Biochemistry - I

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Nucleic Acids III
Lecture # 22

Third lecture on nucleic acids is going to speak about the stability of DNA and what are the certain factors that are going to lead to the denaturation of the DNA or the renaturation of DNA and how that is extremely important for designing of drugs for the cleaving of the DNA, as I mentioned in the last class where we want to consider that the protein synthesis is inhibited or stopped. If we go back to look at the double helical structure of DNA, we have our sugar phosphate backbone and the specific bases linked together.

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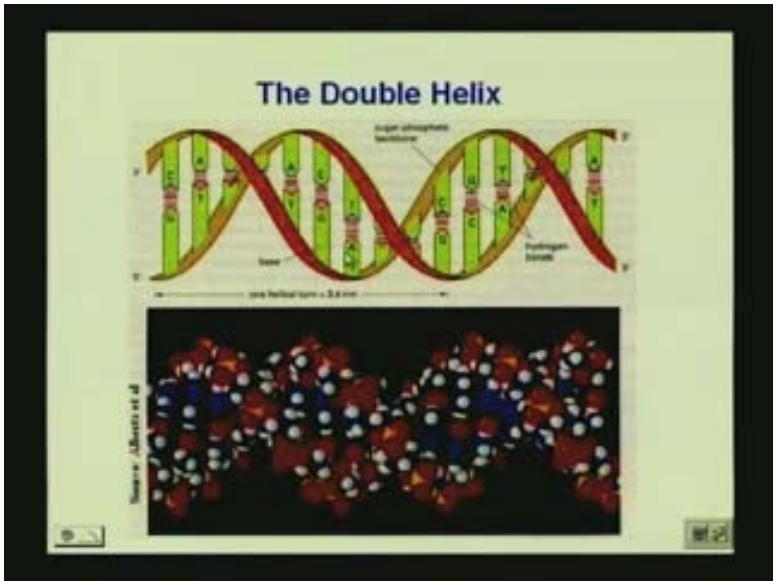

Apart from the hydrogen bonding present here we are going to have certain other interactions that are going to be responsible for the double helix to maintain its structure in solution. The forces that actually result in the DNA structure are electrostatic forces that are mainly repulsion forces. Can you guess why we would call them repulsive forces? Why would the electrostatic force 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 back bone? We have sugar phosphate. What is the charge on these phosphates? They are all negative so the strands were tend to be as far as possible which is going to result in an electrostatic force, that is going to be basically repulsion.

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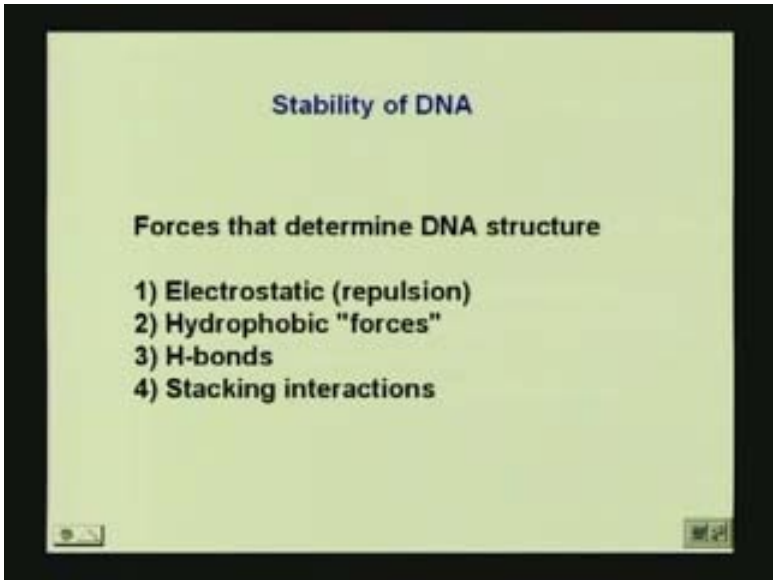

We have hydrophobic forces and hydrogen bonds, obviously between the bases and also possible between the other hetero atoms present in the bases with the water that is around and we have stacking interactions between the bases at different levels. Considering the properties of the Watson Crick base pairing, the base pairings are planar. 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 distracted with any agent that penetrates this region between the bases. We have these four types of forces that actually determine the DNA structure. The base pairs are usually found in the interior of the helix which is going to result in the stacking interactions between the bases.

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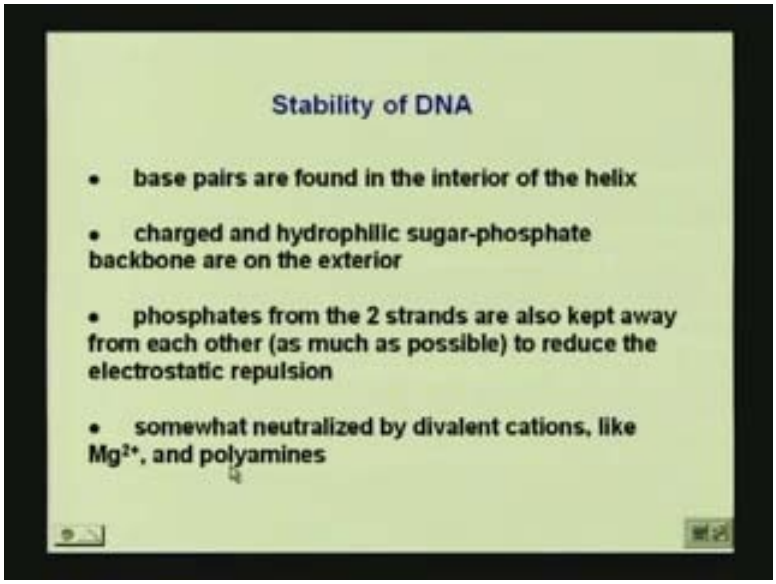

The charged and the hydrophilic sugar phosphate backbone are on the exterior which results in the electrostatic repulsion of the strands. The phosphates from the two strands would obviously be as far away as possible why because they are negatively charged and they would reduce the electrostatic repulsion if they would be as far apart as possible. Some of this is neutralized by poly amines. Why would they be neutralized by polyamines or magnesium? Because they would be positively charged they would counter act the negative charge of the phosphate and result in stabilization. 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 structural DNA.

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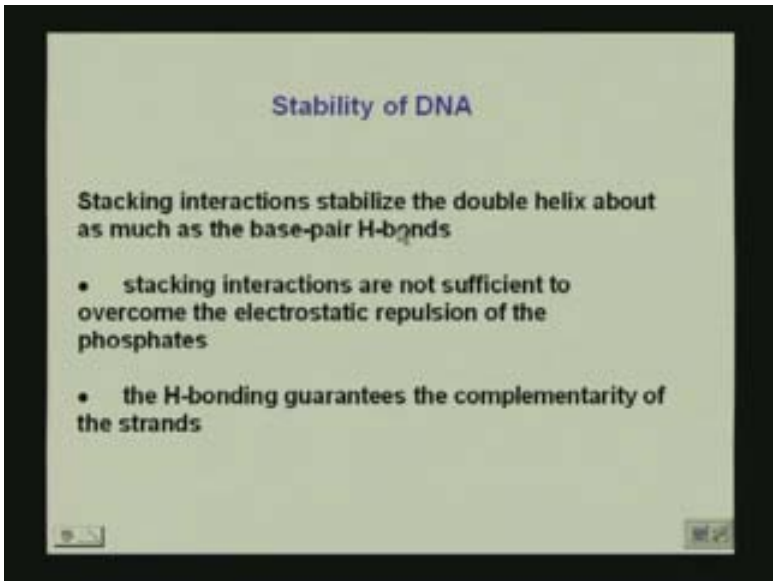

The stacking interactions stabilize the double helix as much as probably close to the base pair hydrogen bonds. Because what do we have in those cases? We have an $\pi - \pi$ interactions. We have a series of such $\pi - \pi$ interactions. The stacking interactions actually stabilize the double helix as much as the base pair hydrogen bonds. The stacking interactions are not sufficient to over come the electrostatic repulsion of the phosphates. The phosphates will remain as far apart as possible that would be stabilized by some positively charged divalent ions or poly amines and the hydrogen bond between the purines and 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 dotted DNA. Then what happens? You have to have perfect complementarity. It is not like RNA where you can have a single strand and you can have a complementarity for some region of the RNA but not for the whole region. So we have these stacking interactions, we have the electrostatic reaction interactions and we have the hydrogen bonding interactions and this is what holds the DNA together.

