Bioengineering: An Interface with Biology and Medicine

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Lecture – 23
Classical Genetics Experiments

So, we have been discussing about Mendelian genetics and we have seen many examples in which way understanding the genetic laws can really help us to do lot of genetic testing. In the same light I think is important for us to learn how new modern biotechnology tools have started making huge difference in day to day medical applications you have just seen one video where you may have realized.

That in which we were personalized medicine is not any more efficient if you understand an individual geno variation what kind of mutation might be happening in this particular individual. So, then you can get some medicine which are very much a could be recognized by the receptors of those mutations and then it will be much more effective for that individual as compared to you are just giving some generic drugs to every individual.

And therefore many times for a given treatment you may realize that you have lot of side effects and those side effects that just result of us we not knowing us exact way of modality of the treatment. So, in molecular basis of inheritance we had discussed about some of the classical experiments and then I will talk to you about some more classical experiment in which some of the fundamentals.

That what is the genetic material in the DNA or the proteins were established and similarly many of the transforming principles as well as how DNA replicates those kind of laws well how they were derived we are going to discuss about those experiments. Can a genetic trade be transferred into bacterial strains?

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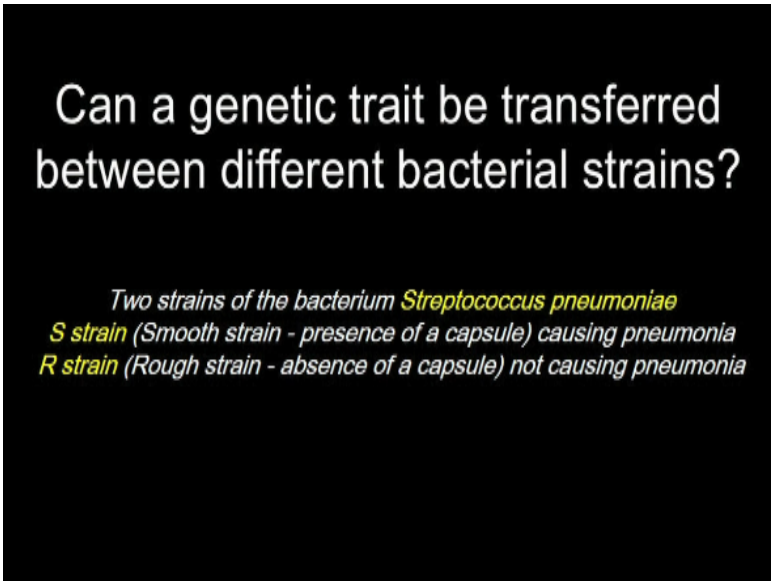

There are two strains one is having capsule with S strain and one is not having capsule with R strain. The one which is having S strain will may cause pneumonia other one is not going to cause pneumonia and this experiment was done by Griffith where he proved the principle of transformation, so we had seen the first condition.

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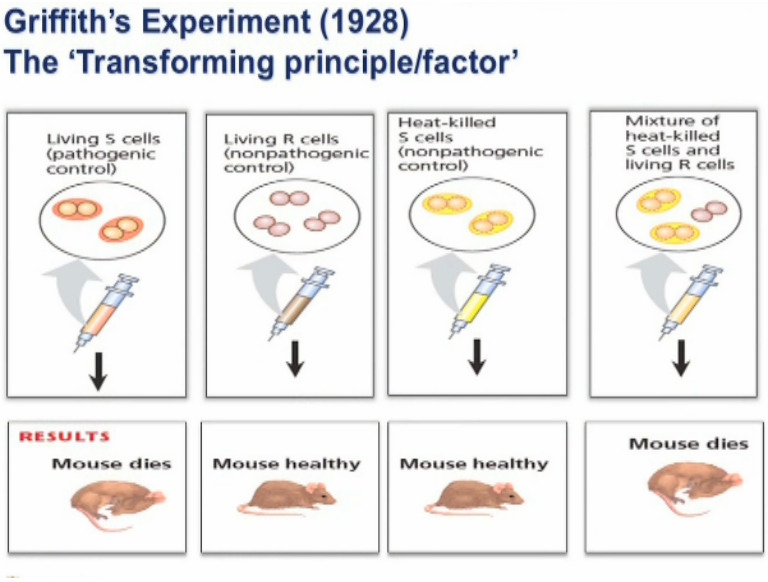

That if you have S capsule which you are now injecting in the mouse, the mouse dies because of the virulence from the capsule. If you have living R cells, then nonpathogenic they will not have any impact, so mouse remain healthy. If you are having the heat- killed S cells killing the capsule layer, then that makes it nonpathogenic and again mouse will remain healthy. If you have lost situation like this when you are adding a mixture of heat killed S cells.

And you are adding the living R cells what are the phenotype for the mouse, the mouse died. And that was because of, **"Professor - student conversation starts"** What happened in the experiment the last one? The last one it gets killed because R cells acquires S cell traits. And how that happen? In which way R cell acquired the S cell characteristics? "**Professor - student conversation ends."**

So, in this case, probably some material, some DNA from the S cell got transferred to R cell and made them pathogenic. And this is what resulted in to this phenotype which was mouse died. So, what Griffith then concluded from this experiment that if we are injecting both non pathogenic and pathogenic. But there is some kind of transformation is happening some substances is moving from the S cells to the R cells.

