

**Introduction to Complex Biological Systems**  
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**Lecture 60**  
**Landmark experiments in biology**

Welcome to the last lecture of the course Introduction to Complex Biological Systems. So in this lecture, I will give you a brief description of what we have seen so far, with an emphasis on some landmark experiments. So we have learned these concepts as if they are known truths. But there has been a lot of work done by some really smart people over the years to arrive at these truths that we now take for granted.

So I will just pick some of the important experiments, which we refer to as landmark experiments, and discuss them. Some of these you have already seen throughout this course, and some will be new to you. So, for example, I will start with the identification of DNA as genetic material and discuss these three experiments. Then I will talk about the discovery of the structure of DNA, which is the double helix structure, and the contributions of all these important scientists in that discovery. Then the discovery of semi-conservative replication, the discovery of codons, when we translate from RNA to protein, we have to go from a 4-base language to a 20-base language.

**CONCEPTS COVERED**

- **Introduction to molecular biology.**
- **Identification of DNA as the genetic material:**
  - Griffith's experiment
  - Avery, Macleod and McCarty's experiment
  - Hershey and Chase's experiment
- **Discovery of structure of DNA:**
  - Contribution of Pauling
  - Contribution of Franklin
  - Contribution of Watson and Crick
- **Discovery of semi-conservative replication of DNA:**
  - Meselson and Stahl's experiment
- **Discovery of codon.**
  - Beadle and Tatum's "One Gene, One Enzyme" experiment
  - Crick and Griffith's concept of commaless triplet-based nondegenerate code
  - Conrat's experiment on RNA genome of TMV

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So that is where codons become so important. Then the discovery of tRNA as adapter molecules, the deciphering of the genetic code, which is the dictionary used to synthesize proteins from the message encoded in DNA or RNA, and finally, the discovery of RNA

splicing. Again, this does not cover all the important experiments in biology, but it will give you a flavor of how real science is done. Let us start with molecular biology because all the experiments I am going to discuss today come under the purview of molecular biology. So what is molecular biology? Here we are talking about important molecules or biological molecules which play an important part in these biological systems. So molecular biology is a branch of biology that focuses on understanding biological activities at the molecular level, particularly the interaction between DNA, RNA and proteins.

**CONCEPTS COVERED**

Landmark experiments in molecular biology.

- **Discovery of tRNA as adaptor molecules.**
  - Crick's Adaptor Hypothesis
  - Zamecnik & Hoagland's contribution
- **Deciphering the genetic code:**
  - Nirenberg's contribution
  - Khorana's contribution
- **Discovery of RNA splicing.**

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So these are the three very important biomolecules that we have discussed about them at length in this course. So molecular biology primarily studies nucleic acids, which are DNA and RNA and proteins and their roles in gene expression, regulation and cellular function. So the flow of genetic information follows central dogma. We have discussed that at length throughout this course and I will again put the central dogma as the central theme of today's lecture, where DNA is transcribed into RNA, which is then translated into protein and proteins are the ones which perform all the cellular functions. To do molecular biology, the tools that we need have been designed through all sorts of very interesting and important experiments throughout the past 100 years. So their techniques like PCR, DNA sequencing, recombinant DNA technology are something that is very important, gene editing, for example CRISPR, etc. So molecular biology has revolutionized genetics, medicine and biotechnology. So it has enabled us to make some real advancement in techniques like gene therapy for development of new drugs, even in forensic science and personalized medicine.