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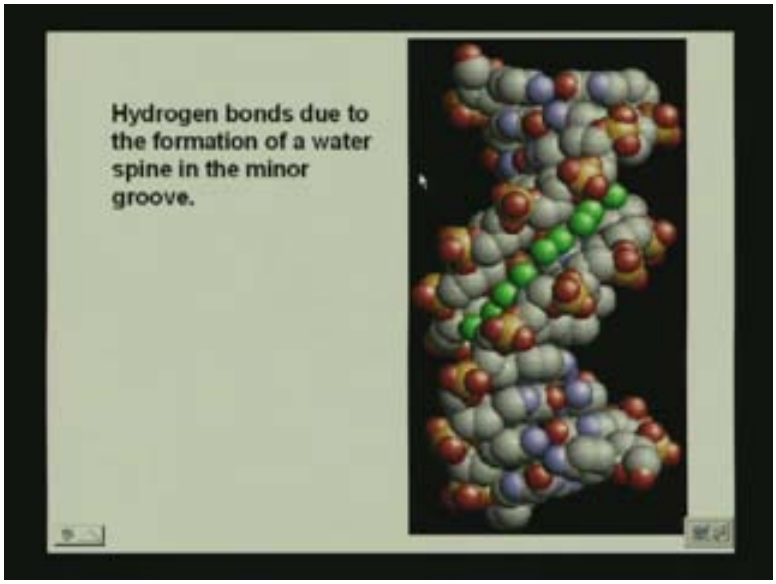

And we also have, this is one picture which we saw previously where we also have an additional hydrogen bonding between water that goes through the minor groove of DNA. We have the major groove that is this large gap here and the minor groove. The minor groove also twists around with the double helical structure. We have these hydrogen bonds also formed in addition to the other types of bonds or the other types of interactions that are observed. DNA usually is quite stable; it actually resists attack in acid and alkali solutions. Can you tell me why DNA is more stable compared to RNA?

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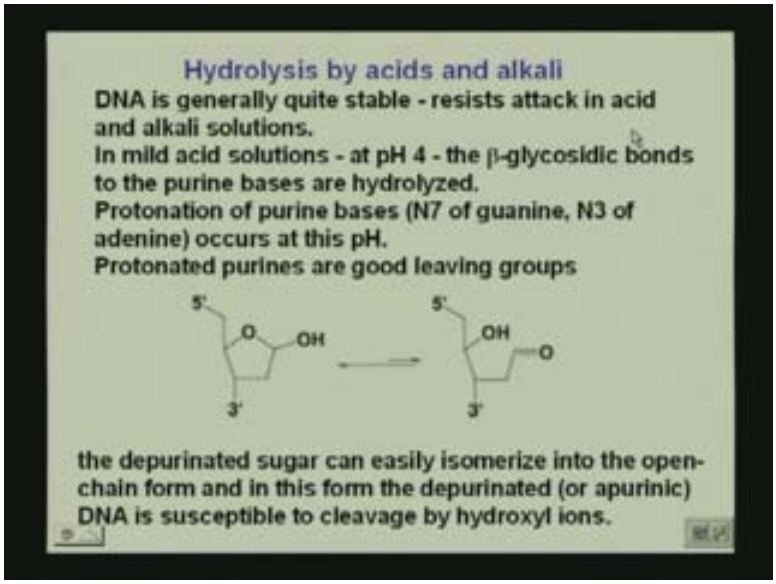

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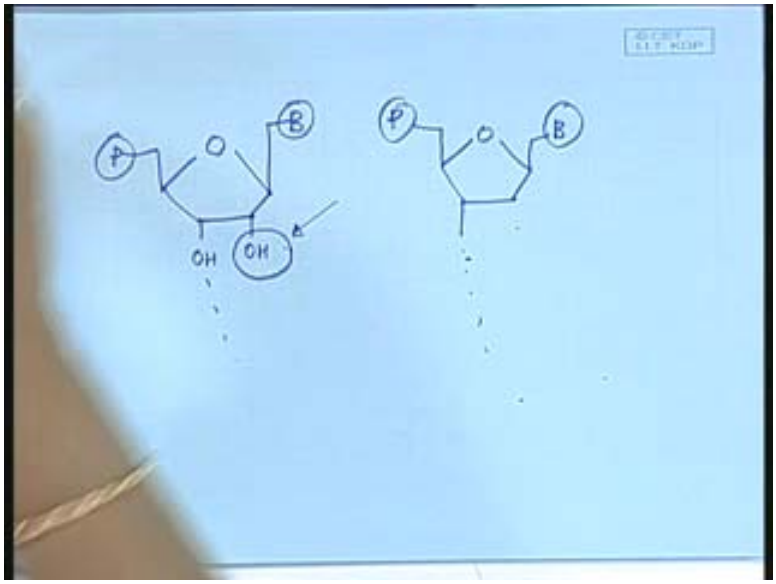

What are we deferring in this? We have our base and phosphate. We have continuation here. What do we have? In RNA we have this; in DNA you are missing that. What is linked here? You have the other strand here, you have the phosphate here then you have the phosphate again another sugar another base and so on and so forth. 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 or enzyme mechanism classes. DNA actually is quite stable and in mild acid solutions at pH 4. In this case we have a different kind of hydrolysis where by the purine bases themselves are hydrolyzed since the OH is absent at the $2^{'}$ positions unlike RNA.

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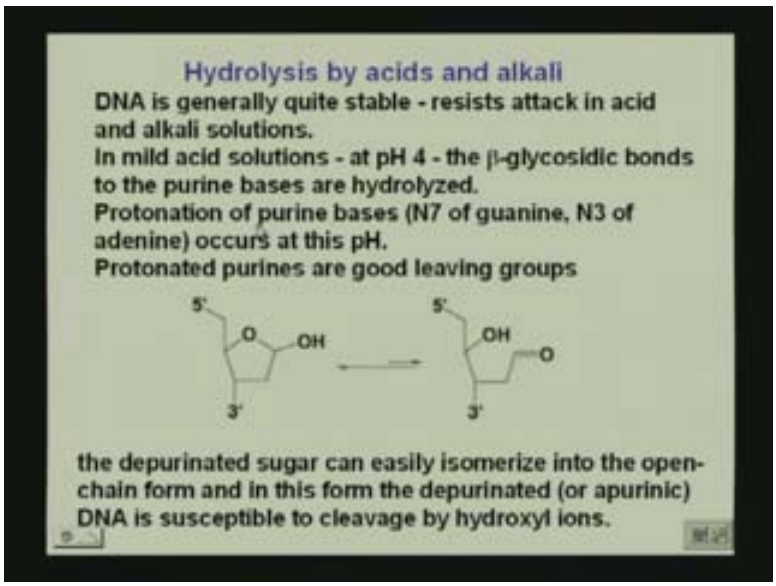

What happens in this case the β glycosidic bond to the purine bases are hydrolyzed. We also have the protonation of the purines bases. What is going to happen if you are going to have the protonation of the purine base bases at the acidic solution? You are going to disrupt the hydrogen bonding. As a result of which you are going to have this hydrolysis which leaves your purines protonated that is going to act as a good leaving group. You are going to have an isomerization of the depurinated sugar. This is the hydrolyzed sugar where you are going to have what is this $5'$ and what does this signify? What is this $5'$ and $3'$ signify? It signifies that this part of a DNA strand.

You do not have the OH here so this is part of the 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 isomerizes to an open chain fall. What have you done in mild acid conditions? You have not only depurinated your DNA; you have also opened up the sugar so that could basically be a problem. What happens in RNA?