And that is giving you the property which is making it much more pathogenic and creating the mouse to die. So, this kind of conclusion from this experiment. So, the living R bacteria got some substance from the heat killed S cells which is like a transformation is happening in this particular type of bacteria and therefore this particular phenotype was observed which is not so much common which was not expected.

And that is where he first time realized that there might be some material could be transformed and that resulted in to Griffith transforming principle or the factors which are known as the DNA or the transforming factors.

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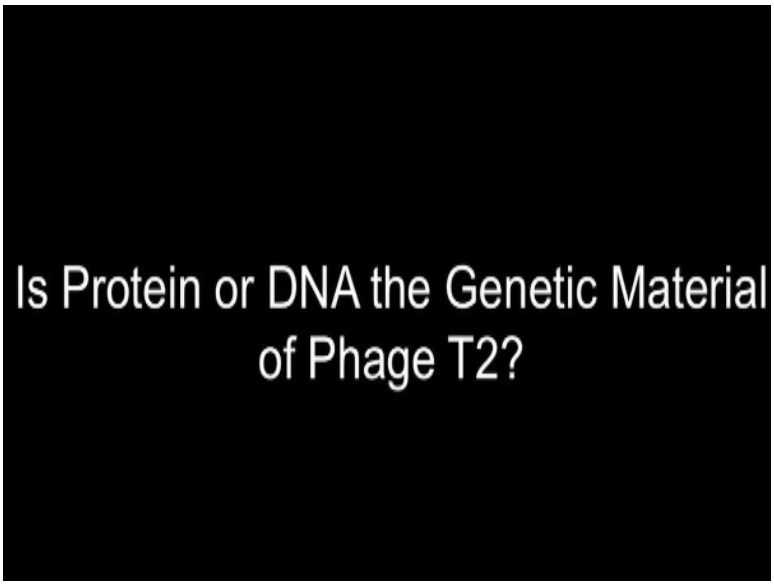

So, now let us come to the next question which we started discussing briefly is protein or DNA the genetic material, and now who will explain this experiment? So, we have been saying that all the genetic based on DNA that DNA is going to transfer from one to the next generation, right? But why by default we assumed that DNA is the only thing which is going to have those property which is going to get transmitted from one to the other generation.

It might be even protein may have the same property, so somebody would have done it first time and thought about this type of this experiment right? And these are the two scientists.

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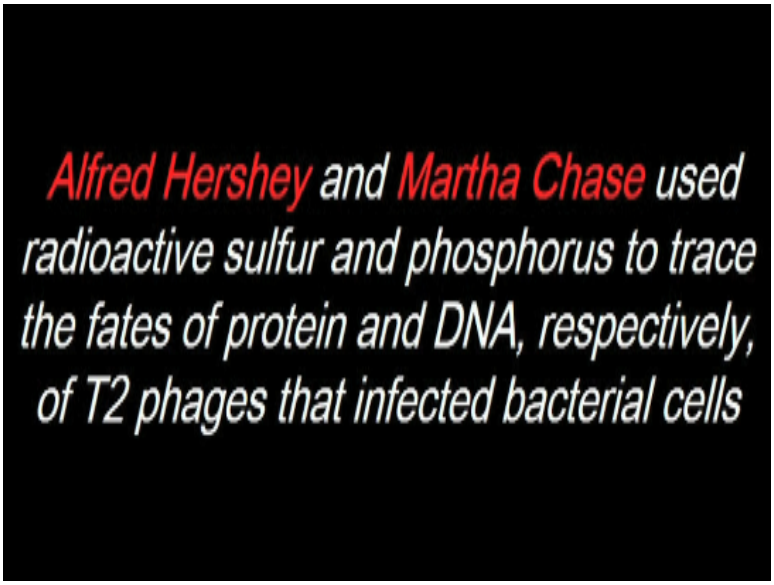

Hershey and Chase if you remember we discuss briefly that one could use some sort of labels to find out or the track these kind of molecules in 1930s and 50s that time most of the experiments were done very elegantly just by using some very basic regions and the labeling strategy then looking at things in the radio activity. So, if you know that you know you can label DNA because you have the phosphorus backbone in the DNA.

With the 32 phosphorus you can label DNA, or you can label protein with some methionine residues are there in (()) (05:48) with the self for you can label proteins so they use that property and let us kind of look briefly.

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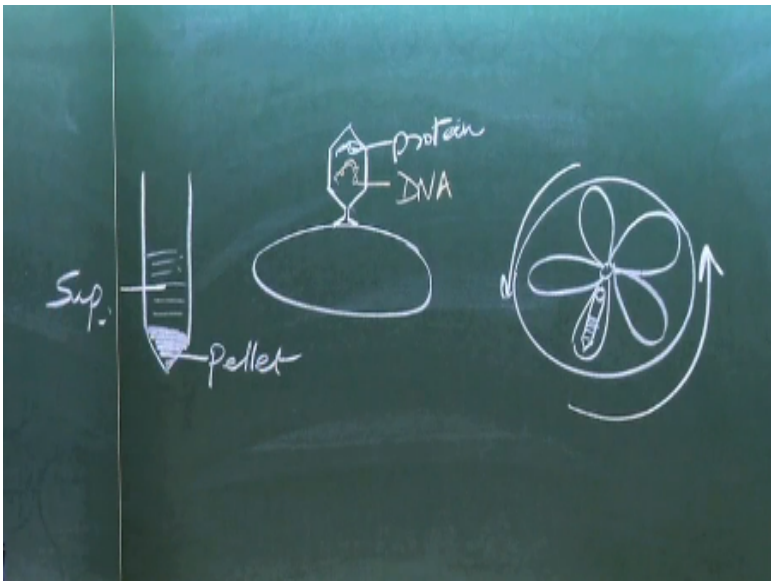

So, just imagine they used one of the bacterial strain and now there is a virus which is phage. A phage which is if it can eat bacteria that is bacteriophage. So, now it is this particular virus is there so virus for its propagation transferred some genetic material to the bacteria and therefore then it can have its multiple property. It can propagate in bacteria and make multiple copies of its own right.

