Introduction to Complex Biological Systems
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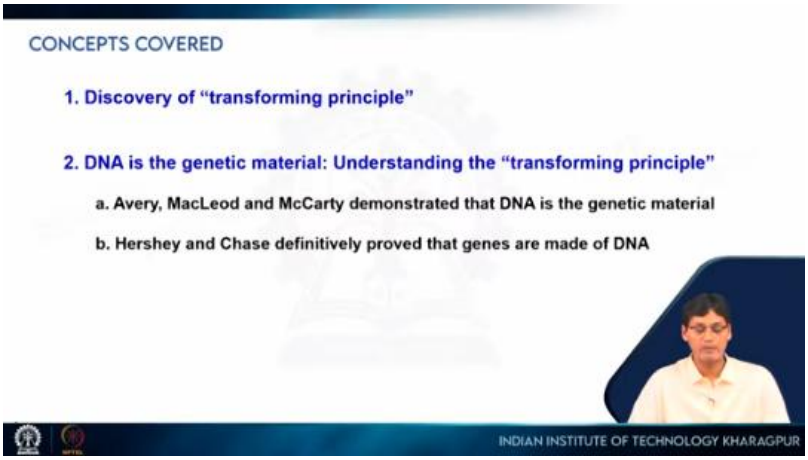
Lecture 2
Discovery of Genetic Material

Hello, everyone. In the last lecture, we discussed the fundamentals of the living system and, particularly, I mentioned that genetic material is one of the most important things that should be present inside all living units. Now in this lecture, I'm going to discuss the discovery of genetic material.



I will particularly explain three classic experiments which help us to realize that DNA is our genetic material. The first one is the discovery of the transforming principle. So this is very exciting and important experiment. All of us know that in 1918, there was Spanish flu. So during that time, like more than 100 million people died because of Spanish flu and that is roughly about 5% of world population and scientists across the world, they are trying to develop some kind of therapeutics. During that time, it has been noticed that although Spanish flu, it's a viral disease, but because of that viral infection, the immune system gets weak and because of that, some secondary infection comes. And during that time, Griffith, a British microbiologist, was working with streptococcus pneumoniae. This is the bacterium which causes pneumonia and he was trying to develop some kind of a vaccine against streptococcus so that people can be cured from pneumonia. So what he found, he found that there are two types of streptococcus pneumoniae. So, one is the R type.

So, R stands for here rough surface something like this rough surface and another strain of bacteria he found S type. So, the surface is smooth. So, why are they smooth? Because they have very thick polysaccharide layer on the outer side and they are smooth bacteria.

Now this R strain of bacteria they are non-virulent non-virulent means they cannot cause the disease. For example, this rough surface bacteria or non virulent strain, if we inject that in some mouse, the mouse will survive. They will not die. So this is called non virulent.

And now the smooth surface bacteria, H type bacteria. So they are virulent. So as a result of that, if we inject this type of bacteria, S strain of bacteria, so the mouse will die. Now the thing is, why is this smooth surface bacteria so virulent? Because they have this polysaccharide coating outside, so they escape the host immune system.

So as a result of that, the host immune system cannot detect properly. As a result of that, those S strains of bacteria, they can multiply and they can cause the disease. As a result of that, the mouse will die. So now Griffith did a series of experiments and he proved some interesting things here. So what happened?

Griffith used mouse as model organism. So in this mouse if we inject what Griffith did, so he injected R strain or the rough surface bacteria here. R strain bacteria so as I already mentioned so if we wait for some time i.e. few days the mouse will survive that I already mentioned because the R strain is non-virulent so as a result of that mouse will survive then in the next step of experiment What he did, he injected S strain of bacteria into mouse and now the mouse will die because the S strain is the virulent strain. This is plain and simple. But in the next interesting experiment he has done, he again injected S strain of bacteria, but it is heat killed. So, that means, he just boiled the bacteria in just like a pressure cooker or some kind of autoclave. This is called heat heat-killed S strain. Since those bacteria are dead they cannot cause the disease. So as a result of that, again the mouse survived. Now, the most interesting experiment is coming here.