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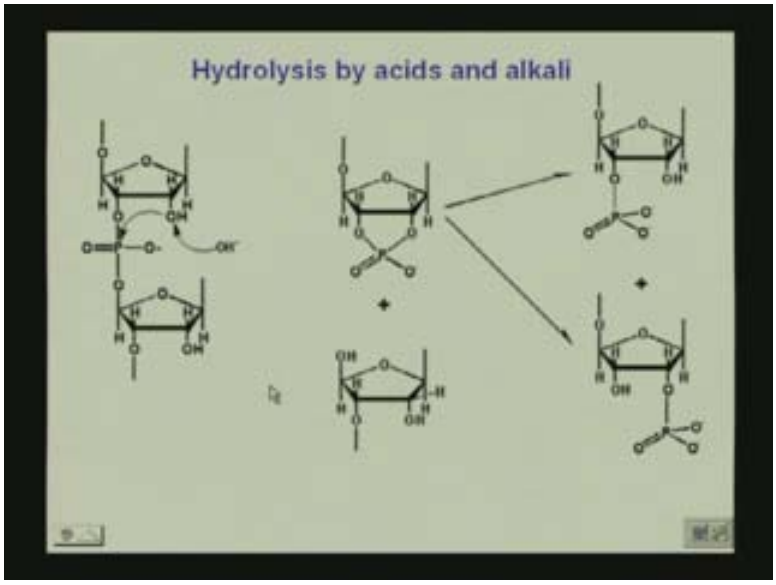

This is RNA now. In RNA what we have here is we have the case where we have the OH at the $2'$ position. The OH at the $2'$ position is going to give you what? This is your OH at the $2'$ position. What is going to happen in that case? You are going to have easy hydrolysis possible with an OH^-. You can have hydrolysis at the OH position. What happened when we considered the ribonuclease mechanism? Histidines 12 and histidine 19 were important in donating a proton. One acted as an acid and one as a base. The roles were reversed in what was called the hydrolysis step. What do we have in a sense? We have a trans phosporlization. This is what is happening. What is this we have a cyclic phosphate intermediate which results in the phosphate being transferred? You see how the phosphate has been transferred.

Where was it originally? You have either it go back to its original form or it is cleaved and so we have this part, which is the rest of the chain and this part is a previous part of the chain. We either have this phosphate remain here or go over to the $2'$ position. This is not possible with DNA, you understand that. Why is it not possible? Simply because you don't have the OH there, so it is not possible but this is easier for the case of ribonuclease. When we look at our RNA, we have it as very unstable and in alkali solution basically because of that $2'$ OH that is present. It results in hydrolysis a possibility of hydrolysis always of the phosphoester diester backbone and this renders your RNA susceptible to strand cleavage.

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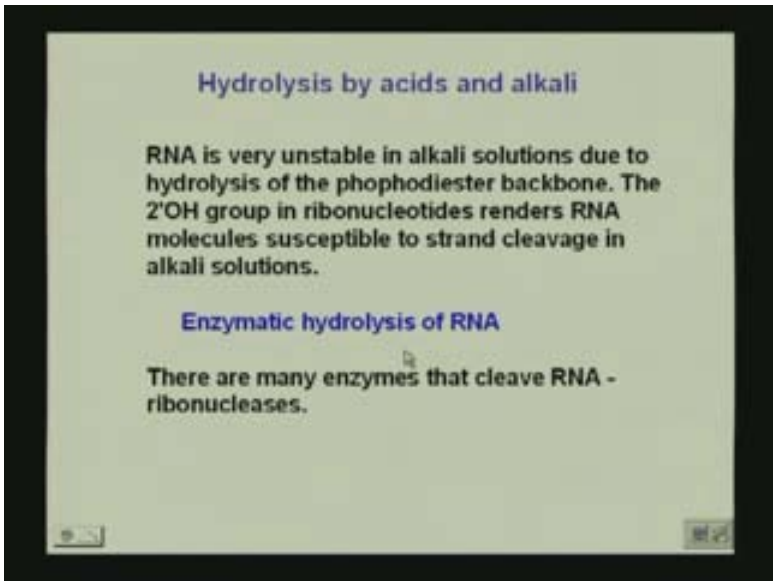

What is going to happen is your RNA strand that was a single strand is going to easily cleave, because of the OH being present there under mild even on the alkali conditions it is going to break up. And we already consider the enzymatic hydrolysis of RNA where there are ribonucleases that are going to cleave RNA in a similar fashion. 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 be your OH^- that is going to attack the $2'$ OH which you cannot do in the case of DNA because it is absent.

We looked at this representation and as I mentioned before when we are looking at enzymatic cleavage we are talking about nucleases. We are talking about ribonuclease which is going to cleave ribo nucleic acid. We are talking about deoxy ribo nucleic nucleases which are going to cleave deoxy ribo nucleic acid. There are two types of nucleases. The two types are exonucleases and endonucleases. Exonucleases chop off nucleotides from the ends, so when you have a nucleotide and you are chopping it off from the end then you have an exonucleases. You have endonucleases when you remove the internal phosphodiester bonds that is something is within the chain. Again you have two types because you have two bonds.

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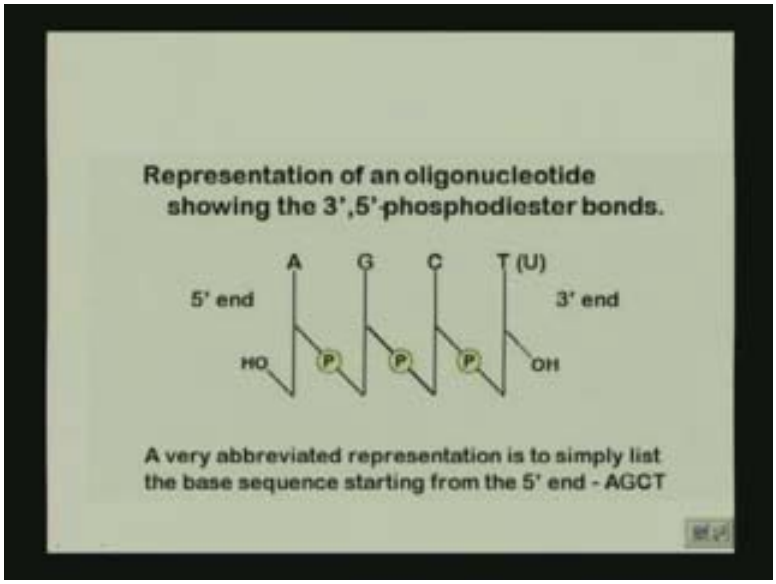

You can remove at the $3'$ positions or you can cleave at the $5'$ position. If you act on the $3'$ hydroxyl group of a nucleotide it is type a, if you if it acts on the $5'$ hydroxyl group it is Type b. So the 2 types of nucleases are exonucleases and endonucleases. Exonucleases are like, what is an analogous case for proteins? An exonucleases, an analogous enzyme for proteins. Remember when we did the c terminal we had a carboxy peptidase. What did that do? Chop off from the carboxy terminal of the poly peptide chain but if you have trypsin or chymotrypsin what did that do? Chop off in the middle it cleaved in the middle. So an analogy for exonucleases in the case of a poly peptide chain would be that for carboxy peptidase.

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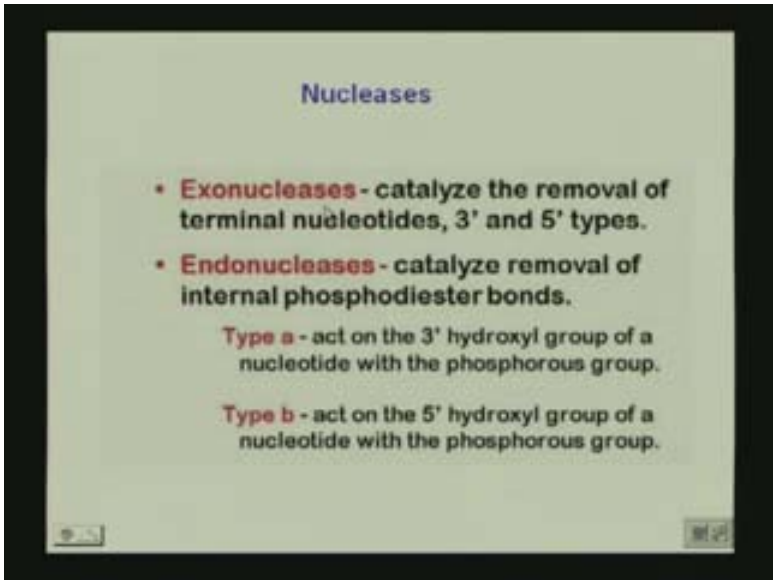

The endonuclease is for two types. We have the 5' end and the 3' end. The 5' we have it acting a type a acts on the 3' hydroxyl group so we have our A G. What is this path? This is the 3' of this. This is the 5' of C. We have A G C T in this sequence we have a 5' end for each we have a 3' end for each. This is the 3' end of the gene but it is the 5'.

