Introduction to Complex Biological Systems
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Lecture 4
Introduction to DNA Replication

Hello everyone I am Dibyendu Samanta from IIT Kharagpur and I am discussing about the course Introduction to Complex Biological System. Today this is the fourth topic Introduction to DNA Replication. So, during the last class we mostly discussed about DNA structure, mostly the chemical and physical properties of DNA. Today I am going to discuss about how the information of DNA gets copied, so that the information can pass from one generation to the next generation.

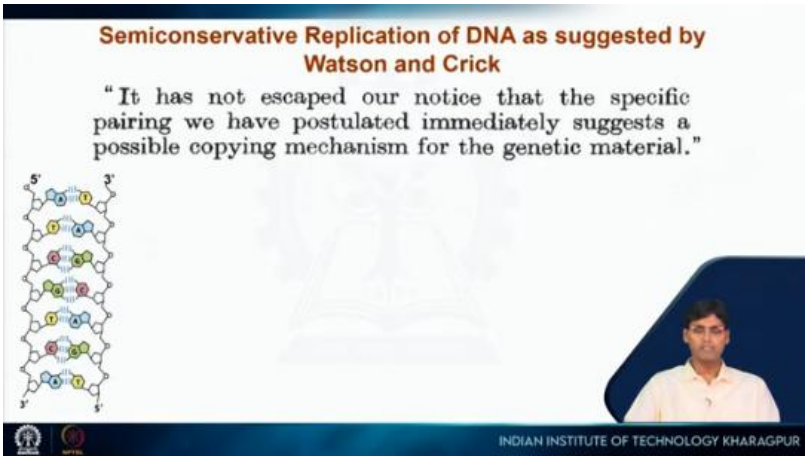
So, this is DNA replication. So, in this topic I will concentrate on basic understanding about DNA replication followed by one classic experiment commonly known as Meselson and Stahl's Experiment that proves the basic understanding of the basic mechanism of DNA replication. So, now I particularly discuss about the differences between DNA and RNA, its chemical composition and everything. So, DNA is deoxyribonucleic acid. So, here most of those nomenclature they are very much pre logical they have some kind of logic.

So, as I explained that doxy means that 2 prime carbon of that sugar, the oxygen atom is not there that is why it is doxy acid and then ribonucleic acid, the sugar is a pentose type particularly ribose that is why deoxyribo then nucleic acid. Acid because whatever we discussed based on that if you see the backbone of the DNA that comprises the sugar phosphate rib. So, because of this phosphate group DNA is negatively charged as well as it has acidic property.

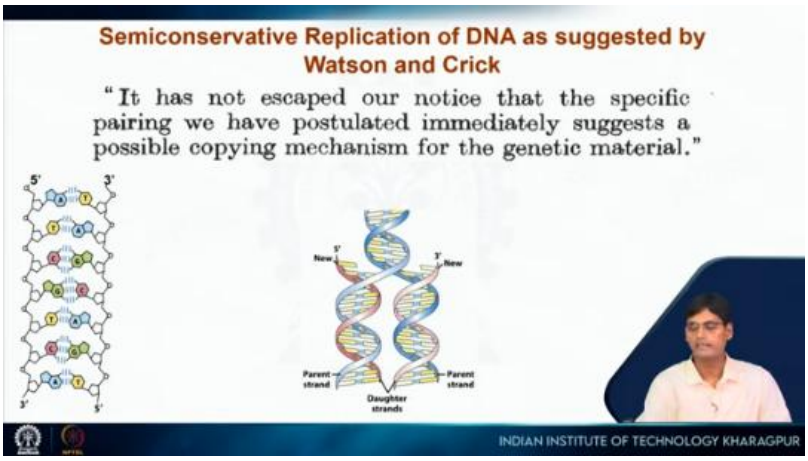
So, nucleic acid, but then what is about the nucleic? So, this is because, if we discuss a little bit about the discovery of DNA then we will understand the name nucleic acid. Many of us know that DNA was discovered by Watson and Crick. That is not right because almost 80 years before the discovery of DNA structure which was done by Watson and Crick. The DNA was discovered by Friedrich Miescher from white blood cell. So, what he observed was that he isolated some substances from the nucleus of those white blood cells and those are very acidic in nature. So, that is why this is called nucleic acid, some acidic substances found

in the nucleus nucleic acid and now I already explained that deoxyribonucleic acid. Similarly, ribonucleic acid, 2 prime position still has the oxygen group, the OH group is present there.