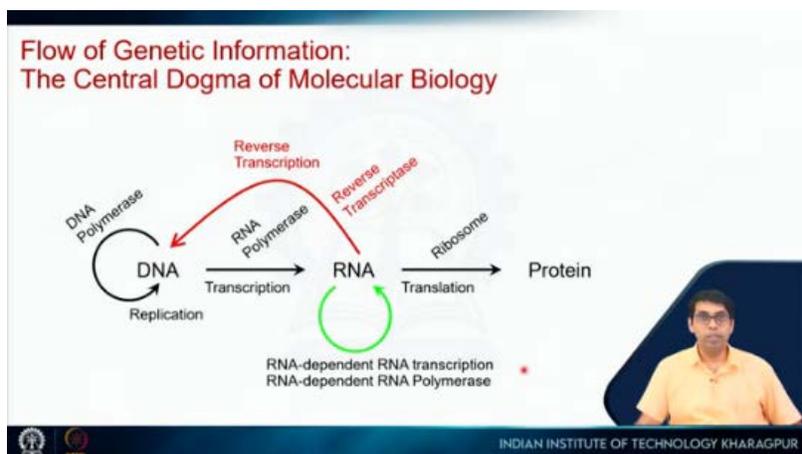
### Introduction to Molecular Biology

- **Molecular biology** is a branch of biology that focuses on understanding **biological activities** at the **molecular level**, particularly the interactions between **DNA, RNA and proteins**.
- It primarily studies **nucleic acids** (DNA and RNA), **proteins** and their roles in **gene expression, regulation and cellular function**.
- The flow of genetic information follows the **central dogma**: DNA is transcribed into RNA, which is then translated into proteins that perform cellular functions.
- Molecular biology employs techniques like **PCR, DNA sequencing, recombinant DNA technology** and **gene editing** (e.g., CRISPR) for research, medicine, and biotechnology.
- Molecular biology has revolutionized genetics, medicine, and biotechnology, enabling advancements in **gene therapy, drug development, forensic science and personalized medicine**.



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So this is something that is going to be really big in the next decade that we are going to see. So major advancement in medicinal science you will see will be in personalized medicine. So let us focus on this central dogma of molecular biology because this will be the central theme of today's lecture. So what we have seen so far, DNA is the one which encodes information in most organisms. When that information is copied, that is replication, it is done by DNA polymerase.



That information is copied into RNA. So part of the DNA is copied into an RNA and a reverse process can also happen which is reverse transcription. So this process is transcription and the reverse process is reverse transcription. So the synthesis of RNA from DNA is done by RNA polymerase and synthesis of DNA from RNA is done by reverse transcriptase. Now RNA can also be synthesized using RNA as a template.

So in this case it becomes RNA dependent RNA transcription and it is done by RNA dependent RNA polymerase. So this is also something that is found in viruses. Now RNA

is used as the messenger from which proteins are synthesized and this last process is called translation and it is done by ribosome and we have seen that for this the information that is encoded in RNA is converted into the amino acid sequence by using of a table called the genetic code, where three consecutive nucleotides code for one amino acid and that interpretation is done by certain adapter molecules called the tRNA.

So, we will talk about the discovery of tRNA and codons in today's lecture. So, the first one is the identification of DNA as the genetic material. So, till this time, all these three molecules were known: there are two types of nucleic acids, DNA and RNA, and there are proteins. But it was not clear which of these molecules is the major genetic material.

The first clue came from this landmark experiment, which was done by Frederick Griffith. So, this was discussed in the first week's lecture. So, I am going to briefly talk about it. Griffith was a British bacteriologist. In 1928, he performed this experiment, which revealed the transforming principle.

So, what is the transforming principle? To do that, Griffith used *Streptococcus pneumoniae*. So, it's a bacterium and this bacterium comes in two strains. The S strain, where S stands for smooth, and the R strain, where R stands for rough.

Now the smooth strain is the one which is infectious. So we call it virulent and the rough strain is not infectious, so it is non-virulent. The major difference of these two is that the S strain had a protective polysaccharide layer on top. So that's why under a microscope it looks shiny, it looks smooth.

But the rough strain, since it does not have this polysaccharide layer, it will not reflect light, it is not shiny, so it is rough and because of this polysaccharide it can escape our immune system and actually can cause virulence. So Griffith observed that when he injected the S-strain into mice, they died, but when he injected the rough strain into mice, they survived. However, the interesting experiment was that when he heated the S-strain, you just boil this bacterium; so it will rise, and everything comes out in the open. If that solution is injected into mice, the mice survive because we have heat-killed the bacteria. But if you mix this solution with live R strain incubated for some time and then inject that into mice, the mice die.

