Experimental Biochemistry
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Lecture - 42
Isolation and Characterization of DNA Summary

Welcome back. So, in this week 9 we have talked about Isolation and Characterization of DNA. In this week we saw how we can isolate DNA either genomic DNA or plasmid DNA from different sources. I specifically talked about plasmid DNA isolation from bacterial source and we also saw that being done in the lab.

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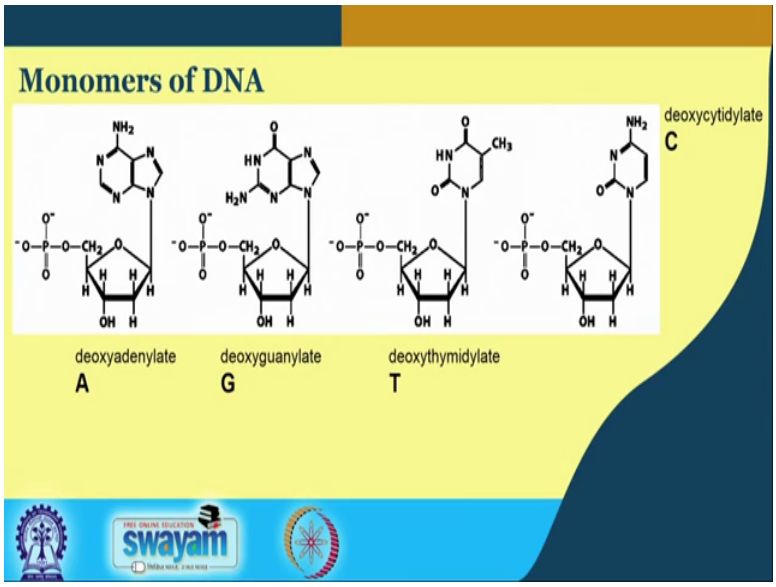

So, just to summarize what we learnt in this week. We saw that DNA is again a polymer just like proteins. DNA is a polymer of nucleotides and we saw that there are two types of nucleotide polymers. One is DNA deoxyribose nucleic acid and then the other one is RNA ribonucleic acid. Chemically they are very similar. Both are formed by four different types of monomers. Here we see the monomers of deoxyribose nucleic acids that is DNA.

We saw that these monomers have 3 different components. One is the base, the nucleic the nitrogenous base which is attached to this deoxyribose sugar and the third one is the phosphate group. The phosphate group is attached to the fifth carbon through a phosphatized link and the nitrogenous base is attached to the first carbon of the

deoxyribose sugar. In case of RNA this sugar is ribose and it has a hydroxyl group at this second position. There are four types of monomers in case of DNA and these four monomers differ in the nitrogenous base. So, this is adenine, guanine, thymine and cytosine. The rest of the nucleotide is exactly the same.

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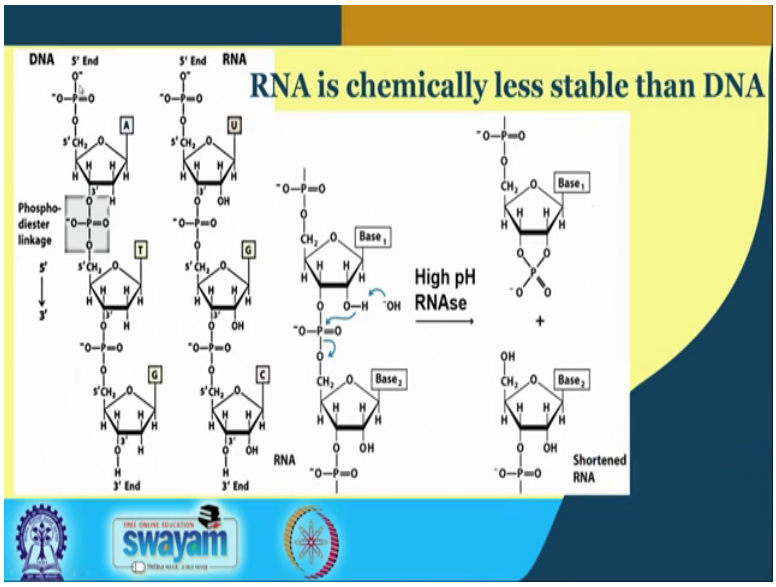

Then we saw that these monomers are linked into a linear polymer via phosphatized linkages where the phosphate group connects the 3 prime OH of one monomer with the 5 prime OH of the next monomer. So, what we get essentially is a linear polymer which has a 5 prime end and the 3 prime end. If you look at these phosphate sugar, phosphate sugar backbone this is chemically exactly the same throughout the DNA; only the bases are different in each monomer.