So, now I will just start today's discussion with basic understanding about DNA replication. The major important thing from Watson and Crick's discovery is their specific base pairing, because if we go back as I just told about the discovery of DNA. Similarly, after that many scientists contributed a lot to understand the overall features of DNA. Like how they are coherently linked to the phosphodiester bond and how nitrogenous bases are attached to the sugar, all those things were known. Then what is the major important thing of Watson and Crick discovery is this specific base pairing, this double helix structure that A always base pair with T and G always base pair with C. So, this is the most interesting part of Watson and Crick discovery because that actually tells us that how the information can go to the next level that means how the information can be copied to the next generation.



So, that is what I would say is the most important thing. So, here in that paper particularly, this one Watson and Crick published in Nature in 1953. In this paper at the end they mention that it has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material, this is very important because before that how this information is going to the next level was not known.



But here if you see this is one DNA structure as you can see that it runs from 5 prime to 3 prime direction, one strand and the other strand 5 prime to 3 prime direction because DNA strands are antiparallel and as you can see that A always base pair with T, ATGC, it is going like this. Now, there is some advantage of this also, I discussed during the last class that for

example, if you increase the temperature of a solution where you have DNA that those two strands will be separated because this hydrogen bonding will be disrupted as a result of that those two strands will be separated. So, if we can separate those two strands of DNA, then again we can copy the information, because if you know the sequence of one strand of this DNA, then you can automatically put the others sequence of the other strand, because this is always a very strict rule that A base pair with C and G base paired with C, those two strands are complementary in nature.

So, as a result of this DNA, if I write in this way, 5 prime ATCGTCA, 3 prime, exactly whatever is mentioned here on the left hand side. So, as a result of that the complementary strands should be 3 prime TAGCAGT and 5 prime, but here this is double stranded DNA, they have hydrogen bonding between this ATGC.

So, now if there is some mechanism so that these two strands are getting separated then we can synthesize the new strand because of this complementary root. As a result of that in the next stage if these two strands are getting separated, 5 prime ATCGTCA, 3 prime plus this 3 prime TAGCAGT so these two strands can actually make the new strand again from here. So, here TAGCAGT, 5 prime 3 prime this underlined strand. This is the new strand here and also the other strand will be synthesized from 5 prime to 3 prime directions.

So, as a result of that you are getting ATCGT then CA, 3 prime, this underlined strand is the new strand. As a result of that if you see this is the template strand and then we are getting the new strand so one strand is the old strand here or the parent strand and this is the new strand. Similarly, here this is the old strand or parental strand and this is the new strand.

So now we have two DNA molecules, 1 and 2, these two DNA molecules and in each case you have one strand is new and one strand is the old. So, here in this DNA molecule for example, that 50 percent of DNA is conserved, 50 percent old and 50 percent new. So, that is why this model is called semi-conservative model of DNA replication. So, this is semi-conservative model of DNA replication.

So, as you can see in this figure those two strands are referred to in blue colour. So, those are the parental strands. So, if we have some kind of mechanism so that those two strands are getting separated then you can see this new strand.