So, what are their thinking? Can we label both the DNA of it and if there is some DNA component of virus or even the protein if you are labeling both of them. And now let us see what is going inside the bacteria which is going to create multiplication so that will be probably one

which is going to have all the genetic information so that one could be extrapolate information format it whether the DNA or the protein have the genetic information.

So, to do this experiment they use a very simple technique, analytical instrument which is known as centrifuge. In centrifuge I am sure you have seen washing machines, so something like washing machine where you are using a very high centrifugal force and let us say you have these are the rotors in which you are keeping the tubes so let us say one of the tube is kept here and when you are using very high centrifugal speed here.

Let us say it know 10000 rpms etc. so then based on this particular speed If you have this tube here whatever the bacterial membrane and the contents are there, they will just come as a part of the bottom part of it which will be pellet and anything which is liquid, clear part that will remain as the supernatant. So, if whatever is going inside the bacteria along with bacterial membrane and everything will come inside which will become the part of the pellet.

And anything which remains out will become the part of the supernatant that as the assumption and he had labeled both protein and DNA. So, he was trying to see from radioactivity what is going inside the pellet and what is going in the supernatant part when he did this particular labeling experiment, what he found that in the pellet.

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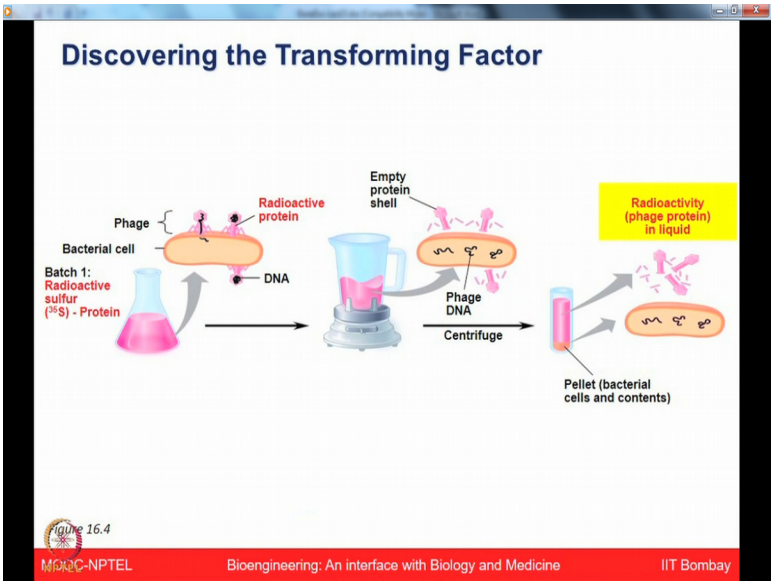

He could see radio activity of the DNA so based on the cell formation and whereas the protein was found in the supernatant part. So, looking at this information he was able to conclude that the DNA is going inside the bacteria from the phage whereas the protein remains outside is not entering inside the bacteria and therefore protein and he can see in this supernatant part and DNA is coming in the pellet part.

Just by doing the simple labling experiment he was able to conclude that the phage DNA entered the bacterial cells, but phage proteins did not. So, these are some very classical experiment done in 1900 that century which has resulted in to lot of fundamental information and if you think about it and I think these are not so difficult thing to do it just need some sort of concepts to be tried out.

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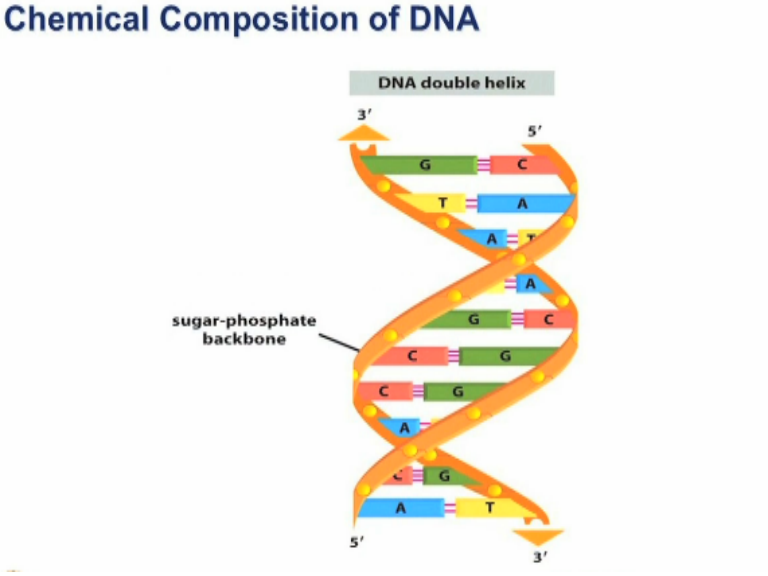

I am sure we have studied about chemical composition of DNA is you have been taught looking at two DNA strand double helix model of DNA.