So what he did he injected heat killed S strain bacteria plus live R strain of bacteria. So, together he injected this. Now, the result was surprising because individually heat-killed bacteria cannot cause the disease and mouse can survive. Similarly only the R strain cannot

also cause the disease but in this case surprisingly he found that mouse died, so this is very surprising at that time.

Because individually, the heat-killed S strain, it cannot cause the disease. At the same time, live R cells, that also cannot cause the disease. So he actually hypothesized that something is present in the heat-killed S cells and that converts the R strain of bacteria into virulent strain so that the mouse can die. Not only that, more importantly, when he took some sample from this dead mouse and culture in the laboratory, he found from this mouse that smooth cells bacteria are appearing smooth cells bacteria, they are appearing in the culture plate not only that this transformation this changes is permanent. If you grow these cells again and again in Petri plates they will give rise to smooth cell bacteria so that means this change is permanent and this is he mentioned that this is transforming principle. This is very important.

But what is the transforming principle? What is the ingredient that was not known during that time? But something is present in heat-killed S-strain that converts R-cells into S-cells and unfortunately he didn't realize the everything and when those things happened later on because this time whatever I mentioned the experiment is 1928 then soon after second world war started and in 1941, he passed away because of bombing in his laboratory. So as a result of that, he didn't see the final conclusion of this experiment. Later on, some other scientists proved that this transforming principle is nothing but DNA and that stored the information. So as a result of that that in heat-killed S cells in S strain although the bacteria is dead but some of the DNA present in this strain that went into the R cells and it convert the R cells into S strain so that they can make this polysaccharide layer on the surface and as a result of that that bacteria can survive, it can escape host immune system and therefore it can cause the disease and it can kill the mouse.

But all these things came later by some other group of scientists. So here I would like to mention that the experiment that proves DNA is our genetic material. Griffith just said this is a transforming principle. It's very important.

But a series of experiments conducted by Avery, McLeod and McAtee demonstrated that DNA is our genetic material. So, briefly I would like to mention here that what they did that they took a cell suspension that means cell free extract of S strain of bacteria.

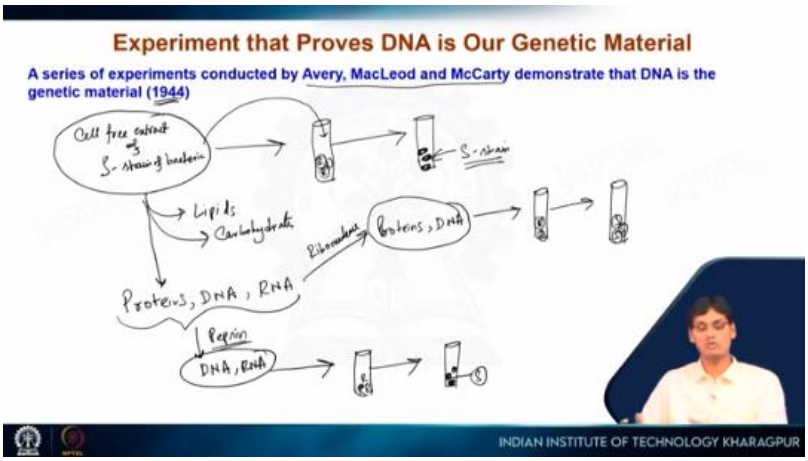
So, cell free extract of S strain of bacteria so there is no cell so only the extract of S strain then what they have done, they were trying to do different types of experiments, they are trying to fascinate it so that from the cell extract they are removing carbohydrate, they are removing a lipid, sometimes they are removing protein or nucleic acid and then they are trying to see what is the chemical nature of transforming principle? That means when you are removing the lipid and carbohydrate and protein, it can still transform this bacteria or not, transform this R strain to S strain or not. Because they cleverly escape or I would say they avoid the use of mouse here.