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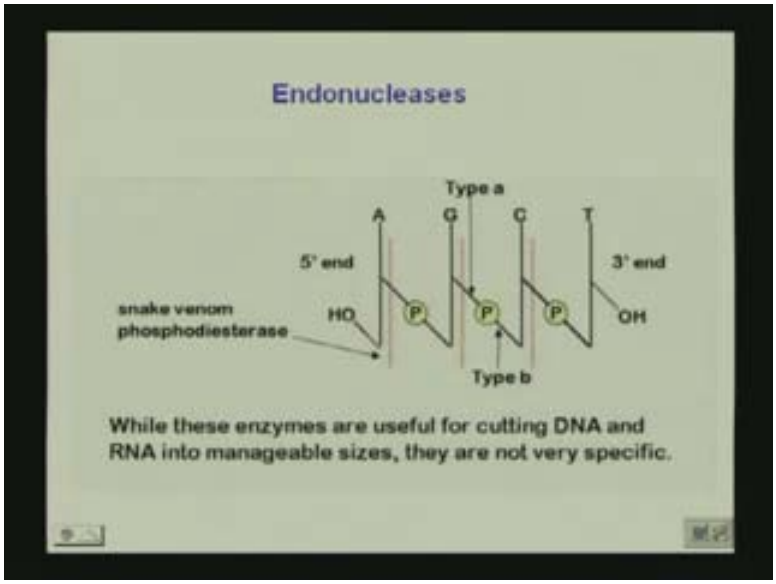

What do we have here? The 5' end and the 3' end. This is 3 so this phosphate, what is this? This is the 3 of C. This is the 3 of G. If you cleave here first of all you are in an endonucleases and type. When you are cleaving this is the 3' end you have to remember this is the 3' end. This is the 3' end of G. what is this? C, so if we now look at the cleavages what do we have here? If the cleavages here it is cleaving at the 5' end so it is Type b. If it is cleaving here where, the red dotted lines are it is cleaving at the 3' end. It is type a for example the phosphodiester present in the snake venom is a type a endonucleases, it just chops off the RNA.

These enzymes are actually used for cutting DNA and RNA into manageable sizes. They are used a lot in microbiology molecular biology, where you have these specific genes that are tailored to what you want to make or 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 bases you want for that amino acid to be made. What you have to do is in your DNA sequence you have to change that set of bases. This is routinely done and it is called side directed mutagenesis. You call it recombinant DNA technology where you have the set of bases that you can change the protein that you are going to synthesized. If you want to 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 what ever you want it to be and then you have the protein expressed in bacteria and once it is expressed in bacteria then you have the mutated protein, 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. You have the DNA; you have changed the particular sequence of the DNA. Once you change that what is going to happen a different protein is going to form, why because it is going 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 a base or a set of bases.

When you use the bacteria to make the protein for you, the protein is going to be the changed protein. A lot of routines 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 alamine and then you check for protein activity, you won't get any activity why? Because histidine 12 is crucial for the ribonucleictic activity of the protein. In this way you figure out which amino acid residues are important in determining the mechanism of the reaction is basically. These are different types of nucleases basically.

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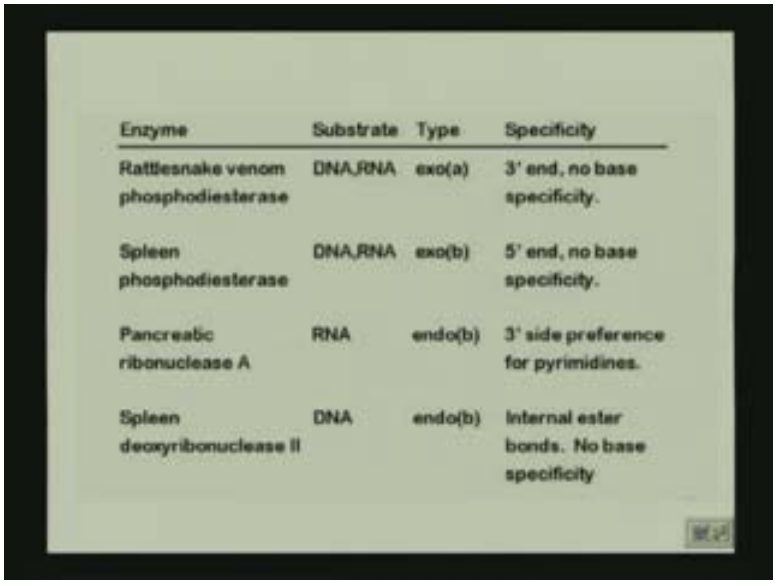

We have rattle snake venom here or snake venom. These are mostly nucleases. This cleaves DNA and RNA that is why it is snake venom in the first place. It cleaves exo (a). What is that mean? The $3'$ end chopping off one nucleotide at a time and there is no base specificity. So it will just chop off your DNA or your RNA. Rendering any protein synthesis impossible, you have pancreatic ribonuclease A that is on the $3'$ end. It has a preference for pyrimidine, it is the type of the endonuclease because it cleaves in the middle. It is a Type b so this is as much as we are going to do about the endonucleases or the nucleases in general because we study the mechanism of ribonucleases in detail.

We are going to study what interactions of DNA can be disrupted by disrupting the chains or by separating out the chains. What we have is there are certain terminologies that they have used here. We have dsDNA. What is this mean? ssDNA, dsDNA Double stranded DNA going to single stranded DNA and because this is what going to happen, 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 from dsDNA to ssDNA. We have the strands, hydrogen bonded separated. The process is that we are rather the terminologies that we are going to see are melting, denaturation, strand separation.

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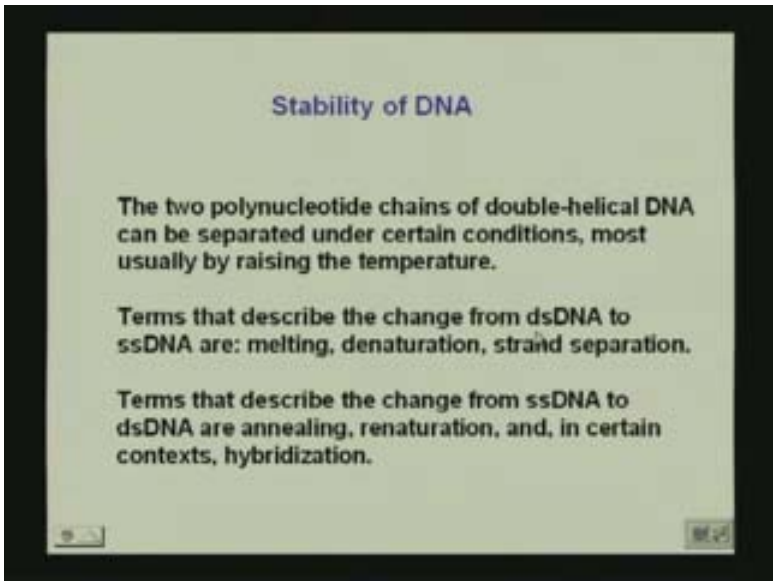

Then the terms that describe the changing from ssDNA to dsDNA. What are we doing then? We are reforming. It is called annealing, renaturation and sometimes hybridization. We even have a process that is called zippering we just have one you start linking one set of bases and the rest zips up by itself. Now how can DNA be denatured? DNA can be denatured under extreme conditions of temperature or pH. What do we do? We want to disrupt any of the interactions that are responsible for stabilizing DNA. 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. 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 in 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 hydrogen bonding or whatever forces are holding it together. So the solution is going to be more viscous. Once you separate the strands, what can happen is within the strands you can get some coiling. 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.