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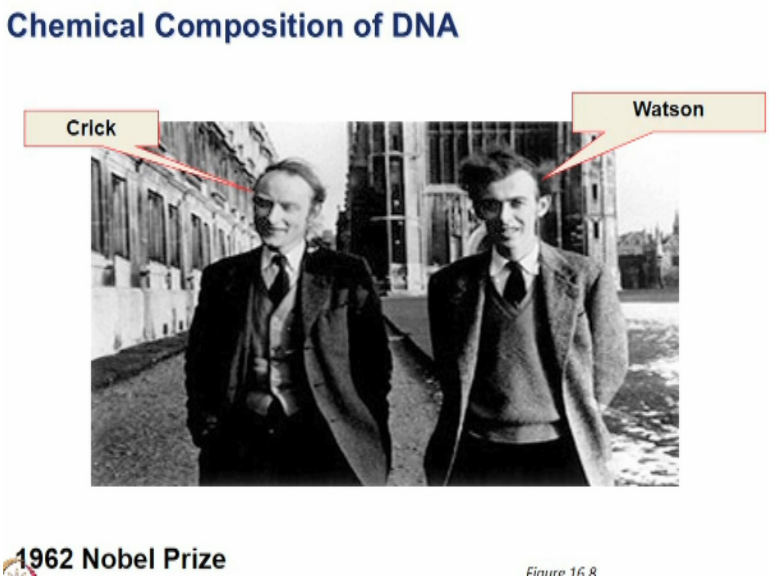

Watson and Crick these are the scientists who illustrated the structure of DNA and they got Nobel prize for it.

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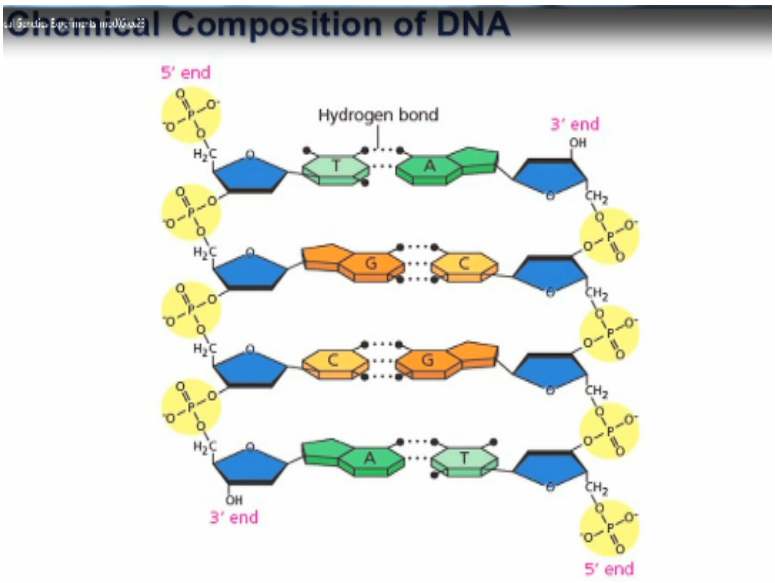

Very briefly you have know 80 and GC base pairs whenever you have A, you will have the T with the two hydrogen bonds and G with the C3 triple hydrogen bonds. So, this kind of pairing will be there complimentary base pairing will be there.

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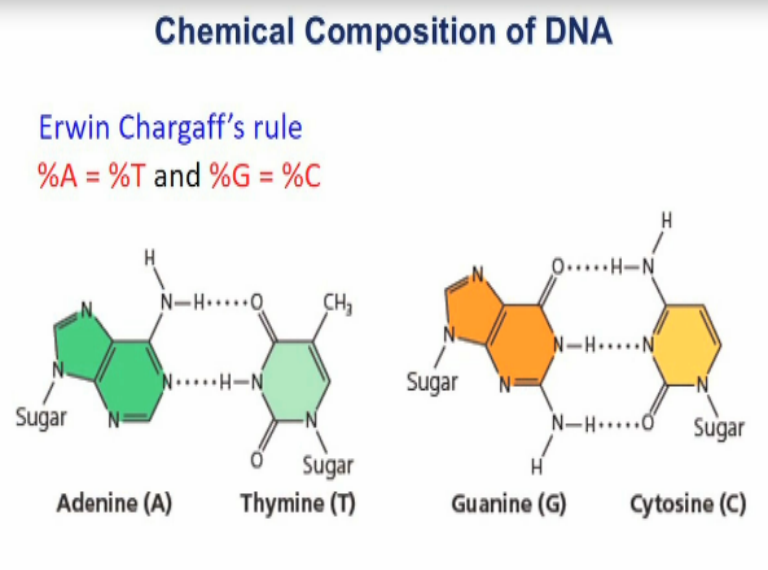

And now whenever a scientist actually Erwin Chargaff he derived the rule that a % A base sphere = % T base sphere whereas % G base sphere = % C base spheres and some of the other basic formation linked to the DNA. Now let us think about one of the properties of the DNA which was DNA replication let us think about how DNA replication happens, so you are making multiple copies of DNA happening inside the cell.

DNA is double stranded, so people have proposed multiple hypothesis what are the possible ways in which DNA replication may happen? And they were have been three hypothesis proposed.