### Identification of DNA as the Genetic Material

**Griffith's experiment:**

- **Frederick Griffith** was a British bacteriologist whose 1928 experiment with bacterium was the first to reveal the "**transforming principle**", which led to the discovery that DNA acts as the carrier of genetic information.
- Griffith worked with ***Streptococcus pneumoniae***, a bacterium that causes pneumonia. He used two strains: **S strain (Smooth) – Virulent** due to its protective polysaccharide coat and **R strain (Rough) – Non-virulent**, lacking the protective coat.
- Observations:
  - Live S strain injected into mice → Mice died.
  - Live R strain injected into mice → Mice survived.
  - Heat-killed S strain injected into mice → Mice survived.
  - Mixture of heat-killed S strain + live R strain injected into mice → Mice died.



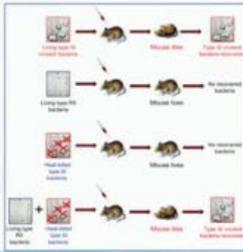
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When he looked into the blood of these dead mice, he actually saw that it contained live S strain. So somehow the R strain got converted into the S strain and that is what is this transforming principle. So it means that when the heat kills the S strain, the molecules that come out, one of those molecules is able to convert or transform the R strain into the S strain.

So this is the transforming principle and then people started looking for the molecule responsible for this transformation of the rough strain to the smooth strain. So this is the summary of Griffith's experiment. So he found live S strain bacteria in dead mice, suggesting that the harmless R strain had transformed into the S strain by acquiring some transforming principle or molecule that resulted in this transformation. So he concluded that a heritable substance was responsible for this transformation.

### Identification of DNA as the Genetic Material

- Griffith found live S strain bacteria in the dead mice, suggesting that the **harmless R strain had transformed into the S strain** by acquiring some "transforming principle" from the dead S bacteria. Griffith concluded that a **heritable substance (later identified as DNA)** from the dead S strain had transformed the R strain into a virulent form.


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So that was the first time he was able to show that there is some molecule responsible for this hereditary effect that we see. The experiment is explained here, as I have just

mentioned. Now, we know that it is mostly DNA, RNA, or protein, one of these is responsible. So to do that, several experiments were conducted.

### Identification of DNA as the Genetic Material

**Avery, Macleod and McCarty's experiment:**

- **Avery, MacLeod, and McCarty's experiment** (1944) built upon Griffith's findings and provided strong evidence that DNA is the genetic material. It was the culmination of research in the 1930s and early 20th century at the **Rockefeller Institute for Medical Research** to **purify and characterize the "transforming principle"** responsible for the transformation phenomenon first described in Griffith's experiment.
- The researchers isolated different macromolecules (DNA, RNA, and proteins) from heat-killed S strain bacteria.



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One of the most important experiments was done by Avery, McLeod, and McCarty. So it was done in 1944. So they built upon Griffith's findings and provided strong evidence that DNA is the genetic material. So this research was done at the Rockefeller Institute for Medical Research, which is in New York City. So they tried to purify the transforming principle responsible for this transformation of the rough strain to the smooth strain.

So, they isolated different macromolecules, DNA, RNA and proteins from the heat-killed S-strain bacteria. Then they performed selective enzyme treatment. So what were they doing? If you add proteinase, it will destroy the proteins. So only the RNA and DNA will remain and they saw that transformation happened.

### Identification of DNA as the Genetic Material

- They performed selective enzyme treatment:
  - Proteinase (Destroys proteins) + R strain + S DNA → Transformation occurred.
  - RNase (Destroys RNA) + R strain + S DNA → Transformation occurred.
  - **DNase (Destroys DNA) + R strain + S DNA → No transformation.**
- DNA was destroyed, the transformation did not occur, meaning the R strain remained non-virulent. This proved that DNA, not protein or RNA, was the "transforming principle" responsible for carrying genetic information



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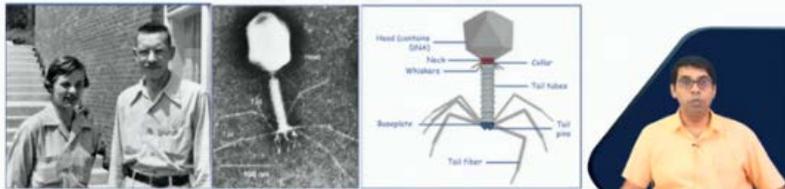
So it means that protein is not responsible. When they added RNAs, it destroys the RNA, which means that protein and DNA remained, transformation happened. So again, RNA is not responsible. When they added the DNAs, which destroy DNA, No transformation happened.