Similarly, in case of RNA the structure is very similar in terms of chemical structure where it is again phosphate sugar phosphate sugar backbone. Only in this case the sugar is ribose sugar instead of deoxyribose and it again runs from the 5 prime end to the 3 prime end. It also has the nitrogenous base which is different for each monomer and in case of RNA the base 3 bases are exactly same as DNA, the one that is different is uracil instead of thymine.

We also saw that RNA is chemically less stable compared to DNA and the reason for that is the presence of this hydroxyl group at the 2 prime position. So, this hydroxyl group facilitates the hydrolysis of this ester linkage through alkaline hydrolysis. So, if the pH is

in the alkalic alkaline region. So, the pH is more than 7 then the concentration of hydroxyl ions are more and then we get this catalytic reaction.

We can also get the same reaction facilitate by certain types of enzymes called RNA. So, these enzymes can cleave RNA into its monomeric units because they are not specific to the base that is attached in each monomer. They attack these phosphatized linkage and this sugar phosphate backbone is exactly the same for all the nucleotides. So, the RNAs are not very specific to the nucleotide type, but so, they will leave all the RNA resulting in monomeric units if they are incubated long enough.

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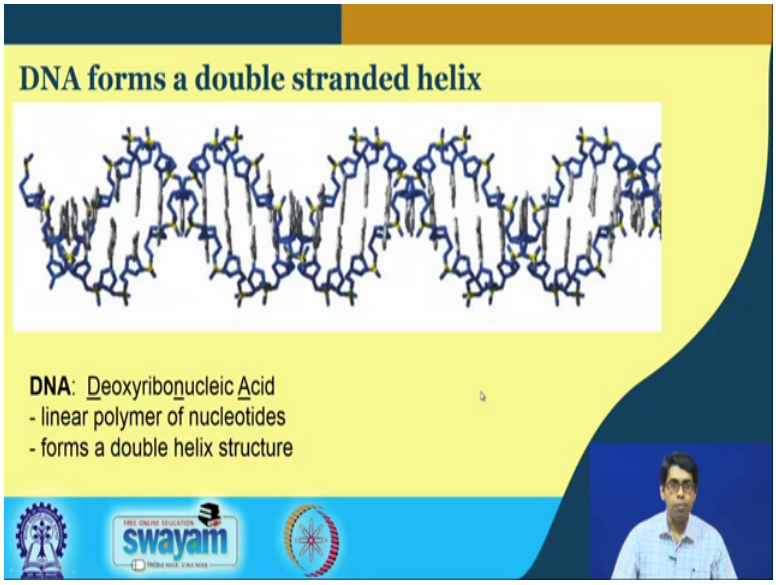

So, we also saw that DNA forms a double helix structure. In the previous slide what we saw is the linear form of the DNA and here what we saw is what we see is 2 strands of DNA are intertwined into a helical structure and this is called the double helix structure of DNA. Unlike proteins, DNA molecules look very similar. So, irrespective of whatever is the sequence the base sequence inside the DNA the double helical structure is more or less the same.