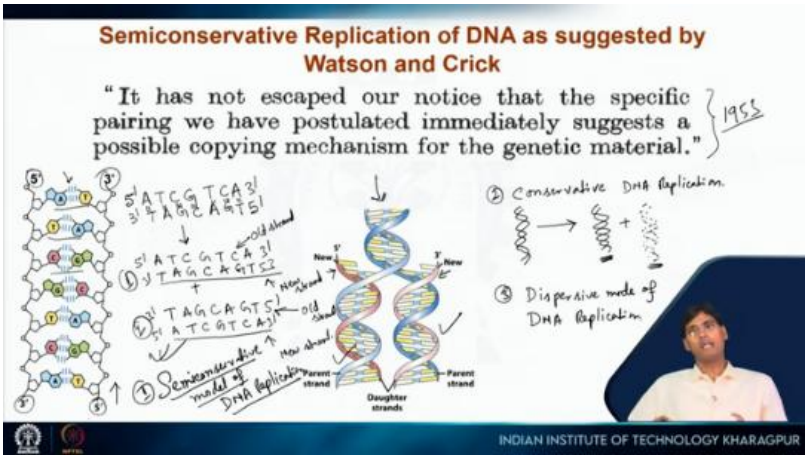
So, in pink colour here are the new strands. So, as you can see this is semi-conservative replication so here in this DNA you have one old strand and one new strand. Similarly, in this DNA also you have one old strand and one new strand, but during this time this is proposed by Watson and Crick that semi-conservative DNA replication based on their DNA structure.

But the thing is that at the same time there are some other models, other hypotheses of DNA replication. I would say this is one semi-conservative replication then the second one is conservative mode of DNA replication. So, based on this hypothesis they are suggesting that if you have just a double stranded DNA, now by somehow after replication in the next round what you are getting is that this DNA is preserved, this is the old DNA plus one new DNA is being synthesized here.

So, this is both strands here new and here both strands were old or the parental strand. As a result of that, the parental DNA is conserved here and the both strands contain the new strands in this DNA. So, that is why this is called conservative mode of DNA replication.

And there was another mode of DNA replication, a hypothesis that is dispersive that means that somehow with this double stranded DNA they get a small break in both strands and from there the new strand will be synthesized. So, as a result of that in the final molecule that means, from one DNA to the next cycle when we will get two DNA molecules, both the strands will have the new and old strand mixed together this is called dispersive mode of DNA replication.

Now we need to come out with some experiment so that it can be proved that which one is right although Watson and Crick suggested based on their solid data that this could be semi-conservative, but after few years Meselson and Stahl did some experiment, Matthew Meselson and Franklin Stahl.



This is commonly known as one of the most beautiful experiments in biology because of the simplicity of the experiment but still clearly demonstrates that DNA replication is semi-conservative. So, this is commonly known as the Meselson and Stahl experiment. So, first I need to explain the background of the experiment, a few things I need to discuss, so that we can know quickly about the details of the experiment. So, what did they do?

So, they work with E. coli. E. coli is a bacteria that is the prokaryotic cell. So in E. coli cells we have just one circular DNA. So, this is E. coli and DNA present in E. coli, now E. coli cells with proper environment, with proper media it can get doubled from 1 E. coli to 2 E. coli cells almost in 20 minutes.

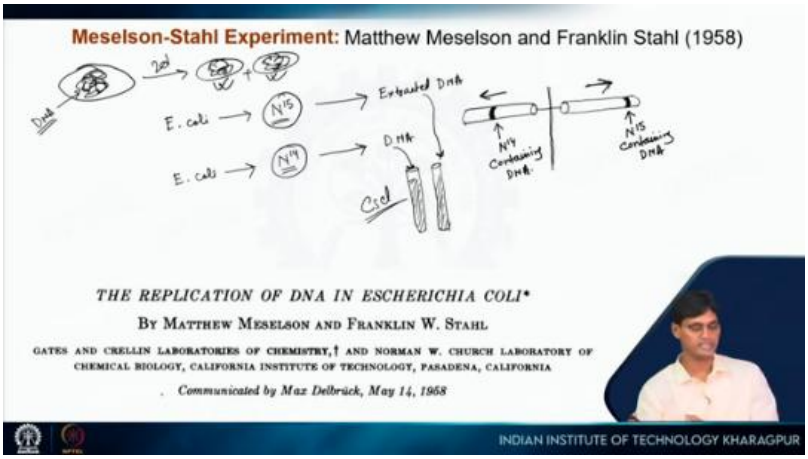
Now that means, by that time the DNA of these E. coli or the chromosome of this DNA is getting replicated and both like these two E. coli cells they are getting DNA. So, they work with E. coli cells. Now in order to understand what the mechanism of DNA replication is, we have to understand or we have to somehow designate which one is the new strain and which one is the old strain. We have to differentiate after the experiment. So, that was the major thing in this experiment. What did Meselson and Stahl do? So, they grow E. coli cells.