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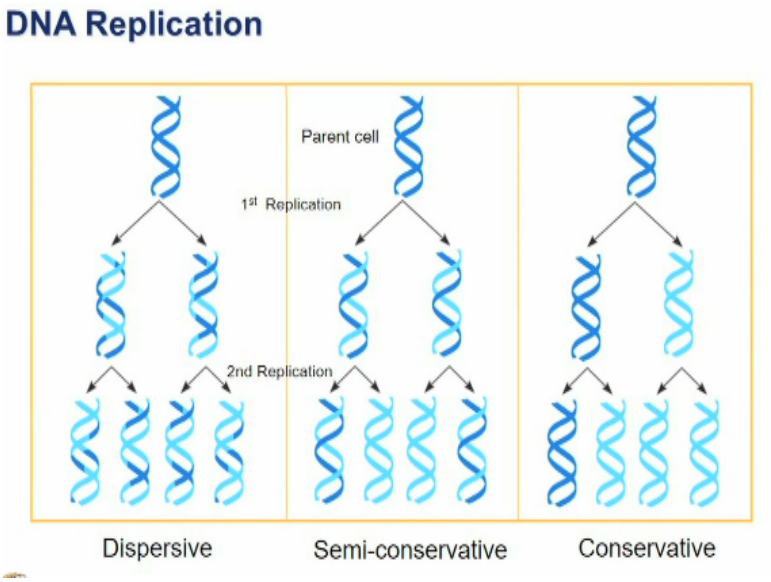

Dispersive, semi-conservative and conservative. Semi-conservative has been most popular hypothesis.

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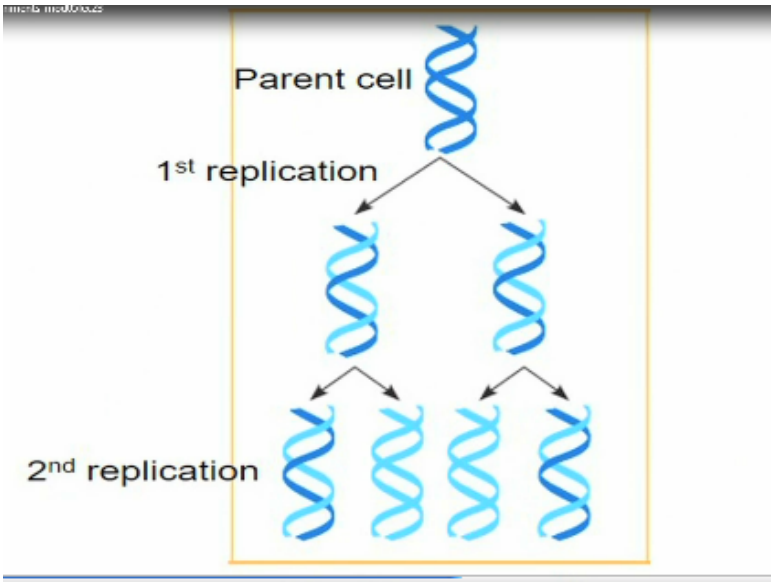

So let us look at this slightly in detail If you have this DNA, let us say dark blue color ones, both double helix Now in the first replication after that you can see one of the dark blue remain there and one of the new light chain new light form has appeared and now the second generation, one of the dark form and the light form remain there and again light forms again is synthesized, which makes it again double helix.

This is kind of DNA, semi-conservative replication the conservative replication is based on that 1 DNA is always going to be like the original parental DNA, even in the after the first and the second replication and the dispersive model says a mixture of both the strands of the DNA will be keep segregating after first replication and the second replication. So, again, these are the hypothesis.

Now people were kind of puzzled at what is the way of DNA replication to happen and a scientist, a group of scientists in fact Meselson and Stahl.

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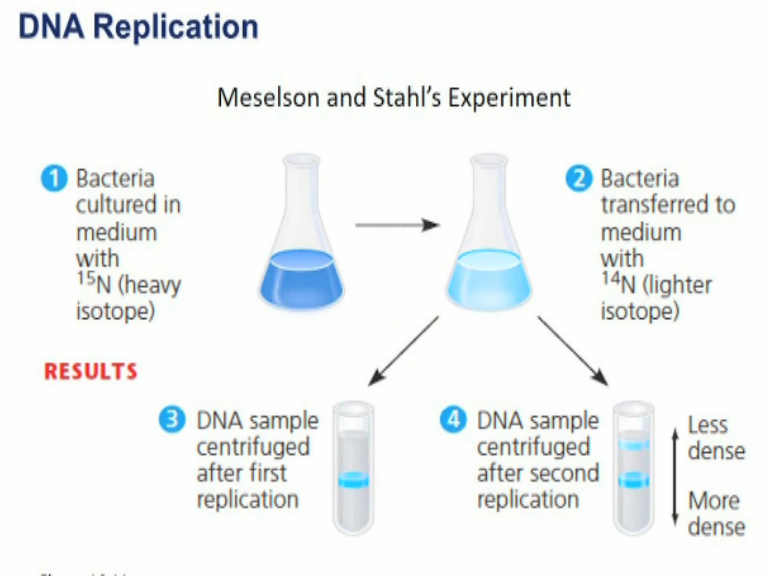

They did a very nice experiment and try to prove in which way the DNA replication may happen so let us assume that we have this DNA in the parental strand.