So there is no DNA, but there is protein and RNA, but no transformation happens, which means that if you destroy DNA, you cannot get this transformation. So it is the DNA which is responsible for this transformation. This was further proved by another classic experiment done by Hershey and Chase. So this is classically known as the Hershey Chase experiment. So this was done by Alfred Hershey here and Martha Chase in 1952.

**Identification of DNA as the Genetic Material**

**Hershey and Chase's experiment:**

- This experiment, conducted by **Alfred Hershey and Martha Chase (1952)**, provided definitive proof that DNA is the genetic material, not proteins.
- Hershey and Chase used the **T2 bacteriophage**, which consists of a **protein coat surrounding DNA**. They used **radioactive labeling** to track which molecule entered the bacteria during infection.
- **DNA** labeled with radioactive **phosphorus-32** (since DNA contains phosphorus, but proteins do not). **Protein** labeled with radioactive **sulfur-35** (since proteins contain sulfur, but DNA does not).



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So this also provided definitive proof that DNA is the genetic material and not proteins. Later, it was shown that RNA can also be genetic material in the case of bacteria. So what Hershey and Chase did was use T2 bacteriophage. This is a virus that infects bacteria. The way this virus infects bacteria is by sitting on top of the bacteria and injecting its DNA, which is present inside.

The virus itself does not enter. It only injects the DNA, which then enters the bacteria. From that DNA, proteins and other components will be synthesized, the whole virus will assemble, and these viruses will bud out from the bacteria by rupturing it. So bacteriophage viruses can infect bacteria by injecting their DNA.

This virus is made up of protein. The coat, or envelope, that surrounds it is made of protein. They used radioactive labeling to track which molecule entered the bacteria during

infection. Which molecule is actually responsible for infecting the bacteria? Is it the protein of the virus or the DNA of the virus?

So at that time, it was not known which was going on. So the DNA was labeled with phosphorus-32 and sulfur was labeled with sulfur-35. So both are radioactive. Now, it is important to note that DNA contains phosphorus.

Normally, if proteins are not phosphorylated because of post-translational modification, then you will not have phosphorus in the protein. So phosphorus will be only in DNA and sulfur will only be in protein because we have methionine and cysteine. So there is sulfur in the protein, but there is no sulfur in DNA. So we can distinguish between DNA and protein based on these two radio-labeled atoms.

So the experiment was that they labeled the phages and they infected the *E. coli*, which is the bacteria and the mixture was blended. So again, it is not very harsh blending. The only way it was mildly blending so that the virus that is sitting on top of the bacteria will fall off.

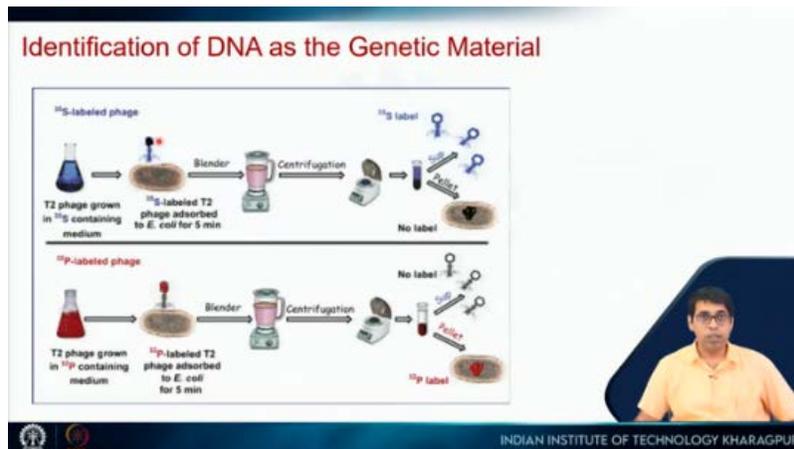
So you will separate the virus from the bacteria and then, if you spin down, the bacteria, which are heavy, will form a pellet at the bottom, and the virus will be in the supernatant at the top. The result they found was that in the bacteria, DNA was found inside the bacterial cell. But the supernatant had the protein. So there was P32 radioactivity inside the bacteria.