So, what happened? They took E. coli cells and they grew in the media. So, we grow E. coli cells generally in liquid media, this is called LB liquid media. So they made some media where they put nitrogen 15. So, the nitrogen source is just N15 not the N14, which is a heavier isotope. So they made some kind of media so that you do not have N14 in that media, only you have N15 and similarly they are also growing E. coli cells in another flask where they have only N14 containing media.

So, they added ammonium chloride where the nitrogen is either N14 or N15. So as a result of that as we already discussed that nitrogen is an important atom in DNA so during replication this nitrogen will be incorporated in DNA. Now, after going several hours they extracted DNA from this bacteria which were grown in N15 media.

Similarly, they extracted DNA from those bacteria which were grown in N14 containing media then they analyzed it with some ultra centrifugation. So simply if I want to say that if this is centrifugal axis here and here you have two tubes here two tubes. So this is the centrifugal force that is why when it is spinning very rapidly those tubes will be like this, but when we stop the centrifugation then those tubes will be just like this because of the centrifugal force it is spreading in this way. Now, what did we did? They loaded DNA samples from this N15 sample containing samples in this tube. In this tube they have some solution which is made up of very high density caesium chloride. So they have very highly dense caesium chloride they have in this tube and now they loaded this DNA which was extracted either from N15 or N14 and then they started this centrifugation for long time. So, this is called equilibrium density centrifugation.

So, as a result of that if you spin it for a long time then since you have a very dense solution in the tube then because of the centrifugal force DNA will migrate in this direction but also you have a very highly dense solution. So, as a result of that the DNA will move based on their weight. Now N15 since it is heavier so I would say if N15 is present here, they noticed that the N15 containing DNA moved towards this direction more compared to N14, those are the same size tube. So as you can see here this is the centrifugal force so they noticed that this is N14 containing DNA and this is N15 containing DNA. That means that when DNA is getting replicated in presence of N15, all those nucleotides have the nitrogenous bases and all those nitrogen are heavier nitrogen N15. So as a result of that overall the molecular weight of those DNA, those are heavier than the N14 containing DNA and that is why they migrated more compared to the N14 containing DNA. So, this is the basis of their experiment. So, that they can basically distinguish between the two types of DNA strand the N14 containing DNA strand and N15 containing DNA strand.



Let us come to their experiment and discuss that. So, here what they did? So, they took E. coli cells and they grew this E. coli in N15 containing media, which is liquid media for long hours for example, for overnight and then they harvested those cells.

So, cells were grown for a very long time, I would say 10 to 16 hours and then they harvested E. coli cells. So how will they harvest? That means, again they took the whole media where you have those equalized cells, they spin it through some kind of centrifugation method. As a result of that equalized cells will settle down at the bottom as they are heavier and now we have to remove the supernatant.

So we are actually trying to remove any solution that contains N15 media. Now you have just the cells without the media. Now few cells or I would say a fraction of those cells were taken to the isolated DNA, DNA was isolated from these cells which were grown in N15 media overnight. But the rest of the cells they transferred into N14 media and rest of the cells transferred into N14 media. So, now, they wait for 20 minutes.

