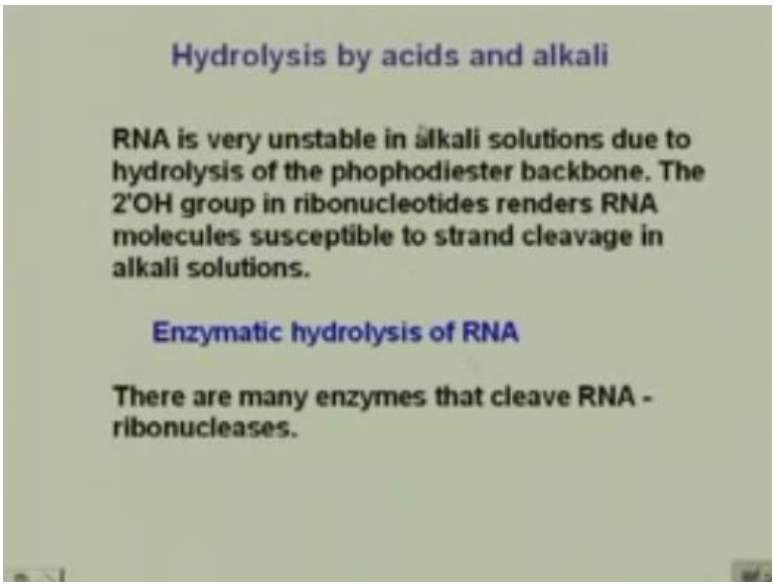

So when we look at our RNA, we have is very unstable in alkali solution basically because of that 2 prime OH that is present, it results in hydrolysis. The possibility of hydrolysis is always of the phosphodiester backbone and this renders your RNA susceptible to strand cleavage. So what is going to happen, is your RNA strand that was a single strand is going to easily cleave right because of the OH being present there even under alkali conditions.

It is going to break up and we already considered the enzymatic hydrolysis of RNA where there are ribonucleases that are going to cleave RNA in a similar fashion, but in this case it is going to be the histidines that are going to be important in the cleavage mechanism whereas in just considering an alkali solution, it is going to your OH minus that is going to attack the 2 prime OH, which it cannot do in the case of DNA because it is absent.

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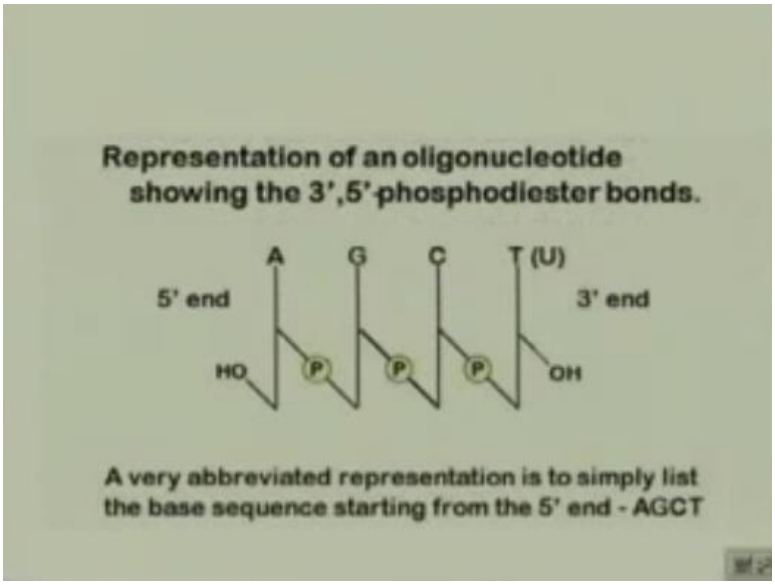

We looked at this representation and as I mentioned before when we were looking at enzymatic cleavage, we were talking about nucleases okay. We were talking about ribonuclease, which is going to cleave ribonucleic acid. We were talking about deoxy ribonuclease which is going to cleave deoxy ribonucleic acid.

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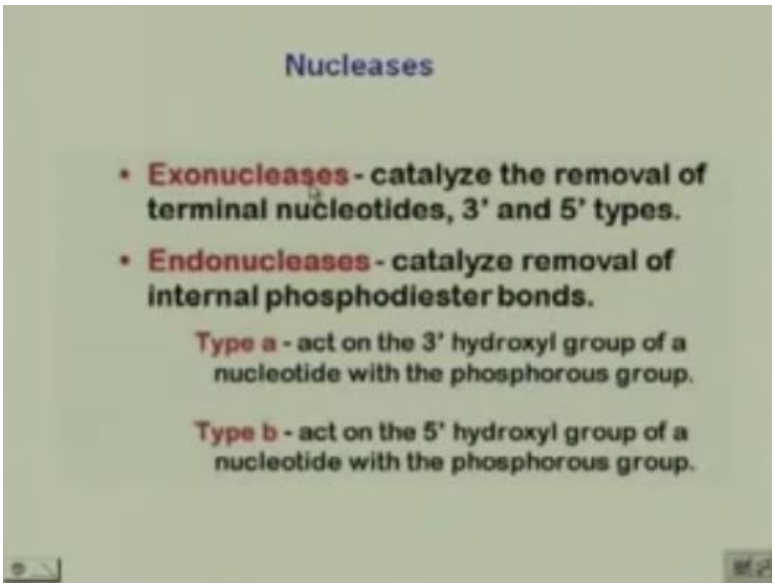

There are two types of nucleases. There are two types; the two types are exonucleases and endonucleases. Exonucleases chop of nucleotides from the ends okay. So when you have a nucleotide and you are chopping it off from the end then you have an exonucleases, you have an endonucleases when you remove the internal phosphodiester bonds that is something that is within the chain again you have two types there because you have two bonds.

You can remove at the 3 prime position or you can cleave at the 5 prime position. If you act

on the 3 prime hydroxyl group of a nucleotide, it is type A. If it acts on the 5 prime hydroxyl group, it is type B okay.