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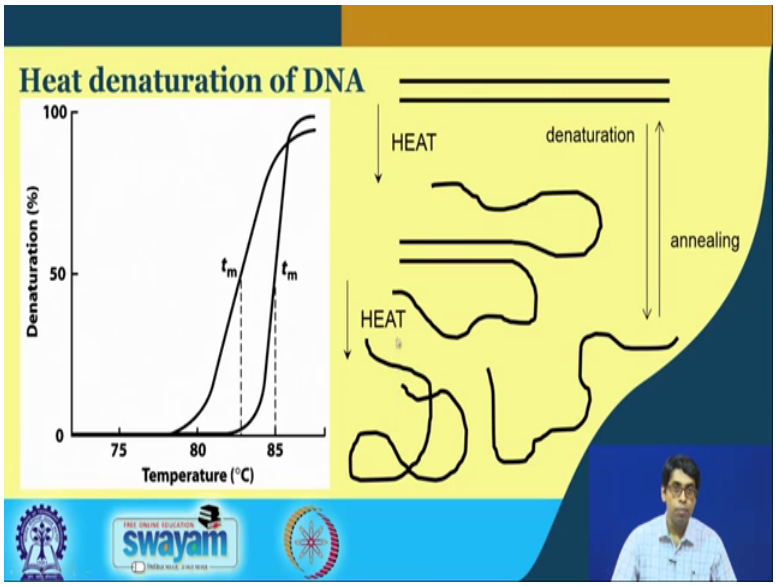

The third thing that we learned was the heat denaturation of DNA. So, just like protein denaturation we saw that DNA can also be denatured upon the application of heat. And if you remove the heat, so, if you cool it down it can go back to form the double stranded DNA. So, when we apply heat the hydrogen bonds between the bases. So, a pairs with b and g pairs with c this hydrogen bonds start breaking down and we get more and more. So, the two strands start peeling off from each other. And when you have and so, when we apply heat long enough or you apply sufficient amount of heat you will reach a state where both strands have completely separated from each others. So, all the hydrogen bonds are broken. So, this is the complete denatured state of the DNA.

If the DNA strands are not too long then if we cool down this solution which contains the denatured DNA strands then it can go back to its native state where it forms a double stranded helix. So, this the forward process is called denaturation just like protein denaturation and the reverse process is called annealing. We also saw that we can quantify the stability of a DNA by measuring the midpoint temperature at which 50 percent of the DNA is denatured and 50 percent of the DNA is still in the native state. So, higher the melting point more stable the DNA is and this t m or the melting temperature depends on the sequence of the DNA molecule and also the length of the DNA molecule.

So, these are these are the important characteristics of DNA and RNA that we exploit when we purify DNA from various sources because we have to purify DNA, we have to

separate them from different cellular components that are present. So, we have to separate the DNA from proteins we have to separate the DNA from RNA also. And what we also saw that if we are purifying plasmid DNA then we have to separate the small plasmid DNA from the large genomic DNA and in that case this denaturation step was very important.

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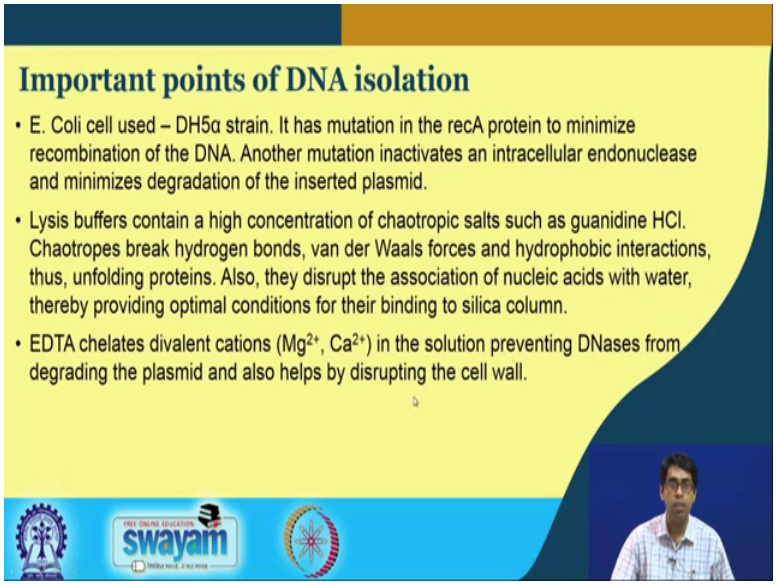

So, the important points that we have covered I have listed them in this last two slides. So, the cell that we used for the purification of plasmid DNA was DH5 alpha cells. So, that is the particular strain that we used as I mentioned before for protein purification we use the different strain called the BL21D3 strain that was more poised towards over expression of protein. But this particular strain is more suitable for maintenance of the plasmid DNA because it has it maintains the high copy number of the plasmid DNA and it has two specific mutations which help in the stability of the plasmid DNA inside the bacterial cell.