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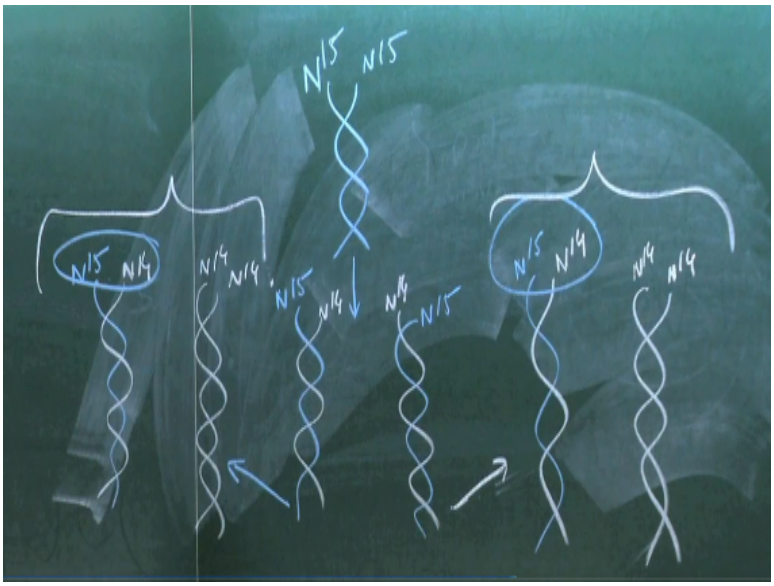

And both of them are N15 labeled after the first generation one of this strand from the parental one remains there and a new form which is from the light, so this is N15. One is strand comes from N15 and 1 Meselson size N 14 and from this one we have one of the blue one and one of the white. And from this again alright same will happen again here as well right. From this part okay now let us think about the % of.

How much we have heavy farm here in the parental 1 for N 15 so that is 100% right. Now if I have grown N15 DNA in a medium which is light medium and 14 medium. Now the second strand of DNA is N14. What is the ratio here for the hybrid? It is a Hybrid. The hybrid means it is having both N14 and N15 mixture of that right. So, in this particular one, in the first after the first replication everything becomes hybrid 100% hybrid forms here.

Now as it goes to the second replication how much is hybrid and how much is the N14? This is hybrid, right? This is a hybrid right so these two are as well as these two these are part of the second replication so what is the %age of hybrid and %age of N14? 50 - 50 right. So, there are ways of doing the density centrifugation where you can separate these based on the density. They use cesium chloride based density to separate this particular bands.

Light or heavy form of band and this is how the experiment was being done, so initially they grow bacteria in and 15 are the heaviest isotopic forms. Then they transferred that to the light form or the N14 medium and now from there they can see the bands you know is that coming in the center or there is some new brand appearing which are less dense than that and when they analyze those particular fractions.

Then they are able to conclude what is the ratio of N 15, what is the ratio of N14 and N15 and what is the from 1 to next generation, how much % they can see the change.

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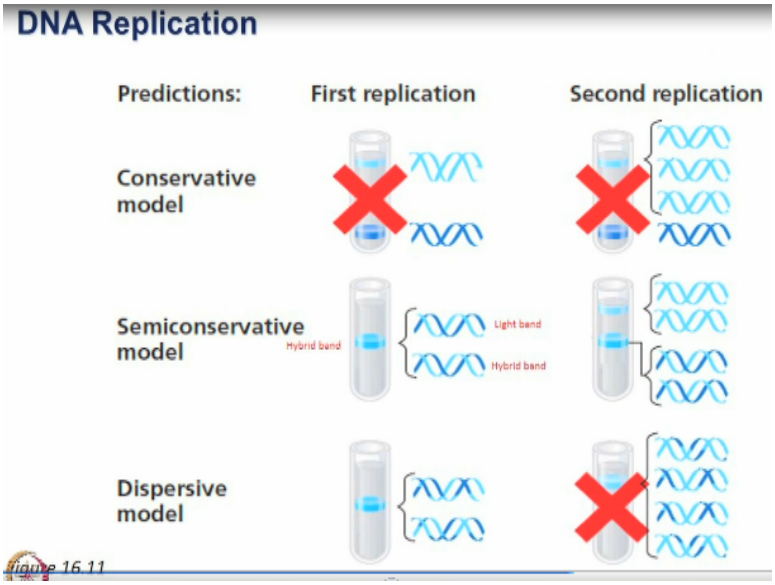

So if you see in this particular experiment here in the hybrid band, which is having both100 % after the both replication and after second replication you have 50 % of the hybrid band and 50 % of the light band appearing. Now if you go to the next generation what will happen? Please draw 25 75 somebody mentioned rightly alright. So, this is pretty much a way for them to prove that among the three models which are known are dispersive model.