So the two types of nucleases are exonucleases and endonucleases. Exonucleases are like, how would you, what is an analogous case for proteins, an exonuclease and analogous enzyme for proteins, remember when we did the C-terminal you had a carboxypeptidase, what did that do, chop off from the carboxy terminal of the polypeptide chain, but if you have trypsin or chymotrypsin, what did that do, chop off in the middle, it cleaved in the middle okay.

So an analogy for exonuclease in the case of a polypeptide chain would be that for carboxypeptidase. The endonuclease is for two types okay.

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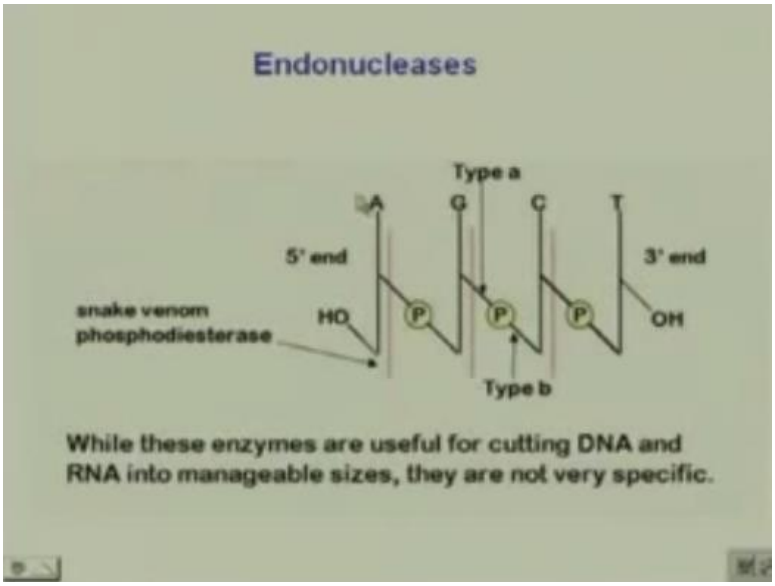

We have the 5 prime end and the 3 prime end. The 5 prime, we have it acting a type A acts on the 3 prime hydroxyl group. So we have our AG, what is this part, this is the 3 prime of this right. This is the 5 prime of C, are you following. We have A G C T in this sequence, we have a 5 prime end for each, we have a 3 prime end for each right.

This is the 3 prime end of the G, but it is the 5 prime, let us go back one, what do we have here, the 5 prime end and the 3 prime end. This is the 3, so this phosphate, what is this, this is the 3 of what, of C. This is the 3 of G. So if you cleave here, first of all you are in endonuclease and type A, is that clear right.

So when you are cleaving this is the 3 prime end and you have to remember. This is the 3 prime end. This is the 3 prime end of G. What is this, of C right. So if you now look at the cleavages what do we have here, if the cleavage is here, it is cleaving at the 5 prime end, so it is type B. If it is cleaving here where the red dotted lines are, it is cleaving at the 3 prime end, it is type A. For example, the phosphodiester is present in snake venom is a type A endonuclease, it just chops off the RNA okay.

So these enzymes are actually used for cutting DNA and RNA into manageable sizes and there used a lot in microbiology, molecular biology where you have these specific genes that are tailored to what you want to make, which protein you want to make. You want to make a mutation in a polypeptide chain. You know the genetic code, you know what amino acid you want and you know what basis you want for that amino acid to be made.

So what you have to do is in your DNA sequence you have to change that set of bases okay. This is routinely done and it is called site directed mutagenesis, you call it recombinant DNA technology where you have the set of bases that you can change, to change what, to change the protein that you are going to synthesise. So if you want of change a specific amino acid, the rest of the chain is all the same. You cleave at a specific position of your original DNA.

You change it to whatever you wanted to be and then you have the protein expressed in bacteria and ones is expressed in bacterial, then you have the mutated protein okay, because the DNA, what you do is you use the machinery of the bacteria to make the protein for you that is what you are doing okay.

You have the DNA, you have changed a particular sequence of the DNA, once you change that what is going to happen, a different protein is going to be formed why because it is going to go from DNA to RNA to protein. So the message that the messenger RNA is going to get is going to be different than the original case because you have already changed the base or a set of bases. Now when you use the bacteria to make the protein for you, the protein is going to be the changed protein okay.

A lot of routine studies are done in protein chemistry to understand the effect of certain amino acids. For example, in ribonuclease, you know that histidine 12 is important for your activity. You change histidine 12 to alanine and then you check for the protein activity. You

will not get any activity why because histidine 12 is crucial for the ribonucleolitic activity of the protein.

In this way you figure out which amino acid residues are important in determining what the mechanism of the reaction is basically okay.

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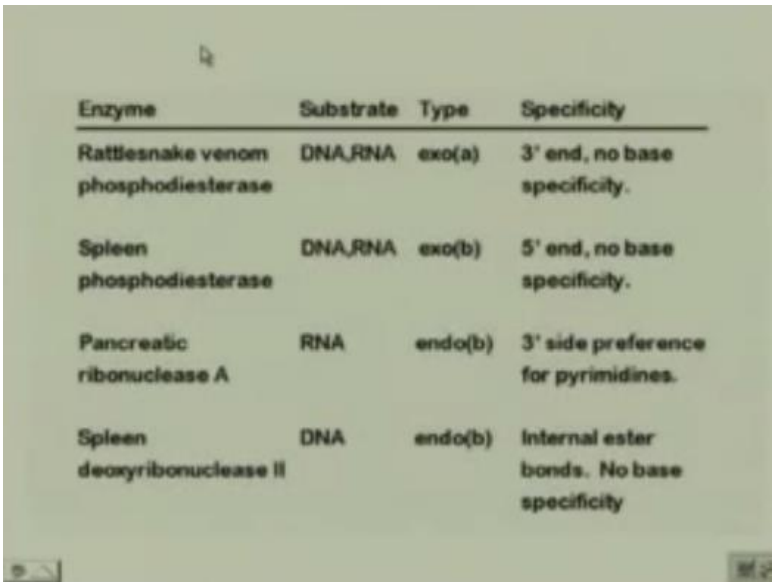

There are these different types of basically nucleases, we have rattle snake venom here or snake venom, these are mostly nucleases okay. This cleaves DNA and RNA, that is why its snake venom in the first place. It cleaves exo(a), what is that mean, the 3 prime end chopping off one nucleotide at a time and there is no base specificity, so it will just chop off you DNA or you RNA okay, rendering any protein synthesis impossible.