It will take longer. What they have done, they took this cell-free extract and then they add this extract in some tube where you have R strain of bacteria and if you culture this R strain of bacteria and after some time they observed that in this tube S cells bacteria also appeared that means they avoid the use of mouse but still you can tell that the cell free extract of this S strain of bacteria, it can convert R strain into S strain. So now this is again S strain. Now what are they trying to do? They are trying to analyze what is the chemical nature of this transforming principle.

So as a result of that, they took this cell extract and then they removed lipid and carbohydrates. lipids and carbohydrates from by some biochemical method so now their hypothesis is in this cell free extract, now they have proteins and nucleic acids so nucleic acids here means DNA and RNA i.e. deoxyribonucleic acid and ribonucleic acid. So, all these three things are present in this cell-free extract.

Now what have they done? So they treated this extract with some protein destroying enzyme. So they used pepsin. pepsin will destroy protein, so they use pepsin so as a result of that they would expect that there is no more protein, so they have DNA and RNA or nucleic acids present there. Now when they use this extract and again they put into a tube where they have R strain of bacteria, they found that this R strain can be transformed into S strain again, that means protein is not responsible for this transformation although they destroyed protein by adding pepsin, still it can transform R strain into S strain then what they have done? They

also added Ribonuclease in a different experiment. Ribonuclease is again another enzyme. So, it will destroy RNA. Then we have here proteins and DNA and this one has the ability to convert R strain of bacteria into S strain, so that means the transforming principle is still present here, therefore they have concluded in 1944 that DNA is responsible here because they have destroyed both proteins as well as RNA, but still it can transform R cell into S strain or smooth version of this bacteria. But we have to be careful here because during that time the scientific field was dominated by protein biochemists. Almost everyone believed that proteins do function for us, not the DNA, because DNA is sometimes thought by scientists to be a very boring molecule, because DNA has only four nucleotides, it's repetitive structure only. But proteins, on the other hand, have 20 different amino acids and their side chains are so different and they can function for us. So as a result of that, it was very difficult for Avery, MacLeod and McCarty to convince the scientific community that this is DNA and DNA is responsible for this transformation. But they also tried to carry out some more experiments. For example, they use some ultracentrifuge, when they centrifuge this cell free extract, they found that something coming at the bottom of the tube.



So that portion should be very high molecular weight and only that bottom fraction of the ultracentrifuge tube can transform R strain into S strain. So therefore, they again predicted that something very large polymer is responsible for this transformation. So, for example, lipids, carbohydrates, and proteins are much smaller compared to long stretches of DNA. So as a result of that, they are again concluding that DNA is responsible for this transformation. But the major problem was during that time that some enzyme which will specifically cleave, specifically destroy DNA was not discovered.

In 1946, McCarty, so he purified DNAs enzyme and then he proved that whenever he is adding this enzyme DNAs, then the transforming principle, it cannot, whatever you know present in this extract, if you add DNAs, then it cannot convert R cells into S cells anymore. So therefore, it establishes that DNA is the genetic material. Whatever the information present in DNA, that actually transforms the character of R cells into S cells and they can produce a polysaccharide layer on the surface and therefore they established or this experiment proved that DNA is our genetic material.

But as I told you, this is in 1944, this was second world war time and most of the scientists didn't pay that much attention here. Part of the reason was, as I said, that during that time, most of the scientists believed protein is the ultimate thing. But again, in another round of experiments done by another group of scientists, Hershey and Chase, they definitively proved that genes are made of DNA in 1952. So this is a very exciting experiment and after this experiment, everybody in the scientific community agrees that DNA is our genetic material.

What Hershey and Chase did, they took some phage virus. So T2 this is bacteriophage, bacteriophage means they will kill bacteria. So what happens is it can infect bacteria, for example E. coli then within 40 minutes or 1 hour this virus will multiply inside E. coli and they will burst E. coli cells and millions of T2 phase will be produced from this E. coli. Now they utilize this system to prove that DNA is our genetic material.