So, one mutation is in the rec A protein. So, this is of recombinants proteins which recombines a two different DNA strands. So, what that might result in is that the integrity of the plasmid will be lost because some part of the genomic DNA might get copied into the plasmid DNA or some part of the plasmid DNA might get deleted or copied into the genomic DNA. So, we do not want that to happen. We want the plasmid DNA to be exactly the same that we have put in there.

So, this mutation helps in maintaining that because it minimizes recombination of DNA. Another mutation is in a endonuclease enzyme which is present in the bacteria and endonucleases are enzymes which specifically cut DNA. We will see examples of endonucleases in the next lecture when we talk about recombinant DNA technology and we will see that they cut at specific sequences. So, this endonucleases can also damage the plasmid that we have inserted in a bacteria.

So, mutation in this endonuclease will again prevent or minimize them. So, we saw that we can grow our bacteria in luria broth overnight and we do not need a lot of cells we grow only 5 ml culture. So, that is one of the differences again between protein and DNA purification. In case of protein, we express the protein in 1 liter minimum 1 liter of l b culture. Here we take only 5 m l of l b culture.

The reason for that is we do not need a lot of DNA because all we need is the information. The actual DNA is not very useful. All we need is the information and we can always amplify the information. Even if the amount of DNA is small we can amplify the information that we need using techniques like polymeric chain reaction.

So, we do not need to start with a lot of material. So, we do not grow one liter of culture because if you do that they will need a lot of reagent to purify your DNA. So, we go with a very small amount that is absolutely necessary and it turns out 5 ml of l b culture gives us enough DNA to work for the subsequent steps in our experiments. The first thing that we do is lies the cells. So, we break open the cells and that is at the lysis buffer is something that is very specially designed because it has several things.

First of all the DNA the lysis buffer contains high concentration of chaotropic salts. So, an example of a chaotropic salt is guanidine hydrochloride. We saw guanidine hydrochloride again in the context of protein folding and unfolding experiments. So, these are salts which interfere with the formation of hydrogen bonds and that is why it denatures protein and the same thing also happens in case of DNA. It breaks the hydrogen bonds, it interferes with the Van der Waal forces and also the hydrophobic interactions. So, all of these results in denaturation of the protein, it also results in denaturation of the DNA.

Since the hydrogen bonding are interrupted this chaotropic salts also disrupts the association of the nucleic acid with water. And this is something that is important in the

last step of purification when we have precipitated out everything else only our DNA is in the solution and we want our DNA to specifically bind to the silica column. So, in that case what happens is the silica column is negatively charged. The DNA has negative charge, but then these two negative charged molecules are mediated by the positive charged ions. So, the DNA sticks to the silica column.

So, a very delicate balance of this chaotropic salts facilitates some of this steps. We also add EDTA. So, EDTA is a chelating agent which binds divalent cations such as magnesium ions and calcium ions. So, this serves two purposes; one is that it prevents different various enzymes which degrade DNA such as deionizes. So, DNAs are enzymes which degrade DNA just like. So, this enzymes are the equivalent of RNAs enzymes. So, RNAs enzymes degrade RNA molecules and DNAs enzymes degrade DNA molecules and those enzymes require these divalent cations. So, if EDTA binds these divalent cations then these enzymes the DNAs enzymes are inactivated and thus it prevents the degradation of our plasmid. Also binding these divalent cations results in the disruption of the cell wall because we want to disrupt the cell wall in order to lies the cell.