You have pancreatic ribonuclease A, that is on the 3 prime end; it has a preference for pyrimidine, it's the type is endo, it is an endonuclease because it cleaves in the middle and it is a B type okay. This is as much as we are going to do about the endonucleases or the nucleases in general because we studied the mechanism of ribonuclease in detail okay.

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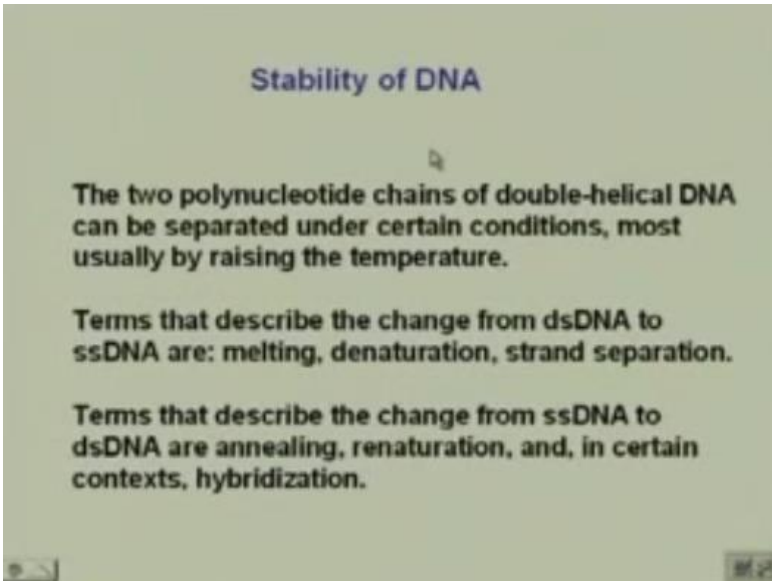

Now we are going to study what interactions of DNA can be disrupted by disrupting the chains or by separating of the chains. So what we have is there are certain terminologies that are used here. We have dsDNA, what is this mean, ssDNA, dsDNA, double stranded DNA going to single stranded DNA okay.

Because this is what is going to suppose we add such an agent that is going to disrupt all hydrogen bonds, what is going to happen, the chains are going to separate. So we are going to go from dsDNA to ssDNA okay. So we have the strands hydrogen bonded separated. The process is that we rather the terminologies that we are going to see are melting, denaturation, strand separation then the terms that describe the changing from ssDNA to dsDNA.

What are we doing then, we are reforming so it is called annealing, renaturation and sometimes hybridisation okay. We even have a process that is called zippering; you just have one you start linking one set of bases and the rest zip sub by itself okay.

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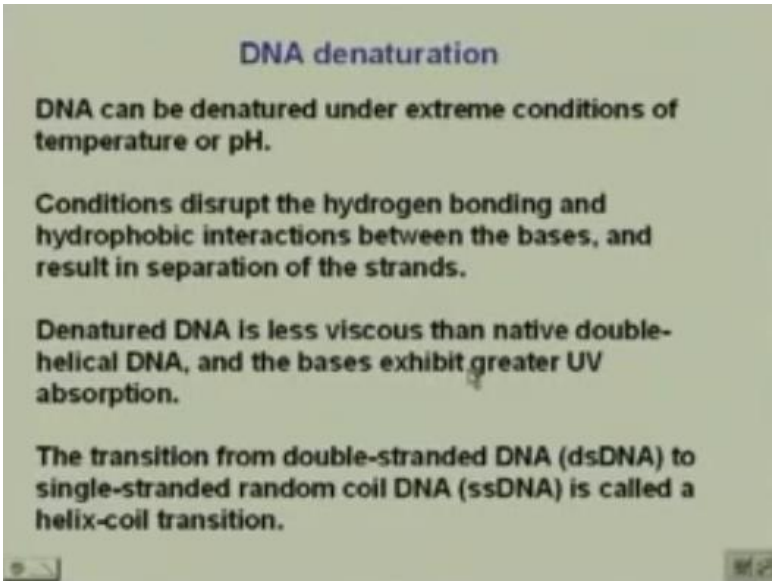

Now how can DNA be denatured. DNA can be denatured under extreme conditions of temperature of pH. What do we do, we want to disrupt any of the interactions that are responsible for stabilising DNA as simple as that. So we want to disrupt either the hydrogen bonding or the hydrophobic interactions or the stacking interactions whatever intercalation just to separate the strands.

Denatured DNA is less viscous than native double-helical DNA and the bases exhibit greater UV absorption. We will see that in a minute. What we have here is denatured DNA is less viscous, why would that be. When we have normal DNA, we have a double stranded DNA that is solution would render the solution more viscous why because you have two strands that have to be kept together always because of the stacking interactions, the hydrogen bonding or whatever forces are holding it together.

So the solution is going to more viscous. Once you separate the strands, what can happen is within the strands, you can get some coiling, so what is going to happen, is your solution of the DNA is going to be easier for it to flow. So it makes it less viscous. The bases exhibit greater UV absorption. Let us go the analogy of a protein. You have a protein a tryptophan says, that has where do you monitor the UV of proteins at 280 nanometres.

You have tryptophan that is embedded in the centre of the protein, you unfold the protein. The tryptophan can be seen right. So your absorption is going to increase the same thing here. The absorption, the UV absorption that you see for the nucleotides is due to the bases. So if the bases are always involved in an interaction within themselves, you cannot see as much

but as soon as you open up the strands, what is going to happen, you will have greater UV absorption.

So we have the basis exhibit greater UV absorption and the DNA is less viscous and this transition from double stranded DNA to single stranded DNA is very commonly called a helix-coil transition okay because you are going from the helix to the coil, so this is exactly what is happening.