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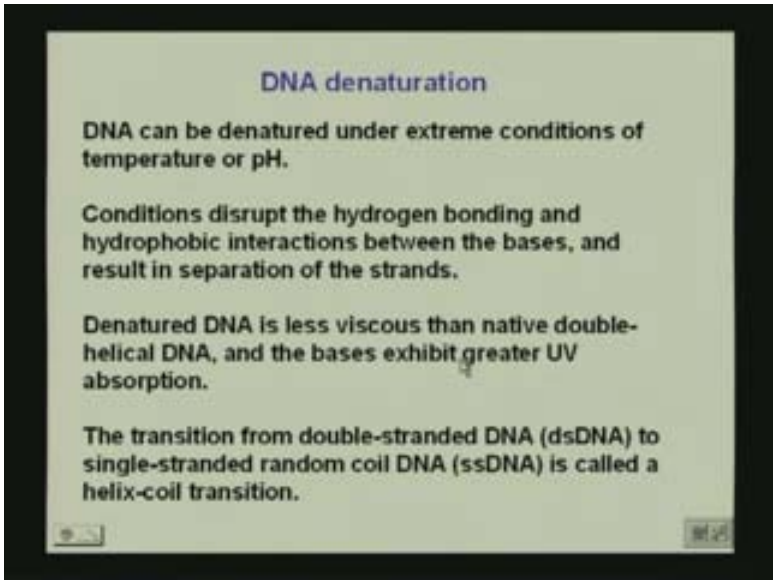

The bases exhibit greater UV absorption. Let us go to the analogy of the protein. You have a protein a tripped fan. Where do you monitor the UV of proteins? At 280 Nano meters. You have a tripped fan that is embedded in the center of the protein. You unfold the protein; the tripped fan can be seen. Your 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. 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. We have the bases exhibit greater UV absorption and the DNA is less viscous.

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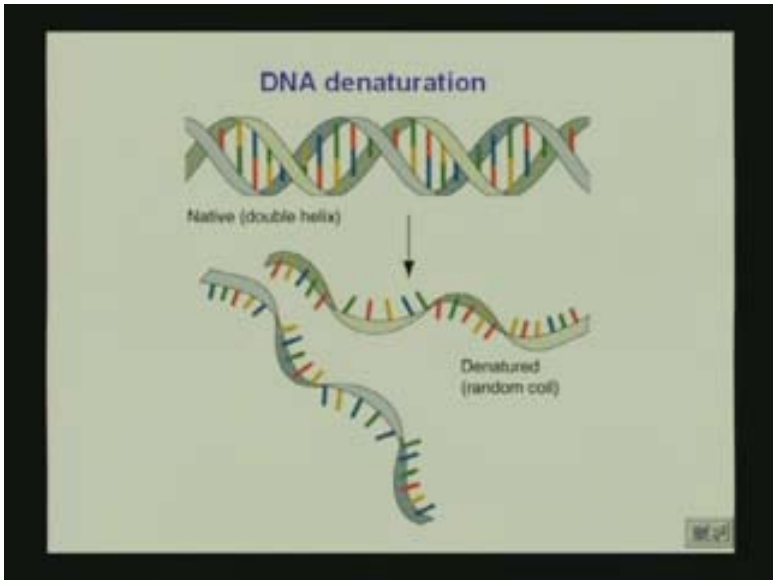

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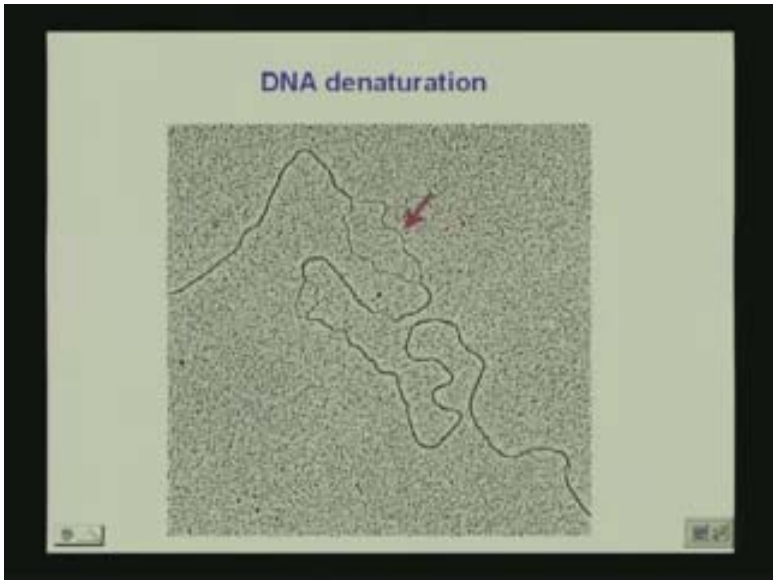

This transition from double stranded DNA to single stranded DNA is very commonly called the helix coil transition because you are going from the helix to the coil. This is exactly what is happening. You have a double helix. You are disrupting it, DNA denaturation and you have a coil. So this is helix to coil transitions, a dsDNA going to an ssDNA. This is an actual picture. 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? 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. So what has happened here? It has denatured. It finds, usually DNA repairs itself. If there is some problem it will form the double helix back again itself.

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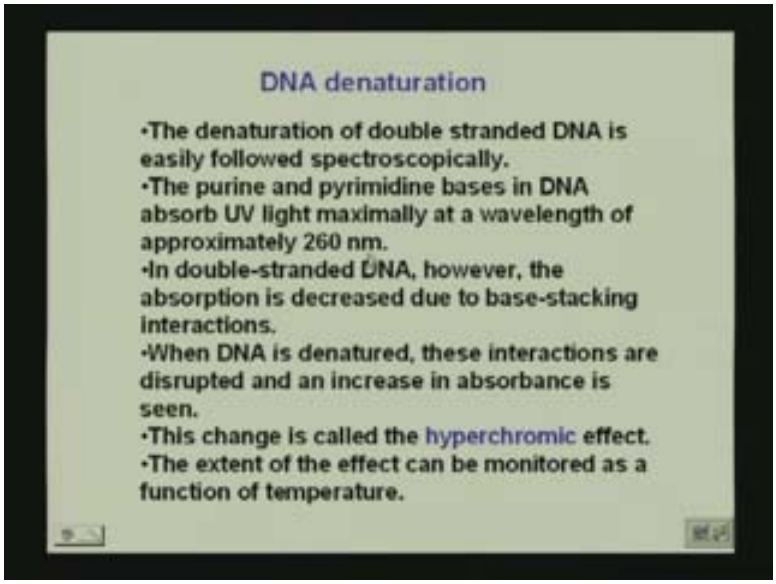

We can follow the denaturation of dsDNA by spectroscopy. The bases have a maximal absorption at 260 nanometers not 280 like proteins. In double stranded DNA the absorption is decreased due to the base stacking interactions because you cannot see as much as you see when they are single stranded. When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

Let us see what we get. So this is native DNA the blue line at 25 degree centigrade. Where is the maximum? 260, if I did the same for proteins what would it look like? I would have something that comes down here and it goes up here. I would get the maximum at 280 nm for a protein. When the DNA is denatured you increase the temperature so you disrupted the DNA interactions. They are now single stranded. You have rendered a helix coil transition. What has happened to the absorption? It has increased and you now know why it increases. You have a relative absorbents of DNA that increases on DNA denaturation because there are stacking interactions, there are hydrogen bonding interactions in the double stranded DNA that are not going to allow a an absorbents as high as it could be in the case of a single stranded.

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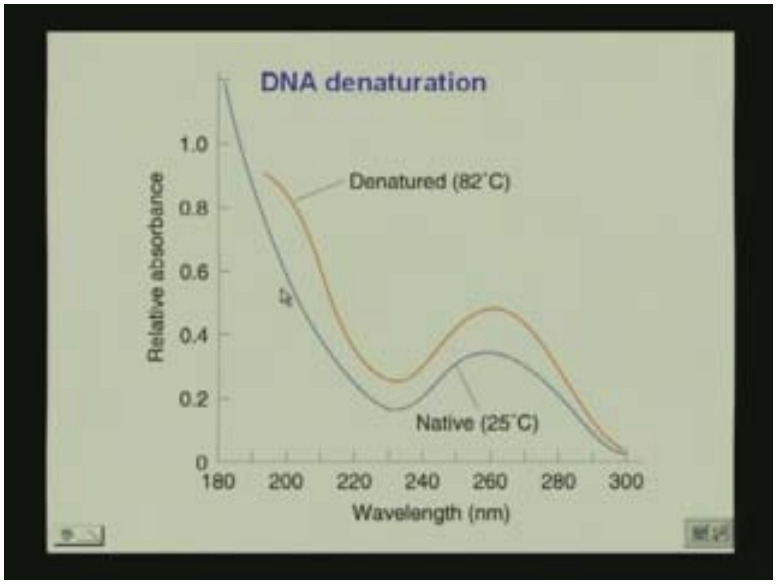

We have denaturation. What happens in some cases is you cannot reverse the situation. It is irreversible denaturation. What happens is if the temperature is rapidly decreased, then the change in the viscosity of the absorption that is absorbed cannot be fully reversed and the change occurs over a broader range of temperatures. We will see what that means and sometimes what happens is because now if you just look at say the strand like this.