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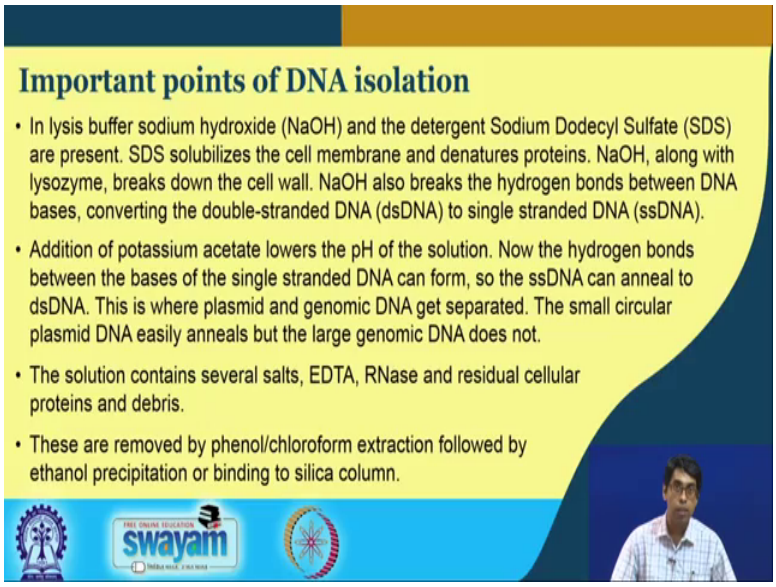

The lysis buffer has high concentration of sodium hydroxide. So, this is a base and when you add sodium hydroxide the pH rapidly increases. So, the pH is. So, it will be alkaline solution. We also add sodium dodecyl sulfate or SDS. This is the same SDS that is used

in SDS page gel electrophoresis. So, SDS it interferes with the cell membrane because it has very similar structure. It has this charged head group and very hydrophobic tail which interferes with the structure of the cell membrane and disrupts it. And then when the proteins are out SDS will denature the protein. NaOH along with lysozymes; so, we also add lysozymes this is an enzyme which breaks down the cell wall.

So, addition of EDTA and addition of lysozyme helps in breaking down the cell wall. The NaOH on the other hand breaks down the hydrogen bonds between the DNA bases. So, it denatures the double stranded DNA and gets and converts it into a single stranded DNA. So, in this case instead of heating the solution we are denaturing the double stranded DNA by the application of alkaline which is NaOH and remember that this same Na OH will also break down RNA because the OH will now catalyze the hydrolysis of the phosphatized group in the RNA molecule and we also add RNAs. So, that whole process is speeded up.

Once all of these happens, we rapidly increase the pH sorry decrease the pH by adding potassium acetate. So, acetic acid this is the salt of acetic acid and when we add this the pH drops and it becomes acidic. Now this is a very important step because the addition of NaOH disrupted the hydrogen bonds in the double stranded DNA converted into single stranded DNA. So, if we want to isolate plasmid DNA at this step in this step both the genomic DNA and the plasmid DNA are single stranded.

Now, when we add potassium acetate the solution becomes acidic, the hydrogen bonds now can form. So, that the single stranded DNA can anneal to form the double stranded DNA. In case of plasmid since it is small much smaller than the genomic DNA, it can easily find the right basis to form the hydrogen bond and forms the circular double stranded DNA. But the genomic DNA is very very large and it is almost impossible for the genomic the 2 strands of the genomic DNA to find the correct basis and form the double stranded DNA. So, the genomic DNA remains denatured. It is just a probability factor that does not allow the genomic DNA to go back to the double stranded state. So, it remains denatured and it becomes and it remains insoluble.

Now, what we do is we simply precipitate all this insoluble material that are present in the solution only our plasmid DNA remains soluble. The soluble part is extracted out, but it contains several salts. It contains EDTA, it contains RNAs; RNA s is a very stable

protein and it might also contain some residual cell proteins and debris. So, all of this have to be removed and that is done either by one of these two steps we can use phenol chloroform extraction and separate out the DNA in the aqueous phase and then precipitate it using chilled ethanol and then again do this washing several times to get a very pure DNA.

Or if the amount of DNA is very small we can use the silica column where we add the add this solution directly to the silica column and wash it so that everything else goes out. And, our DNA remains bound to the column and we can then elute the DNA from the column using water or TE buffer which is tress EDTA buffer and pH 8.

So, that is all for this week. In the next week we will see that once we have purified this DNA how we can use that for our recombinant DNA technology experiments.

Thank you.