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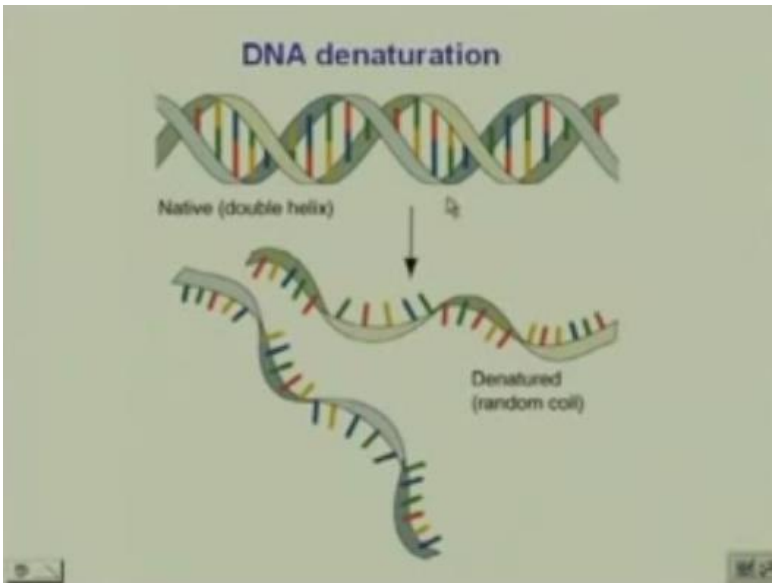

You have a double helix, you are disrupting it, DNA denaturisation and you have a coil. So this is helix to coil transition okay, a dsDNA going to an ssDNA.

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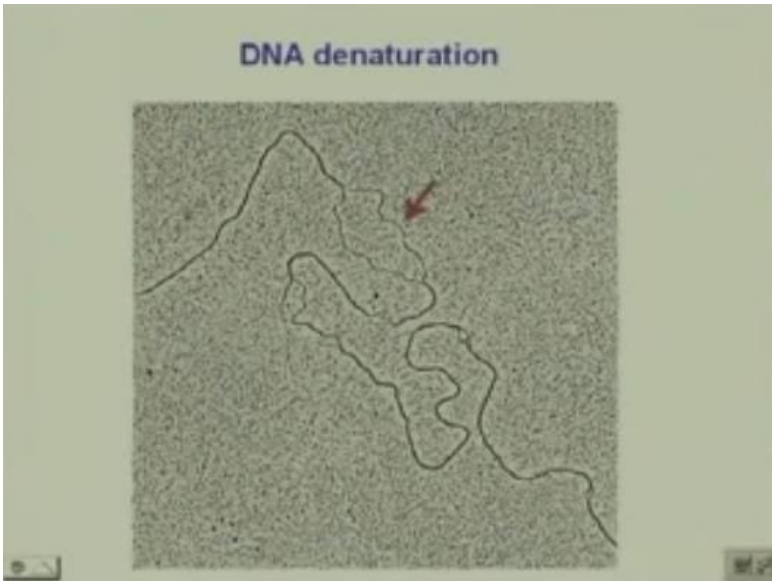

This is an actual picture of, you see how this strand, this is actually the double helix and how

the double helix has opened up here where the arrow is, can you see that, the double helix (()) (28:05) there is a strand going down here, then it is single helix again and then slightly double helix, not well, all of it is double helix, it looks like a single thread here, but it is opened up here okay. So what has happened here, it has denatured okay. It finds usually DNA repairs itself okay. If there is some problem, it will form the double helix back again itself okay.

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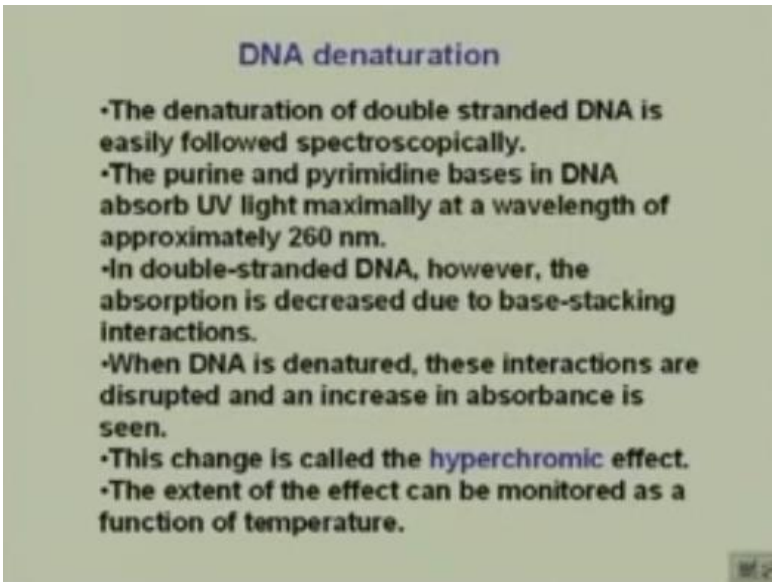

Now we can follow the denaturation of dsDNA by spectroscopy. The bases have a maximal absorption at 260 nanometres not 280 like proteins. In double stranded DNA, the absorption is decreased due to the base-stacking interactions because you cannot see as much as you would see when they are single stranded. When DNA is denatured, these interactions are disrupted and you see an increase this is called as hyperchromic effect why because it is more and the extent of the effect can also be monitored by a function of temperature.