**Identification of DNA as the Genetic Material**

- The labeled phages were allowed to **infect *E. coli*** bacteria and the mixture was blended to separate the **viral protein coats** from the **bacterial cells**. It was then centrifuged, where **heavier bacterial cells formed a pellet** at the bottom, and **lighter viral coats remained in the supernatant**.
- Results:
  - In bacteria: **32-P (DNA) was found inside the bacterial cells, showing that DNA had entered.**
  - In supernatant: 35 S (Protein) was found outside the bacterial cells, in the viral protein coats, showing that protein did not enter.

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There was no S35 radioactivity. The S35 radioactivity was only in the supernatant, which means that the protein did not enter. It is the DNA that entered the bacteria and this DNA is responsible for producing new viral particles inside the bacteria. So this is the schematic of the experiment.



So we are growing these bacteria with two different labeled viruses. In one case, sulfur is labeled; in the other case, phosphorus is labeled and then you infect the bacteria with the virus. So the virus attaches on top of the bacteria like this and then it will inject the DNA.

So this is something that we know now, but it was not very clear at that time. So to see what goes inside the bacteria, is it the protein or the DNA they blended this with? So what they did was, it will just detach the virus from the bacterial surface. Then you spin it down in a centrifuge.

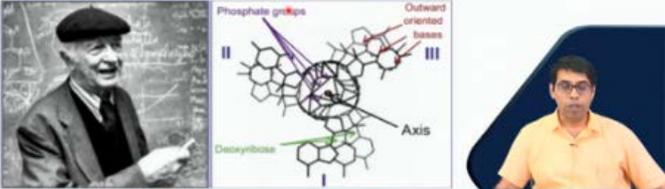
So the bacteria are heavy. They will form a small pellet at the bottom. But the virus, since it is so small, will be in the supernatant. So it will still float in the water. So if you separate the supernatant and the pellet and check for radioactivity, for S35 labeling, they found all the radioactivity in the supernatant and nothing in the pellet, which means that the protein did not enter the bacteria. But when they used P32 labeling, they found that all the P32 was in the pellet. There was nothing in the supernatant, which means that the DNA is the one that entered the bacteria, not the protein. So this again conclusively proved that DNA is the genetic material.

It is the transformative principle. So during that time, while all of these experiments were being done, people were also thinking about; so it was more or less clear that DNA is the genetic material, but it was not clear how it looks. So how it stores information and how it passes on information, how the information is copied. So these were all very important questions and to answer that, it was very important to determine the structure of the DNA.

**Discovery of DNA structure**

**Contribution of Pauling:**

- Linus Pauling (1901–1994) – Incorrect Triple-Helix Model
- A brilliant chemist, Pauling proposed a triple-helix DNA structure in early 1953. His model was incorrect because:
  - It placed phosphates inside instead of on the outside of the helix.
  - It did not account for Chargaff's base pairing rules (A-T, G-C).
- His work still influenced the race to correctly determine DNA's structure.



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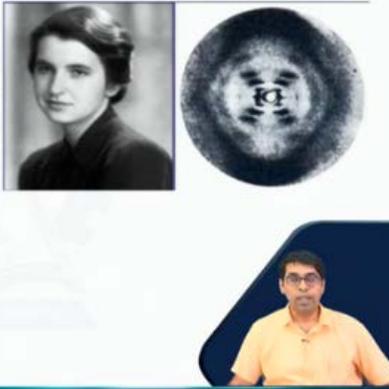
So there was a big competition that was going on to determine the DNA structure between various labs. Two prominent labs where one was led by Linus Pauling. So he was in Stanford at that time in the USA. He published the first structure in 1953, which was this triple helix model. So, where the phosphate groups are inside and the bases are outside like this and it turned out to be a wrong model, but then this model was something that also led to the publication of their model by Watson and Crick in the same year 1953 later on and we will see that that was the correct structure.

So, even though this was not the correct model, he was able to influence this sort of race for discovery or explanation of the DNA structure. So, Rosalind Franklin had worked a lot on DNA fibers, not crystal fibers, and she had collected a lot of very beautiful X-ray pictures like this. So these are the diffraction spectrum of X-ray. This one is famously called the photo 51 and this type of pattern was something that eventually led Watson and Crick, specifically Crick, to believe that DNA forms some sort of helical structure.