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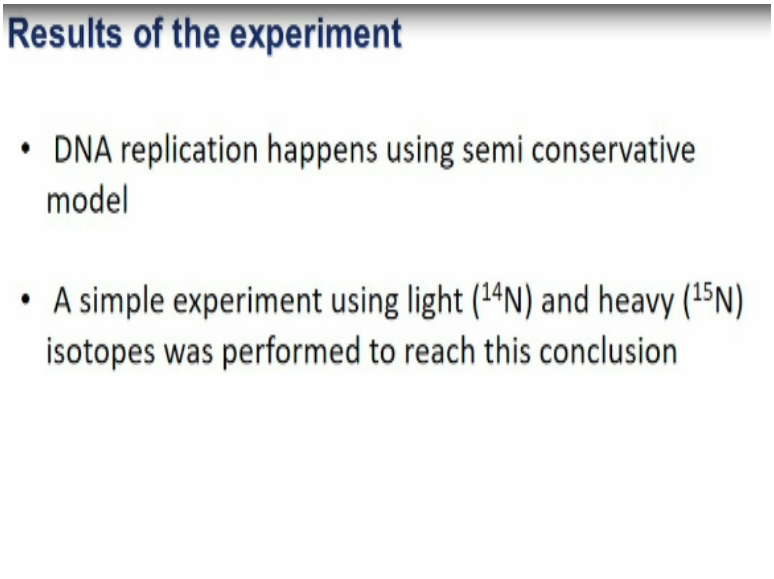

Semi conservative conductor, even conservative probably the DNA replication happens using semi conservative model and just by growing DNA from N15 and N14 medium and analyzing the DNA contents they were able to make this conclusion.

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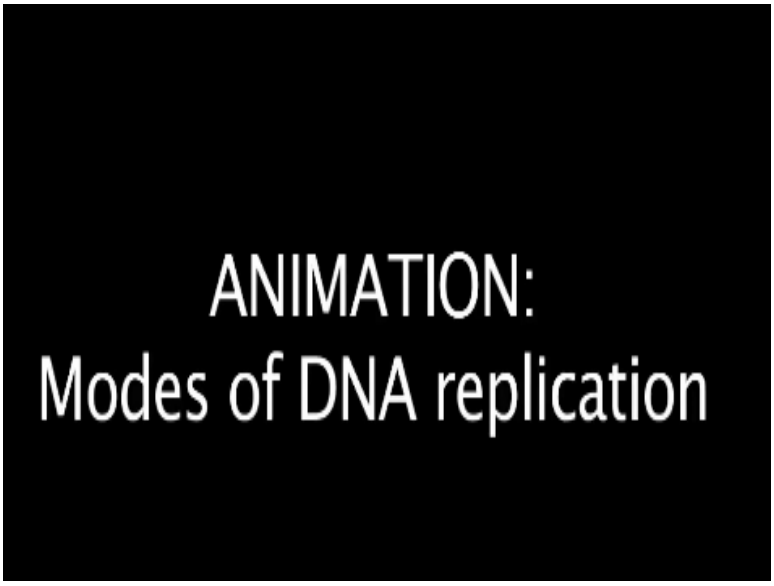

Let me explain more detail in the following animation.

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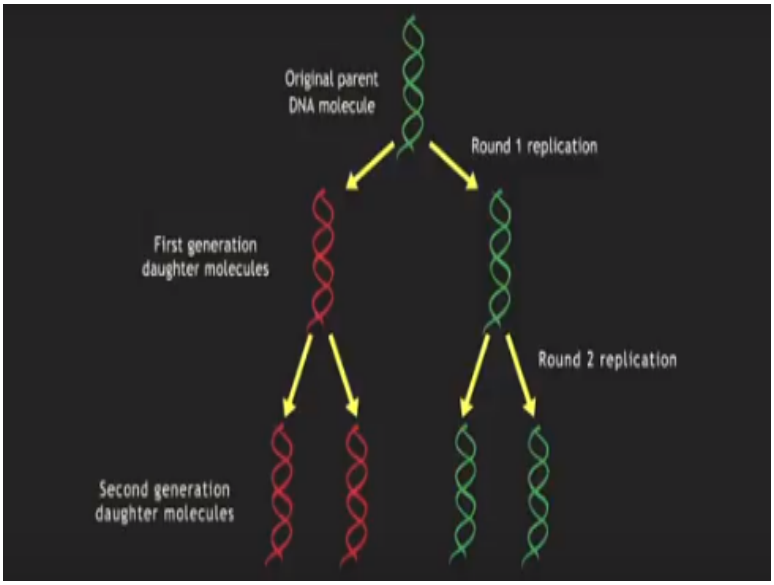

According to the conservative model, the two parental strands of DNA as a whole, serve as a template for the synthesis of progenerity and A molecules thus one of the daughter DNA molecules is actually the parental DNA while the other daughter DNA consists of two newly synthesized strand from fresh nucleotides. The dispersive model of DNA replication hypothesizes.

That the parental DNA molecule is cleaved into smaller double stranded DNA segments. Which serve as a template for synthesis of new DNA strands. The segments then reassembled into

completed DNA double helix with parental and daughter DNA segments interspersed. The content of parental DNA is a double helix goes on decreasing with each generation. According to the semi conservative model of replication.

Each parental strand act as a template for the synthesis of a new strand of DNA which is complimentary to the parental strand. Each daughter DNA molecule always has 1 parental DNA strand and one newly synthesized daughter strand.