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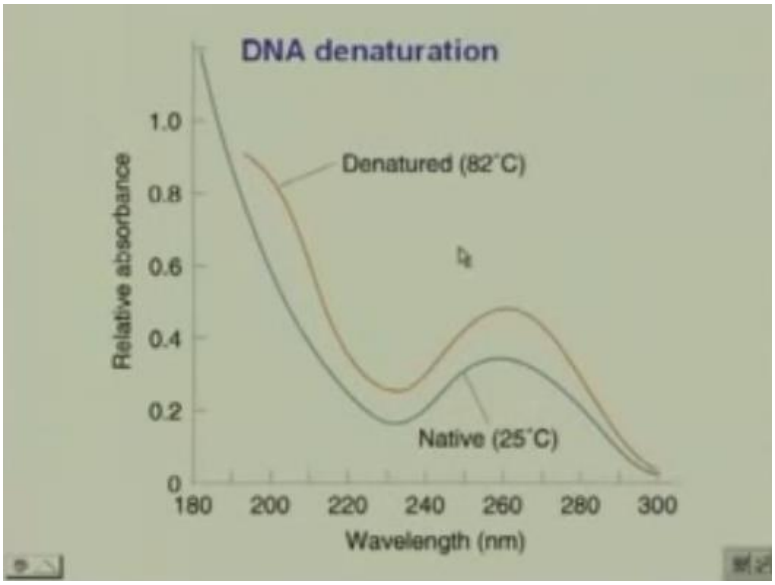

So let us see what we get. So this is native DNA, the blue line at 25 degrees centigrade where is the maximum 260. If I did the same for proteins, what would it look like, I would have something that, it comes down here and it goes up here and I would get a maximum at 280 right for a protein. When the DNA is denatured you increase the temperature so you have disrupted the DNA interactions, they are now single stranded, so you have rendered a helix-coil transition, what has happened to the absorption, it has increased and you now know why it increases okay.

So you have a relative absorbance of the DNA that increases on DNA denaturation okay, because there are stacking interactions, there are hydrogen bonding interactions in the double stranded DNA that are not going to allow an absorbance as high as it could be in the case of a single stranded state.

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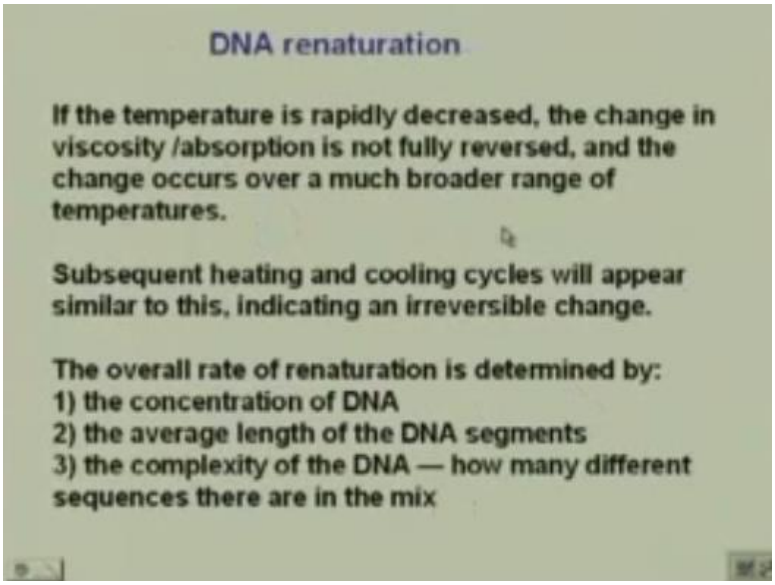

So we have denaturation. Now what happens in some cases is you cannot reverse the situation, its irreversible denaturation. Now what happens is if say the temperature is rapidly decreased, then the change in the viscosity of the absorption that is absorbed cannot be fully reversed and the change occurs over a broader range of temperatures. We will see what that means.

Sometimes what happens is because now if you just look at say the strand like this, now what are you intending, if you separate the strands out altogether then what is going to happen and if you result in a cleavage of the strands to, it is unlikely that they will coming together right. So what happens is with increase in temperature with certain agents also it is not possible for the renaturation of DNA.

The overall renaturation actually depends upon the average length of the DNA segments okay. If the segments are small there is a possibility that they are going to find the complementary base strands and join up to form the double stranded helix. Then it also depends upon the concentration of DNA okay. If the strands are too far apart, then it is unlikely that they are going to find the partner DNA and also the complexity of the DNA.

What do we mean by the complexity of the DNA, we have to remember look for complementarity in the bases right? So for looking for complementarity in the bases, it might not always be possible to find the same stretch of DNA that is going to act or form the double-helix back together again okay.

So we have the overall rate of renaturation determined by these specific factors, the concentration of the DNA, the average length of the DNA segments and also the complexity of the DNA.

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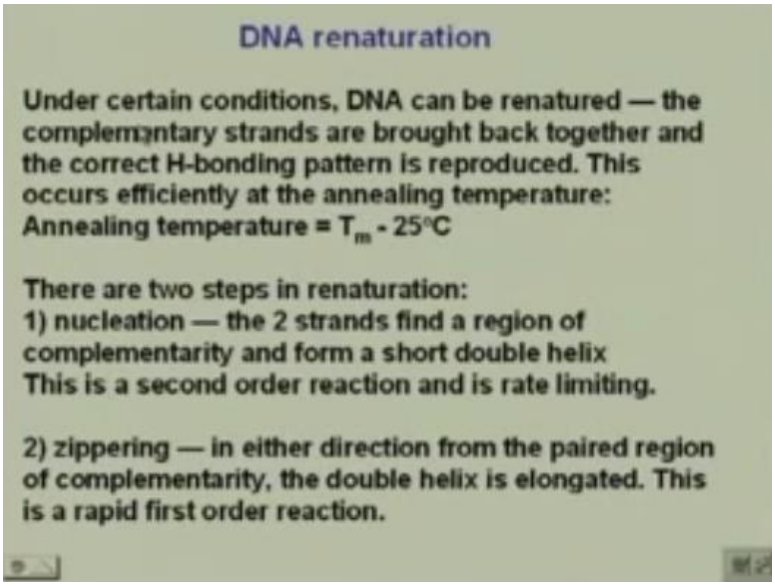

Now under certain conditions DNA can be renatured. When we say renatured, in this case what do you mean, how is it different from the renaturation of proteins. When we denature a protein we are unfolding the polypeptide chain right. But when we are denaturing DNA, we are separating the strands right.

But for the protein, the amino acids are still linked to one another. So when we remove the denaturing agent, for example urea, whatever has been used usually temperate denaturation is not always not renaturable, but suppose we have urea and the solution when we have denatured the protein, we have just prepared the polypeptide chain back again. We remove the denaturing substance what is going to happen.