### Discovery of DNA structure

**Contribution of Franklin:**

- Rosalind Franklin (1920–1958) was an expert in **X-ray diffraction techniques**.
- She produced **Photo 51**, a crucial X-ray image of DNA fibers, which revealed the helical structure of DNA.
- Her precise measurements suggested that **DNA had a uniform width and grooves in the helical structure**.
- Her work, formed the basis of the correct helical model of DNA proposed by Watson and Crick.



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So that formed the basis of their model, and they were able to show that the two strands of DNA are intertwined together and form this double helix structure. Also, certain pitches that you can see here also told them that DNA has a uniform width and grooves in the helical structure so all of these were important clues in determining the final structure of DNA.

So, using this data from Franklin and Chargaff's base pairing rule, where AT and GC were present in a certain ratio, Watson and Crick were able to describe the accurate double helix model of DNA in 1953.

### Discovery of DNA structure

**Contribution of Watson and Crick:**

- Using **Franklin's Photo 51** and **Chargaff's base-pairing rules**, **James Watson** and **Francis Crick** built the first accurate double-helix model of DNA in 1953.
- Their key findings:
  - DNA has two **antiparallel strands** forming a **right-handed double helix**, and has two grooves – major and minor.
  - Complementary base pairing:** A always pairs with T, and G always pairs with C.
  - The **sugar-phosphate backbone** is on the **outside**, with **bases inside** like rungs on a ladder.
- They published their findings in *Nature* (1953), revolutionizing molecular biology.



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This was published in their *Nature* paper in 1953. Their key findings, as we have already seen, are that there are two antiparallel strands. It has a right-handed double helix. It has two grooves: a major groove and a minor groove. A always pairs with T, and G always pairs with C. The sugar-phosphate backbone is on the outside, and the bases are on the inside.

Once this structure was published, this is the famous double helix structure, and it was very clear to everyone that this had to be the right structure because it explained many things. It is important to note that this double helix structure was actually drawn by Francis Crick's wife, Odile. So, there is a very interesting paragraph. This was a very small paper. You can actually go and see it; there is this very interesting paragraph, which I have reproduced here. It says, 'It has not escaped our notice of the specific base pairing.'

So, this base pairing in the double helix structure that we have postulated immediately suggests a possible copying mechanism for the genetic material. Since you have two strands which are complementary to each other, that was the basis of this double helix structure. You can imagine that if you open up the two strands like this, you can use one strand as a template to copy the other strand. So, this copying mechanism became obvious because of this double helix structure. So, it actually provided a huge impetus in the field of molecular biology at that time. So, based on that, as we have already discussed how DNA replication happens, all of those things were discovered.

The slide features a scan of the original 1953 *Nature* paper on the left, with a red arrow pointing to the DNA double helix diagram. To the right is a photograph of Crick and Watson with the caption: "Crick and Watson after the publication of their letter to Nature". Below the photo is the text: "This famous diagram was drawn by Crick's wife Odile". On the far right, a quote from the paper reads: "It has not escaped our notice that the specific base pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." A small inset image shows a man in a yellow shirt, likely the presenter. The slide footer includes the IIT Kharagpur logo and name.

In 1953, the DNA double helix structure was published and within three years, Francis Crick also published this hypothesis, which he pronounced as the central dogma of molecular biology so 'once information has got into a protein, it can't get out again'. Information here means the sequence of the amino acid residues, or other sequences related to it. This is, we may be able to have.

### Ideas on Protein Synthesis (Oct. 1956)

The Central Dogma: "Once information has got into a protein it can't get out again". by Francis Crick

Information here means the sequence of the amino acid residues, or other sequences related to it.

That is, we may be able to have

but never

DNA ← RNA ← Protein

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So, what he proposed was that DNA has information which can be copied to make more DNA. That information can be used to make RNA. Information from RNA can be used to make DNA. Information from RNA can be used to make more RNA. Information from DNA can be used directly to make protein, or information from RNA can be used to make protein.