The beauty of this system is this T2 phage or virus, they don't have so many complex things inside their body or inside the virus. So, they just have proteins, generally that form the coat of surface of the virus and they do have nucleic acid or nucleic acid. or DNA proteins and nucleic acid. Now, what they did was they labeled T2 they labeled T2 bacteriophage with radioactive phosphorus 32p in some culture they developed this radioactive label T2.

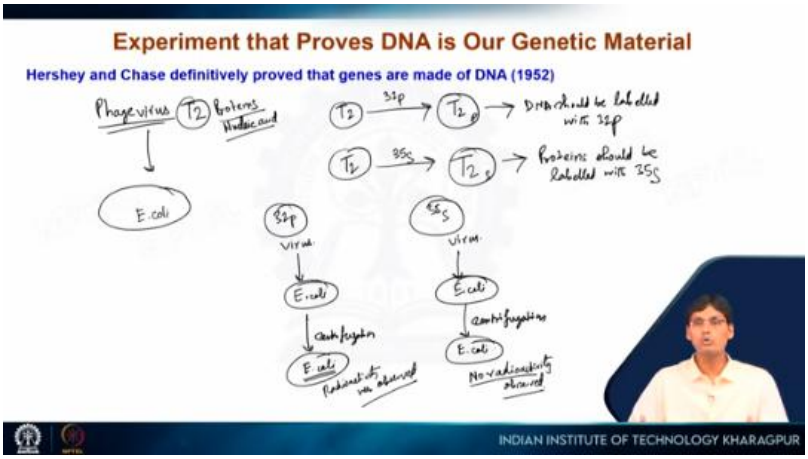
And now in another experiment they labeled this bacteriophage with radioactive Sulphur 35S radioactive sulphur. So, again they got T2, but it is radioactive sulphur labeled. So, this is sulphur and this is phosphorus radioactive. Now, the thing is as I already told before that phosphorus is present in DNA or nucleic acid.

So, as a result of that in this case DNA should be radioactively labeled. So, DNA should be labeled with 32p and in this case proteins should be labeled because proteins should be labeled with 35s or radioactive sulphur because phosphorus is not present in protein. So now what they have done is, they use this 32p labeled virus, they infect E. coli cell or bacterial cell with this 32p labeled virus and they don't wait for 40 minutes or one hour. After just a few minutes, they remove the viruses which are just stuck outside the surface of bacteria and then they centrifuge it and they found the bacterial pellet after centrifugation. for example after infection, after few minutes they just shake the solution vigorously so that whatever sticks to the surface of the bacteria that will fall apart and then they centrifuge and small viruses they will be in the supernatant and bacteria they are much heavier compared to this T2, so they

will be at the bottom and when they after centrifugation, they found the E. coli cells at the bottom and they found radioactivity there inside E. coli cell.

They found radioactivity. But when they use 35s labeled virus and then they infect E. coli. Similarly, after few minutes, they shake the solution to remove the virus which is still stuck to the surface of bacteria and then they carry out centrifugation. Then they found an E. coli cell at the bottom, but no radioactivity observed here.

So, therefore, it proved that when this T2 infect bacteria and they can multiply, they can make huge amounts of phage virus from the E. coli cell. So there should be some gene and that is playing this role. But from this experiment, it has been proved that it is when the 30 phosphorus is labeled, that can be stress inside the bacteria. That means the nucleic acid is entering, the DNA is entering inside the cell and that causes multiplication of this virus inside the bacterial cell.



Whereas when we are labeling, when they have labeled the sulphur, It was not entering. The protein is not entering inside the bacteria. So as a result of that, it definitely proved that DNA is our genetic material. And all these three experiments together, Griffith's experiment, followed by Avery, MacLeod and McCarty's experiment, and the last one here, Hershey and Chase experiment, all this experiment, they definitely proved together that DNA is our genetic material.

That's all for this class. Thank you very much.