The protein will fold back. In this case, the DNA has to find its complementary strand right. So under certain conditions DNA can be renatured where the complementary strand can be brought back together why because only then are you going to get the proper double stranded linear double-helix right or rather the double-helix structure because you have to have that ladder formation first, where you are going to have complementary base pairs.

And you have to have the correct hydrogen bonding pattern reproduced again, only then can you renature the DNA back to where you started from. Now this occurs at a temperature

called the annealing temperature, very efficiently that is TM minus 25 degree centigrade. Now what the TM is, it is called the melting temperature of DNA.

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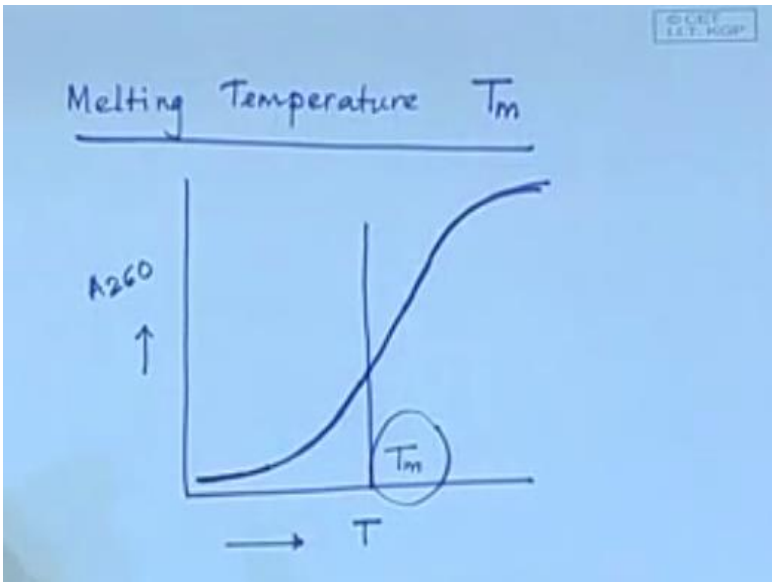

The melting temperature of DNA is referred to as Tm. What are you monitoring here, we are monitoring the melting of DNA. The melting of DNA is basically the separation of the strands helix-coil transition that is going to render the DNA structure disrupted. So we have to have a specific measure of how we can do the, what can we measure here, tell me what can we measure here.

What did we use when we measure the denaturation at for the temperature cases, we measured the relative absorbance, so I can measure the absorbance and now what nanometre, what wave length am I going to use, 260 right? This is going to be my temperature scale. I am going to monitor the absorbance with temperature as I increase the temperature what is going to happen to my double stranded DNA, it is going to come apart right.

As it comes apart, what is going to happen to the absorbance, it will increase, that is exactly what happens. It will increase, but will it keep on increasing, it will come to a point where all the bases are exposed, so what will happen to the absorbance then, it will not increase anymore basically. So we will get something like this. Now so what sort of a DNA do I have here, a helix, a dsDNA and what do I have up here a single stranded DNA, a coil.

So I have a transition, the midpoint of this curve is what is your DNA okay. So basically somewhere here would be the Tm of this. So what you are doing this, when you increase,

now what did we do in the previous one that I showed you, we had the DNA curve, you are monitoring it at different wave lengths right and we found out that the maximum absorbance was at 260 nanometres right.

Now this maximum absorbance at 260 nanometres increases, when we increase the temperature, so what is now done is we are increasing the temperature and I am monitoring the absorbance at 260. We know that the strands are being separated so because why because the hydrogen bonds are being disrupted between the bases. You are disrupting the structure; you have this increase. The midpoint of this is the Tm.

What we are going to look at is factors that are going to affect the Tm okay. Now before we get into that, we have the renaturation that we were talking about. So we have a Tm minus 25 degree centigrade that is going to be an efficient annealing temperature, where the strands are brought back together okay. So whatever the Tm is minus 25 degrees centigrade, it is kind of a rule of thumb that is the annealing temperature.

Now in the, there are two steps in the renaturation. First, there is a nucleation, this nucleation is where the two strands find a region of complementarity and they form a short double helix. Then there is zippering, in either direction from the paired region of complementarity, the double helix is elongated and you understand that only if it finds a correct set otherwise what will happen, it will form a bulge right.

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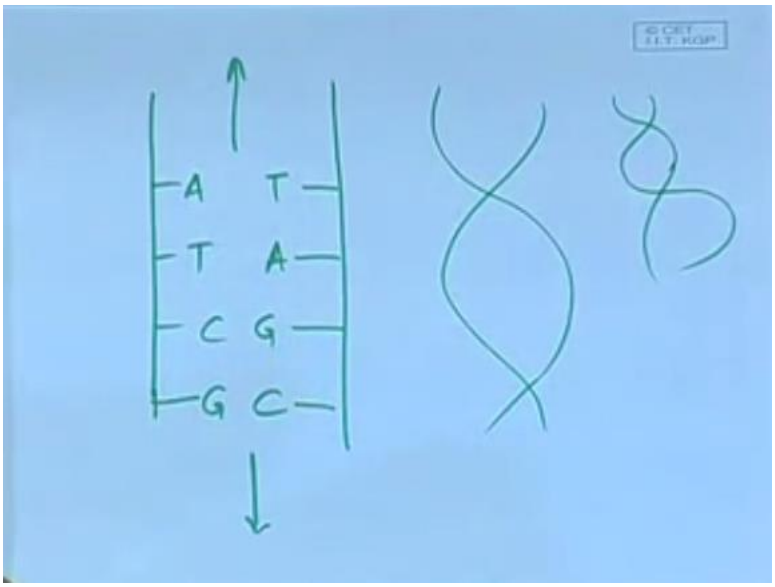

Suppose what are we looking at here, we have two strands, we have two strands A T C G. We

have other strands floating around, but say it is found this part that is complementarity. So we have T, what do we have here, A G C, so this part it forms the double-helix within this part and then it zips up in both directions right. So then it is going to form something like that once it zips up.