But no protein can be copied into protein; protein can be copied into RNA, and protein can be copied into DNA and so far, this has stood the test of time. This was proposed in 1956, almost 75 years ago, and still, it has stood the test of time. So, all of these steps have been discovered now. So, this is the modern version of the central dogma of molecular biology, and it looks exactly as Crick had predicted 75 years ago.

### Flow of Genetic Information: The Central Dogma of Molecular Biology

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So one important step in DNA replication is the semi-conservative replication of DNA. So, Watson and Crick's structure showed that you can separate the two strands and copy them, but what was not known was if there are two strands and they are copied into two new

strands, do the two original strands remain in the parent cell, and do the two newly synthesized strands go to the daughter cell? Or do both cells inherit a hybrid strand where one copy is original and the other is newly constructed? So, that is semi-conservative DNA replication and that was proved by Meselson and Stahl in their famous experiment.

Again, this has been described, so I will briefly go through it. So, in 1958, they demonstrated this using bacteria, bacterial replication, and nitrogen isotopes. So, there are two isotopes of nitrogen that they used, N14 and N15. So N15 is heavier than N14.

Bacterial culture in a medium containing this heavy isotope of nitrogen-15 was grown for several generations. So after several generations, bacteria were transferred to a medium containing the lighter nitrogen, N14. So if you grow it for several generations, all your DNA bases will have N15. Now if I transfer it to N14 medium and let it grow once, so there is only one replication, I will know whether I am getting N14 incorporated in some cells or I am getting a hybrid of N14 and N15 in all the cells.

So how do you do that? They tested that by using ultracentrifugation because the density of both strands as N15 will be different from both strands as N14, which will be different from one strand as N15 and one strand as N14. So they can actually separate out these three types of strands. That is exactly what they did using a cesium chloride gradient density.

So, in the first generation, both were double-stranded, and initially both were double-stranded. In the first generation, they found this intermediate band, which was a mixture of 15N and 14N and then from the second generation onwards, they found a new band where they found both strands were N14, but they never found both strands with N15. That is shown here. So, this has been explained in detail in the previous lecture.

## Discovery of Semi-Conservative Replication of DNA

### Meselson and Stahl's experiment:

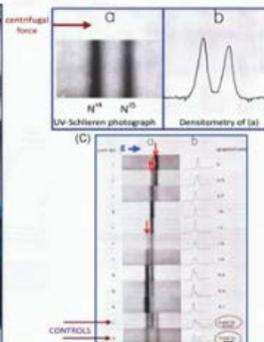
- The Meselson and Stahl experiment (1958) provided strong evidence for the **semi-conservative model of DNA replication**. This experiment was conducted by Matthew Meselson and Franklin Stahl using *E. coli* bacteria and **nitrogen isotopes**.
- Bacteria were cultured in a medium containing the heavy isotope of nitrogen ( **$^{15}\text{N}$** ) for several generations. This made the DNA of the bacteria "heavy" because the nitrogen in the nitrogenous bases of DNA was replaced with  $^{15}\text{N}$ .
- After several generations, the bacteria were transferred to a medium containing the lighter isotope of nitrogen ( **$^{14}\text{N}$** ). As the bacteria replicated in the new medium, they incorporated  $^{14}\text{N}$  into newly synthesized DNA strands.
- **DNA samples** were extracted at different generations and **centrifuged in a CsCl density gradient**. This allowed DNA of different densities to separate.



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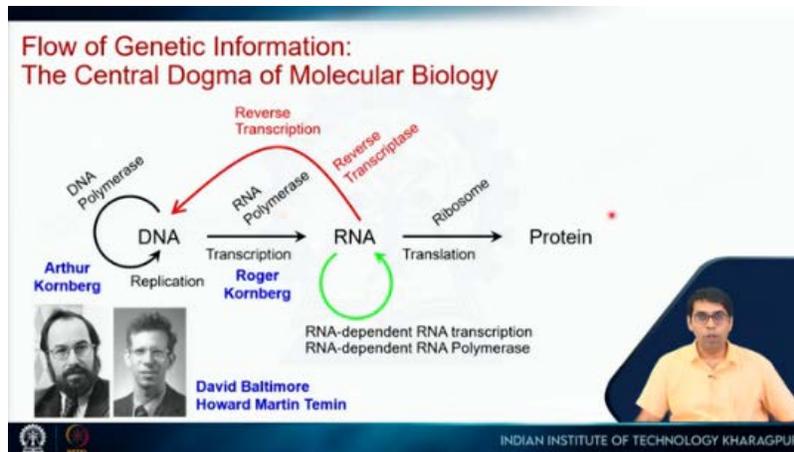
So, I am not going to go through this too much, but what they found was that the original double strands where both were  $\text{N}^{15}$  labeled never again showed up. In the first generation, they found this hybrid, and then from the second generation onwards, they found both were  $\text{N}^{14}$ , and the hybrid  $\text{N}^{15}\text{-N}^{14}$  started decreasing. So, this showed that DNA replication happens in a semi-conservative manner.