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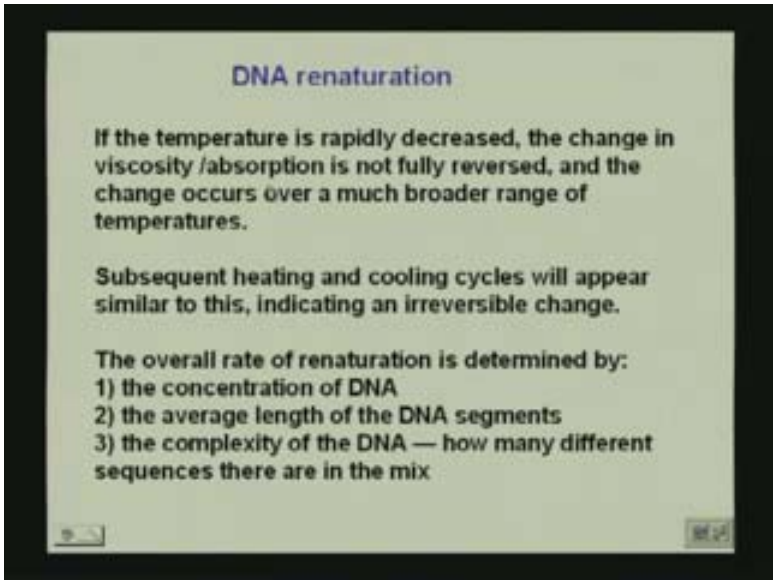

What you are intending? If you separate these strands out all together then what is going to happen and if you result in a cleavage of the strands 2 it is unlikely they will be coming together. What happens is with increase in temperature or with certain agents also

it is not possible for the renaturation of DNA. The overall renaturation actually depends upon the average length of the DNA segments. If the segments are small, the possibility that they are going to find a complementary base strands and join up to form the double stranded helix. Then it is also depends upon the concentration of the DNA. 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?

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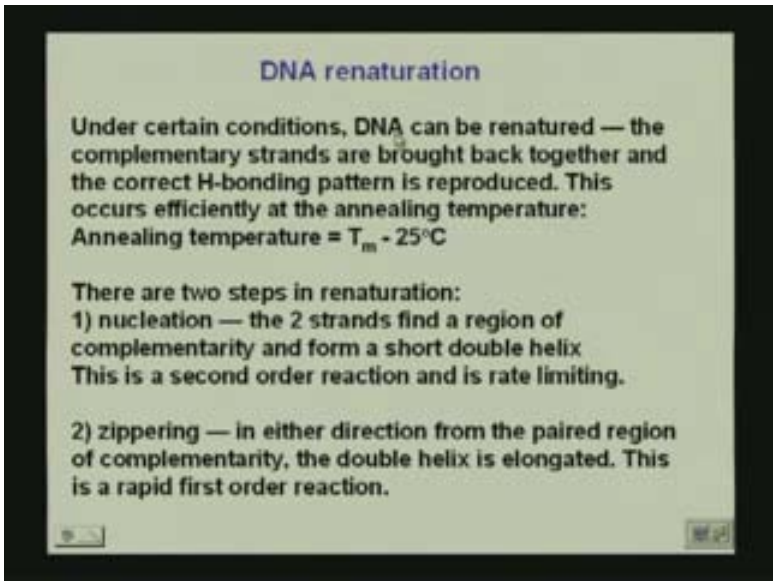

We have to remember for complementarity in the bases, so for looking for complementarity in the bases, it might not always be possible to find the same stretch of DNA that is going to act or form the double helix factor together again. We have the over all rate of renaturation determined by these specific factors. The concentration of DNA, the average length of the DNA segments and also the complexity of the DNA. Under certain conditions DNA can be renatured. When we say renatured in this case what do we mean, how is it different from the renaturation of proteins? When we denature the protein, we are unfolding the poly peptide chain but when we are denaturing DNA, we are separating the strands but for the protein the amino acids are still linked to one another.

When we remove the denaturing agent for example urea what ever has been usually temperated denaturation is always not renaturable but suppose we have urea in this solution and we have denatured the protein. We have just prepared poly peptide chain back again. We removed the denaturing substance. What is going to happen? The protein will fold back in this case the DNA has to find its complementary strand. So under certain conditions 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 rather the double helix structure because you have to have the 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. This occurs at a temperature called the annealing temperature very efficiently that is T_m -25 ^{0}C. What the T_m? It is called the melting temperature of DNA.

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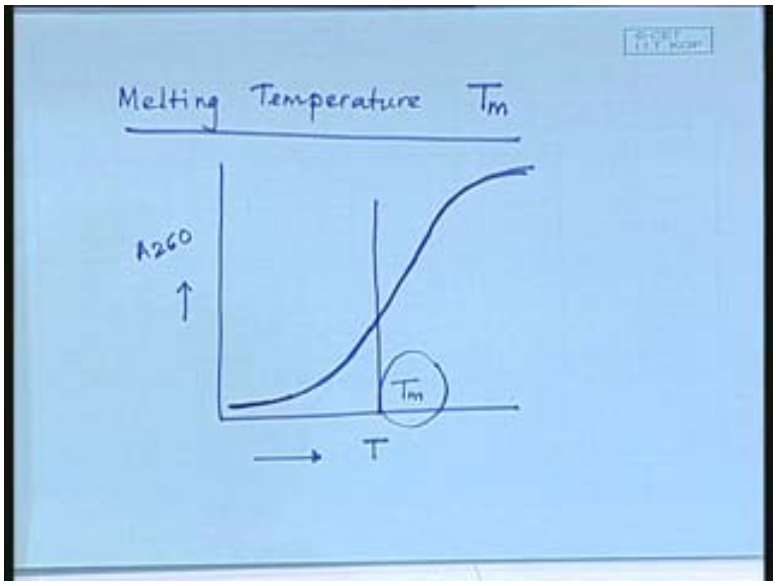

The melting temperature of DNA is referred to as the T_m. What are you monitoring here? We are monitoring the melting of the DNA, melting of DNA is basically the separation of the strands helix coil transition that is going to render the DNA structure disrupted. So we have to have a specific measure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

What nanometer wavelength am I going to use? 260 nm. 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 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. What will happen to the absorbance then? It will not increase any more basically. We will get some thing like this.

What sort of a DNA do I have here? A helix, a dsDNA. What do I have up here? A single stranded DNA, a coil. I have a transition, the midpoint of this curve is what is your T_m. Basically somewhere here would be the T_m of this. What did we do in the previous one that I showed you? We had the DNA curve, we were monitoring at different wavelengths and we found out that the maximum absorbance was at 260 nm. This maximum absorbent at 260 nm increases when we increase the temperature. What is now done is we are increasing the temperature and I am monitoring the absorbance at 260 nm. We know that the strands are being separated so because the hydrogen bonds are being disrupted between the bases. You are disrupting the structure. You have this increase. The mid

point of this is the T_m. What we are going to look at is factors that are going to affect the T_m.

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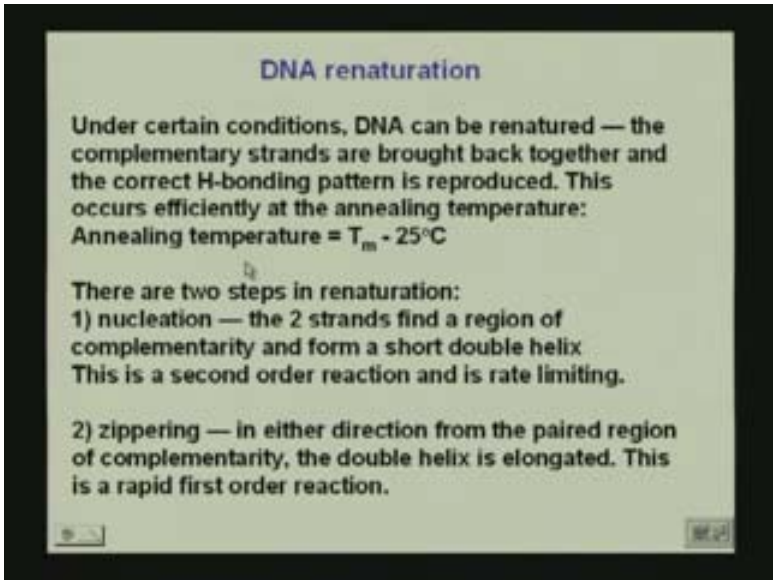

Before we get into that we have the renaturation that we were talking about. We have a T_m -25 ^{0}C that is going to be an efficient annealing temperature where the strands are brought back together. Whatever the T_m is -25 ^{0}C, it is kind of a rule of thumb. There are two steps in the renaturation first there is a nucleation, this nucleation is where the two strands find a region of complementarity and then they form a short double helix.