But if it so happens that this part is complementary, but his part is not, then what are you going to have here, you are going have some part that looks like a proper double-helix, but some parts that sticking out like that right. So then you would have a bulge, you would not have a proper renaturation okay.

It has to find the nucleation step is important, that is rate limiting in finding the two strands where you have the complementary region and then it zips up in both directions and if it finds a right kind of base pairs obviously it is going to be a very fast and it is a first order reaction and it is pretty rapid okay.

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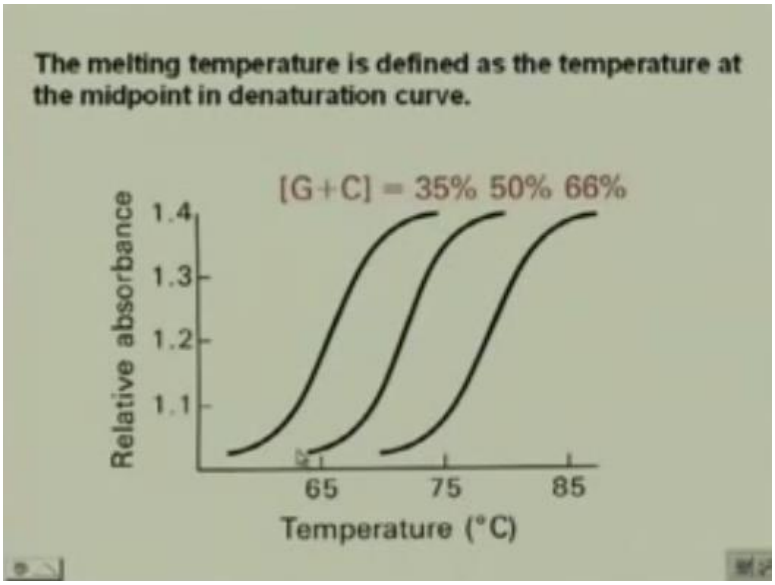

Now we have different melting temperatures. This is what we would call then a denaturation curve like the one I showed you here.

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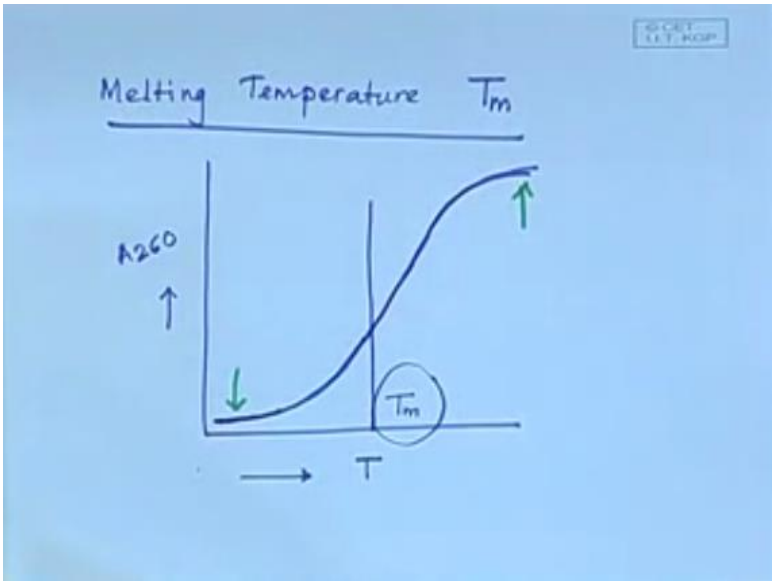

What do we have here a denaturation curve, why is it called a denaturation curve, what do we have here, we have helix, here we have coil, here we have double strand, here we have single strand. So we have denatured it. So this is a denaturation curve and the midpoint of that will give me the Tm.

Now look at what I have three denaturation curves here. So I have three corresponding melting temperatures of three different DNA samples that have differences in their G plus C content. What is a G plus C content; a G-C base pair content. The G-C percentage has increased from left to right and this has resulted in an increase in the Tm why.

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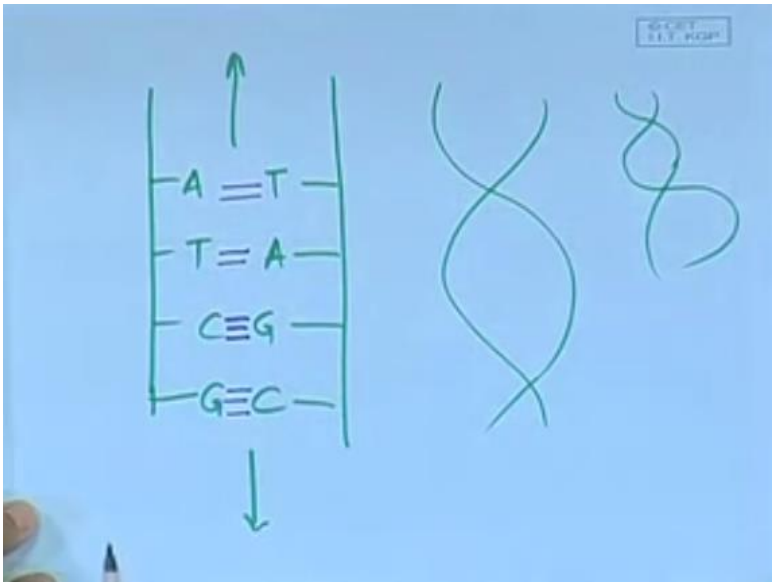

What happens in the G-C pairing? In the G-C pairing, I have triple bonds. I have double bonds in my A-T pairing. So the higher amount of G-C that I have it is going to be more

difficult for me to (()) (43:18) the strands, so the melting temperature is going to be higher okay.

So the G-C content is important in determining what the melting temperature is okay. So the higher that we are going to see other factors also of DNA, but the higher the G-C content, the higher the Tm why, because it has to disrupt in that case three hydrogen bonds to separate the strands.