So I would say here that they transferred here and you have N14 media in this flask and then they took samples. So here after transferring into N14 media they took a sample 20 minutes later. They took some cells and then they extracted DNA and then still you have cells in the media then again they took cells at 40 minutes, again they isolated DNA and then another sample 60 minutes and they isolated DNA. So now we have 4 DNA samples. So, here which were directly harvested, that were grown in N15 containing media followed by those cells we transferred only into N14 containing media and we waited for 20 minutes and then we took another DNA sample. We have extracted and then after 40 minutes again we extracted DNA and after 60 minutes again we have extracted DNA. Now when they analyze these 4 DNA samples to the same centrifugation technique, which I discussed in the last slide.

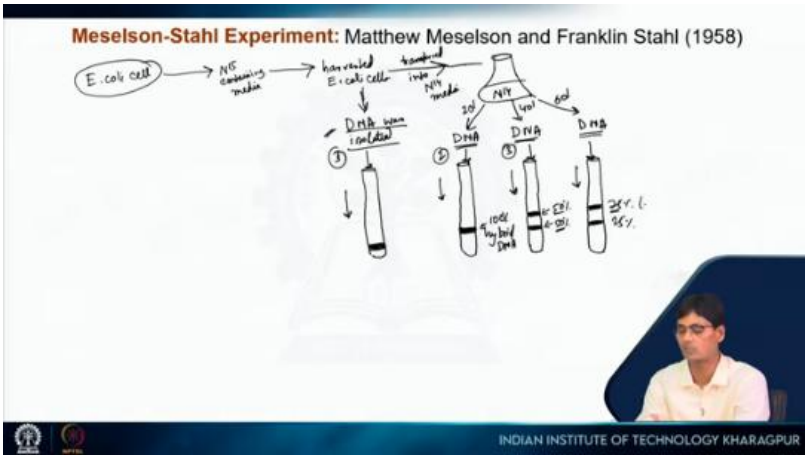
So, here I am just drawing 4 similar tubes here, they do not look exactly similar, but you just imagine that they are exactly the same tube and all of them contain caesium chloride. They loaded this DNA sample in this tube and what they did? They just centrifuge for long time whatever I discussed during the last slide and they observe that in this first tube here, the tube number 1 that has the DNA band somewhere here they settle here is the DNA. So, I already explained that since these E. coli cells were grown overnight in media containing N15. So, these are very heavy DNA, all of their nitrogenous bases are labelled with the N15 atom. Now in the second tube here after 20 minutes, they found that DNA is present a little bit at a higher position not exactly at the same position where you have in the first tube.

And then interestingly in the third tube, the DNA was taken after 40 minutes. So, now, this DNA is present somewhere here and here. So, see in tube number 1 100 percent DNA, they are heavy that is why they migrated maximum. So, this is the centrifugal force in this direction.

So, as a result of that they migrated maximum. Now in tube number 2 if you see they again get a single band, but this is a little lighter than tube 1. So, as a result of that, a different species here is not completely heavy nor completely light, this is some kind of hybrid DNA and 100 percent here so 100 percent hybrid DNA. Why is it a hybrid? Because see in the next tube you have here this hybrid DNA, which is 50 percent and this is the light DNA again 50 percent.

Why is light DNA? Because this is you know migrating minimum. So, this is the light DNA and based on their density they could conclude that this is 50 percent light DNA and 50 percent hybrid DNA. Now in the next tube they found that here only you have 25 percent and here 75 percent. So 25 percent is hybrid and 75 percent is light.