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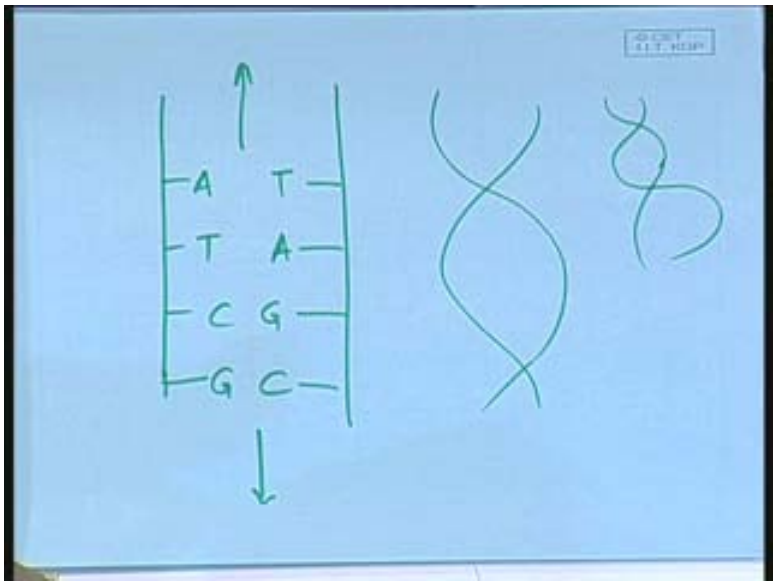

Then there is zippering in either direction from the paired region of complementarity the double helix is elongated and you understand that only if it finds a correct set, otherwise what will happen? It will form a bulge suppose what are we looking at here. We have two strands A T C G. We have other strands floating around but it is found this part that is complemented. We have T, what do we have here? A G C so this part it forms a double helix within this part and then it zips up in both directions. It is going to form something like that once it zips up but if it so happens that this part is complementary but this part is not then what are you going to have? You are going to have some part that looks like a proper double helix but some part that is sticking out like that.

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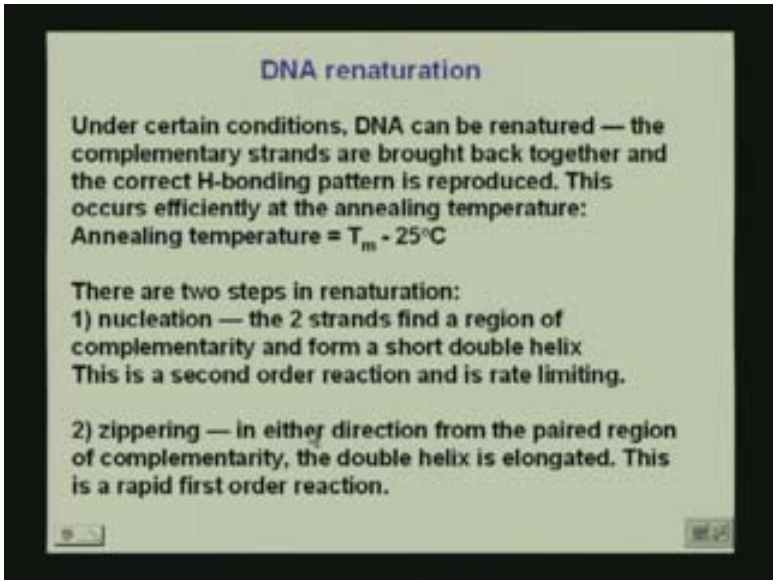

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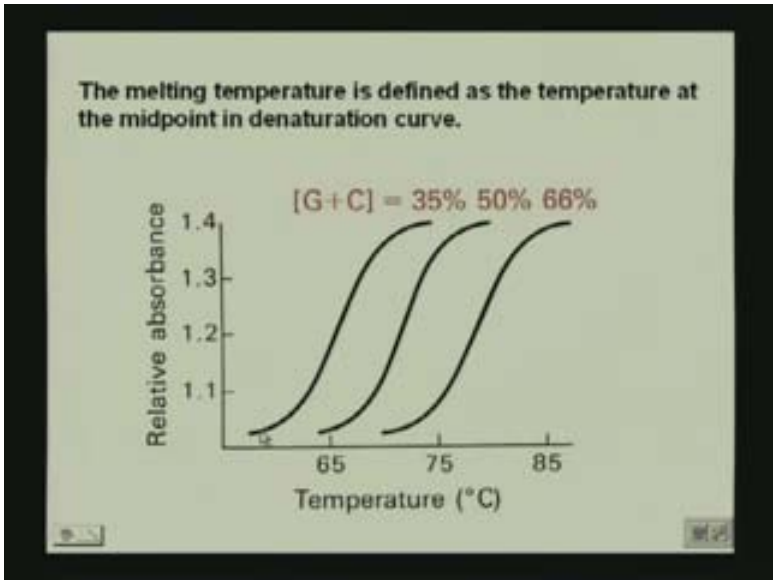

Then you would have a bulge, you would not have a proper renaturation. It has to find, the nucleation step is important that is rate limiting in finding the two strands where you have the complementary region and it zips up in both directions and if it finds the right kind of base pairs obviously it is going to be very fast and it is the first order reaction and it is pretty rapid. We have different melting temperatures. This we would call then a denaturation curve like the one I showed you here. What do we have here? A denaturation curve. Why is it called a denaturation curve? What do we have here, helix. We have coil here. We have double strand here and single strand. We have denatured it, so this is a denaturation curve and the midpoint of that will give me the T_m.

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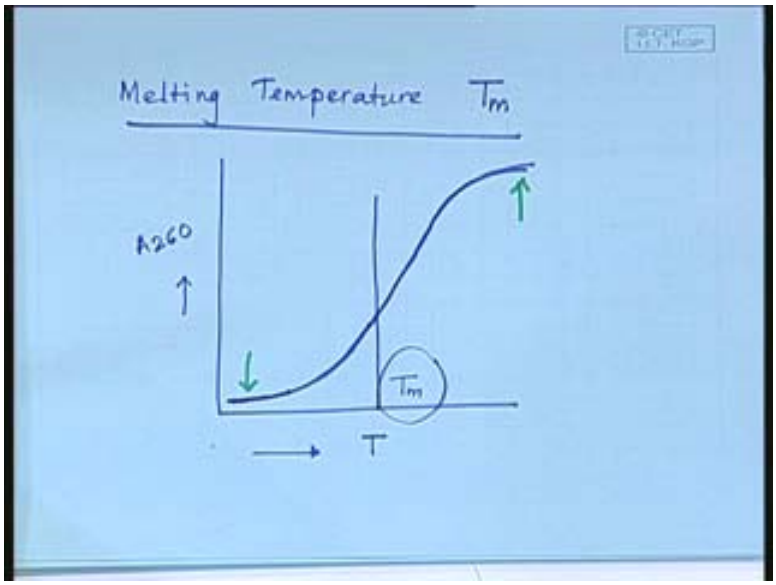