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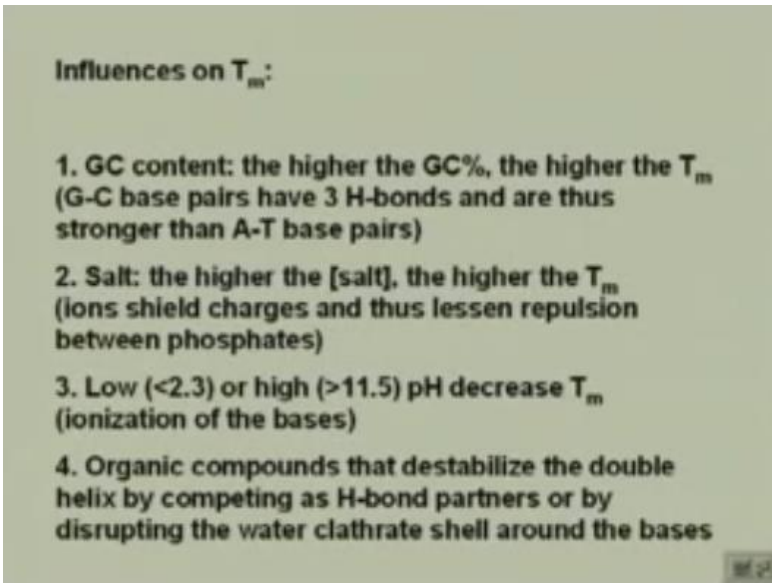

So we have to look at the influences on the melting temperature, the G-C content. Now we know why okay. The higher the G-C percentage, the higher the Tm because the G-C base pairs have three hydrogen bonds and are thus stronger than A-T base pairs that have two hydrogen bonds.

What are the factors do you think might be important? You know what interaction stabilise the DNA right. We know what interactions stabilise the DNA, so the basic idea in this case is going to be disrupting those interactions right. So what else is stabilising in the DNA. We have electrostatic repulsion right so salt, what is salt going to do. The salt is going to shield the charges, lessen the repulsion between the phosphates and the higher the salt concentration, lesser the repulsion than higher the Tm.

What is going to happen, the higher the salt concentration, suppose you have a large amount of magnesium, what is going to happen, the ions are going to shield the phosphate charges on the backbone, this backbone is going to remain as far apart as possible right because you have

the phosphate.

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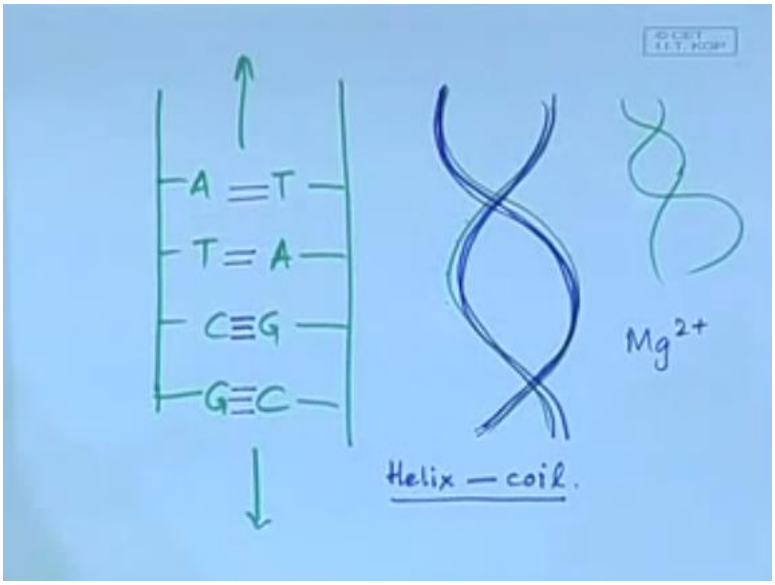

Now if you have salt ions that are going to stabilise this, what is going to happen, it will not be very easy for you to separate the chains because this chains if say you have for the negative charge here, what is going to happen, there is going to be repulsion between the chains right. Repulsion between the strands is what is going to take it a part, because it is essentially what you are doing, is you are going you are resulting in a helix-coil transition that is what you are looking at.

If you are looking at a helix-coil transition what you want is you want destabilisation of your strands right. Now you want this repulsion to be minimum what is going to happen to the repulsion here, if you add salt, if you add Mg 2+, the higher the salt concentration, the higher the Tm right, because you are stabilising the strands. When you have pH, what is going to happen when you have different pH, at low pH what are you doing.

You protonate the bases, as you protonate the bases what is going to happen, you are disrupting the hydrogen bonding right. Again the same thing when you increase the pH, you are disrupting the hydrogen bonding, how is that going to effect the Tm, it will decrease the Tm because you are resulting in a larger disruption. All you have to think about is what effect each of these is going to have on the forces that are stabilising the DNA right.

Then we have organic compounds, organic compounds can act in different ways. One thing is they can intercalate between the bases and what are they going to do, they are going to

disrupt then in that case, the stacking interactions. They can also form more favourable hydrogen bonds with the bases and in that case what are they going to do again disrupt the stability of the DNA, disrupt the hydrogen bonding either the electrostatic repulsion or the hydrogen bonding or the stacking interaction.

Whichever type of interaction is disrupted will result in the separation of the chains okay. So what we learnt was, we learnt how the overall structure of DNA and RNA is formed by the different nucleotide basically coming together. We have the sugar phosphate backbone in both cases, just the sugars being different in the deoxy type and deoxy ribose type and the ribose type.

The bases are the purines and the pyrimidines for both cases; we just have a change from thymine to uracil when we go from DNA to RNA. Then we looked at the hydrogen bonding patterns in each cases where we found that we had two hydrogen bonds between A and T and three hydrogen bonds between G and C and we saw how the stability of DNA can be disrupted by certain factors and what the melting temperature was and what effect the G-C content.

And the A-T content had on the overall DNA stability and other factors like we have temperature also that we have to look at. We have the G-C A-T content that we have look at. We have the pH. We have the addition of ions and the addition of organic solvents. This completes our discussion in nucleic acid. We will begin bioenergetics in our next class. Thank you.