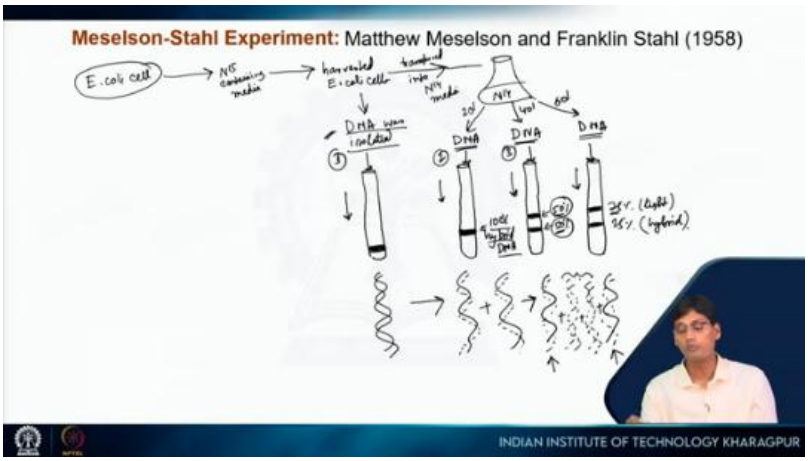
So, this is light DNA that means, DNA containing N14 and this is hybrid DNA. So, after getting this result actually it completely ruled out or I would say it is completely convinced that DNA replication is semi-conservative because now if I draw here, this DNA both the strands are labelled with heavy atom, heavy nitrogen that is why they are heavy. But now if DNA replication is semi-conservative then what will happen? In the next round of replication we have one strand, which is heavy nitrogen and this is the new strand with a dotted line.



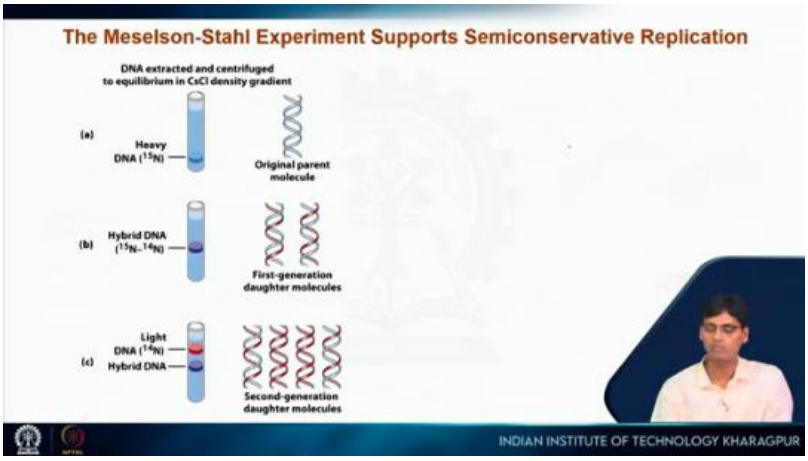
Similarly, here the new strand with a dotted line. So as a result of that, this DNA is now hybrid. They have one heavier strand and one light strand and they are settled here. So, as you can see here 100 percent hybrid DNA. This is because of semi-conservative, if it is conservative then what we would say is that again we will see some are completely light and some are completely heavier DNA molecules. In the next round of replication here we would see because now we do not have any more N15 containing DNA.

So, here we have 4 DNA molecules, again here you can see this one with one heavy strand and this one light strand and here both these two DNA they have both strands are light. So, as a result of that 50 percent DNA here they are hybrid and 50 percent DNA, these two 50 percent DNA they are light. So, these experiments together proved that DNA replication is semi-conservative then why we are taking samples after 20 minutes because I explained that in favourable conditions E coli gets double in 20 minutes. So, as a result of that we could say

that this is just after one round of replication you have sample here in tube 2 and then another round of replication in tube 3 and the next of replication tube 4.



So, the same thing we are showing from some images from some textbook. So, as you can see this is the original or the parent molecule both stands are labelled with N15 and here the first generation both stands are hybrid. So, that is why you can get this hybrid band here and similarly here the second generation as I just explained.



So, you have two strands: two DNA molecules which are hybrid and two DNA molecules which are completely light. So, all those kinds of classic experiments and many scientists believe this is the most beautiful experiment in biology and it is a very simple experiment, but it clearly demonstrates that DNA replication is semi-conservative that is all for this ah class. For further reading you can refer to any standard textbook including this Lehninger Principles of Biochemistry by Nelson and Cox or ah Essential Cell Biology by Alberts.

REFERENCES

1. Lehninger Principles of Biochemistry by Nelson and Cox (8th Edition)

2. Essential Cell Biology by Alberts et al., (4th Edition)