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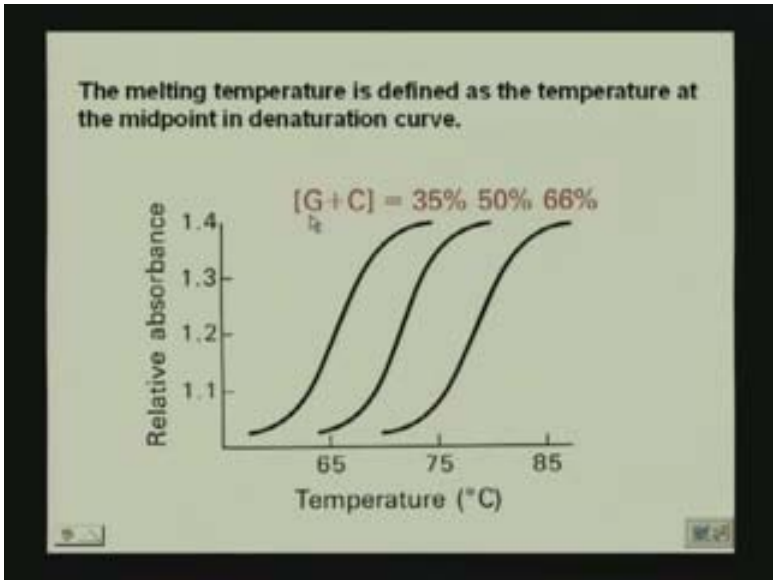

What I have 3 denaturation curves. I have 3 corresponding melting temperatures of 3 different DNA samples that have differences in their G + C content. What is the G + C content? G C base pair content, the G C percentage has increased from left to right and this has resulted in an increase in the T_m. Why? What happens in the G C pairing? In the GC pairing I have triple bonds. I have double bonds in my A T pairing. The higher amount of G C that I have, it is going to be more difficult for me to disparate the strands so the melting temperature is going to be higher. The GC content is important in determining what the melting temperature is. So the higher that you are going to see other factors also of DNA but the higher the GC content, the higher the T_m. Because it has to disrupt in that case, free hydrogen bonds to separate the strands.

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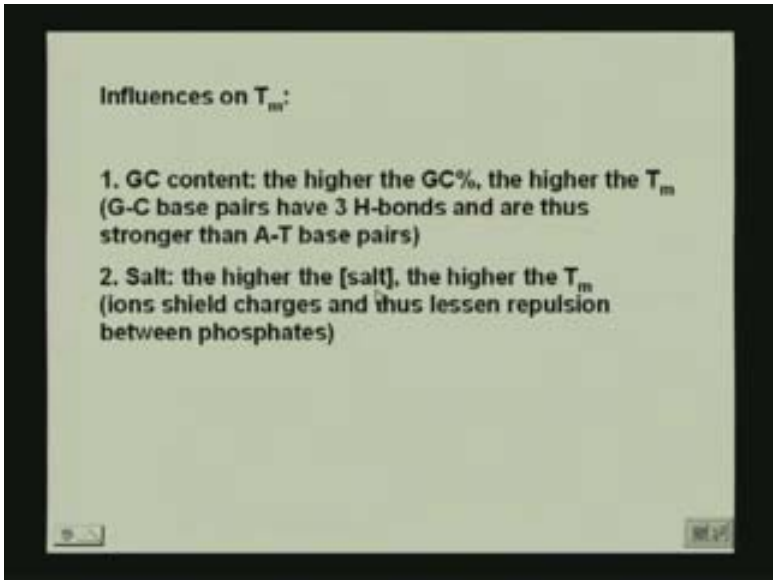

We have to look at the influences on the melting temperature, the GC content. We know why? The higher the GC percentage the higher the T_m because the GC base pairs have 3 hydrogen bonds and are thus stronger than AT base pairs that have 2 hydrogen bonds. What are the factors do you think might be important? You know what interactions stabilize the DNA. The basic idea in this case is going to be disrupting those interactions. What else is stabilizing the DNA? We have electrostatic repulsion. 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 then higher the T_m. 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 and this backbone is going to remain as far apart as possible, because you have the phosphate. If you have salt ions that are going to stabilize this, what is going to happen?

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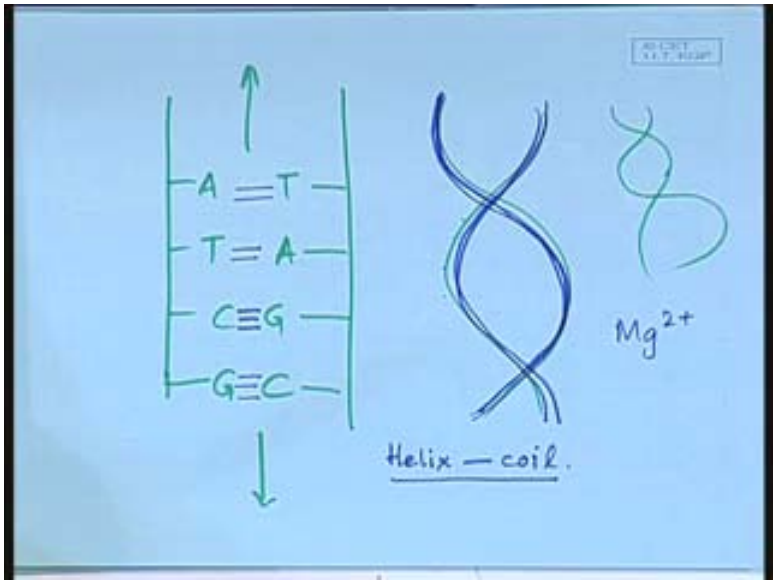

It will not be very easy for you to separate the chains because these chains if say you have further negative charge here. What is going to happen? There is going to be repulsion between the chains. Repulsion between the strands is what is going to take it apart, because essentially what you are doing is you are going and you are resulting in a helix coil transition that is what you are looking at. If you are looking at a helix coil transition is what you want is you want destabilization of this. You want destabilization of your strands. 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 T_m because you are stabilizing 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. Again the same thing when you increase the pH you are disrupting the hydrogen bonding. What is that? How is that going to affect the T_m? It will decrease the T_m because you are resulting in a larger disruption. All you have to think about is what effect each of these are going to have on the forces that are stabilizing the DNA.

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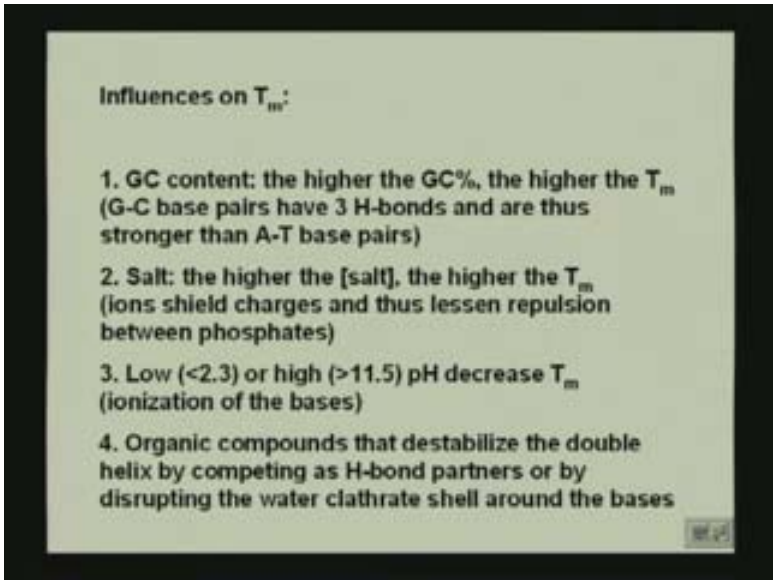

We have organic compounds. Organic compounds can act in different ways. One thing is they can intercalate between the bases. What are they going to do? They are going to disrupt them in that case, the stacking interactions. They can also form more favorable hydrogen bonds with the bases and in that case. 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 interactions which ever type or which ever type of interaction is disrupted will result in the separation of the chains. What we learnt was, we learnt how the overall structure of DNA and RNA is formed by the different, the nucleotides basically coming together.

We have the sugar phosphate backbone in both cases just the sugars being different in the deoxy type, the deoxy ribose type and the ribose type. The bases are the purines and the pyramidines for both cases. We just have a change thymine to urosil, when we go from DNA to RNA. Then we looked at the hydrogen bonding patterns in each cases, where we have 2 hydrogen bonds between A and T and 3 hydrogen bonds between G and C. We saw how the stability of DNA can be disrupted by certain factors and what the melting temperature? What affect the G C content and the A T content had on the overall DNA stability. Other factors like we have temperature also that we have to look at, we have the G C A T content. We have the pH. We have the addition of ions and the addition of organic solvents. This completes our discussion on nucleic acids, we will begin our bio energetics in our next class.