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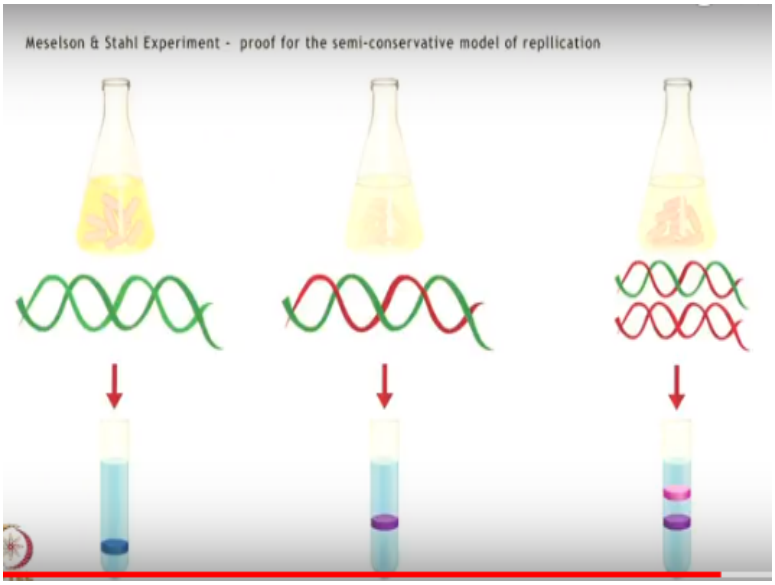

Of the three replication models suggested Meselson and Stahl proved that the semi conservative modern was correct for this. They grew E coli culture for several generations in 15 containing medium. So, that the basis in DNA contained N15 instead of N14. Next and they transferred and grew the cultures for several generation in and N14 containing medium. Throughout the period of growth samples were taken cells listed the DNA analyzed.

By centrifugation and in CSCL gradient. The parent DNA showed 1 band in CSCL gradient corresponding to N15 DNA. The first generation daughter molecules also showed 1 band which was not at the same position as parent DNA. This correspondent to N14 N15 DNA while the second generation showed two bands. One of N14 N 15 and the other of N 14 light DNA. These results exactly matched the semi conservative replication model.

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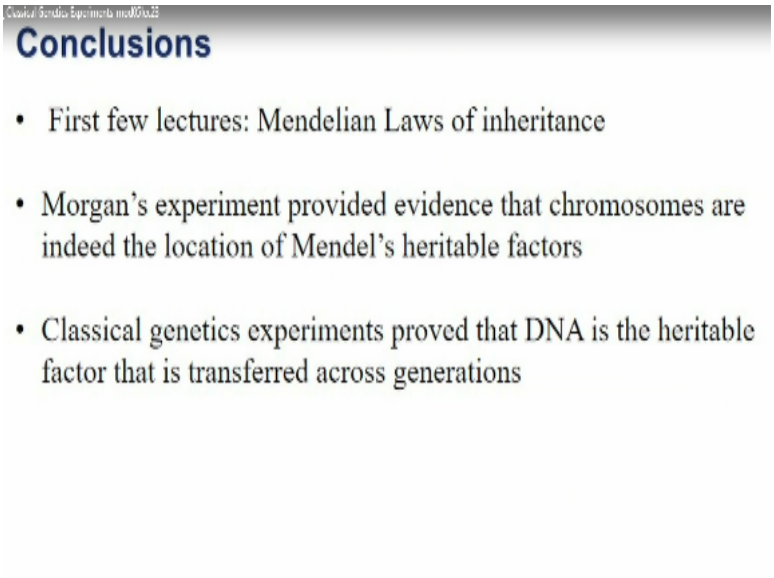

So, this brings the end off this entire section on genetics, are you now familiar with Mendelian law based on Mendels experiment on the classical pea plant. You now also know how a Morgan independently tested A Mendels observation, in mendel experiment using another model system which was drosophila melanogaster or the fruit fly which provided the evidence that the chromosomes are indeed the location of Mendels heritable factors.

Then we have discussed about the classical genetics experiment which proved that the DNA is heritable factor and it is transferred across generations.

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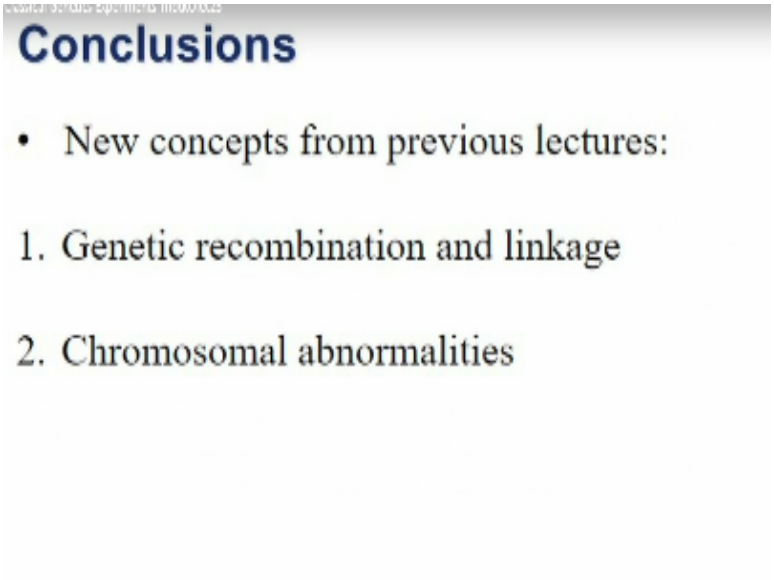

You are also introduced to the concepts of genetic recombination and linkage between genes and how it affects the inheritance of characters. We then discussed about few chromosomal abnormalities and looked at you know various examples and syndromes. How alteration of chromosome numbers and even structure could cause some of the genetic disorders.

In the next lecture we will start about you know a new topic thinking about the bacteria and other prokaryotes and then we will talk about some of the applications which are linked to those microorganisms. Thank you.

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