## Discovery of Semi-Conservative Replication of DNA



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So, here is a summary of all the major discoveries. Watson and Crick showed the double helix structure in 1953. Semi-conservative replication was shown in 1958 by Meselson and Stahl, and then Arthur Kornberg worked a lot on the DNA polymerase, and he explained how DNA replication happens or is catalyzed by DNA polymerase. DNA to RNA transcription was shown by his son, Roger Kornberg. RNA to DNA, the reverse transcription, was shown by David Baltimore and Howard Temin and then, RNA-dependent RNA polymerase discovery was also made.



Now, I will discuss this last step, which is the translation. So, before that, some more important discoveries were made. One of them is the One Gene, One Enzyme experiment. So, one gene codes for only one enzyme, and that was shown by Beadle and Tatum.

So this is famously called the one gene, one enzyme experiment. They were present at Stanford, and what they did was irradiate X-rays on the haploid strains of the fungus *N. crassa*. So, if you take the wild-type fungus and this irradiated fungus and then cross them, what they found was that they were able to produce mutants. So, they were selecting these mutants. They could find mutants that had lost their ability to synthesize the amino acid arginine, which means that if you want to grow this mutant, you have to supply arginine in the growth medium. So you have to supply arginine in the growth medium, and only then will it grow; otherwise, it will not. So that way, you can select for this type of mutant. Now, crosses between these types of fungi produced four distinct mutant strains that were defective in different steps of the arginine biosynthetic pathway. So if you think of the arginine biosynthetic pathway, let us say there are four enzymes that are important, and they found that there were four different mutants defective in arginine production, which means these mutations were in one of these four enzymes.

That is why you have four different types of mutants. So, each mutant lacked a specific gene that encoded one of the four enzymes involved in the production of arginine. That meant that one gene produces only one enzyme because if one gene caused defects in multiple enzymes, you would see many more defects in those mutants.

## Discovery of Codons

### Beadle and Tatum's "One Gene, One Enzyme" experiment:

- These two Stanford scientists **irradiated with X-rays** haploid strain of the fungus *N. crassa*.
- **Crossing wild type** with irradiated fungi produced mutants that **lost their ability to synthesize the amino acid arginine** and that had, therefore, to be grown in arginine-containing medium.
- Crosses between mutant fungi identified **four distinct mutant strains** that were **defective in different steps of the arginine biosynthetic pathway**. From these results, Beadle and Tatum reasoned that **each mutant lacked a specific gene that encoded one of the four enzymes that participated in the production of arginine**.



George W. Beadle  
(1903–1989)



Edward L. Tatum  
(1909–1975)



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Now, based on all this information produced at that time, Crick and Griffith came up with the concept of a commaless, triplet-based, nondegenerate code that there must be some code by which the information stored in DNA and RNA is converted into protein. In 1957, Francis Crick, John Griffith, and Orgel published a paper proposing their scheme of a non-degenerate code, under which each amino acid was encoded by only a single codon.

## Discovery of Codons

### Crick and Griffith's concept of commaless triplet-based nondegenerate code:

- In 1957, Francis Crick, John Griffith and Orgel published a paper where they proposed their scheme of nondegenerate code under which each amino acid was encoded by only a single codon.\*
- Crick, Griffith, and Orgel proposed two basic tenets for the code:
  - Code words(codons) are comprised of **three nucleotides**.
  - The code is probably **nonoverlapping**.



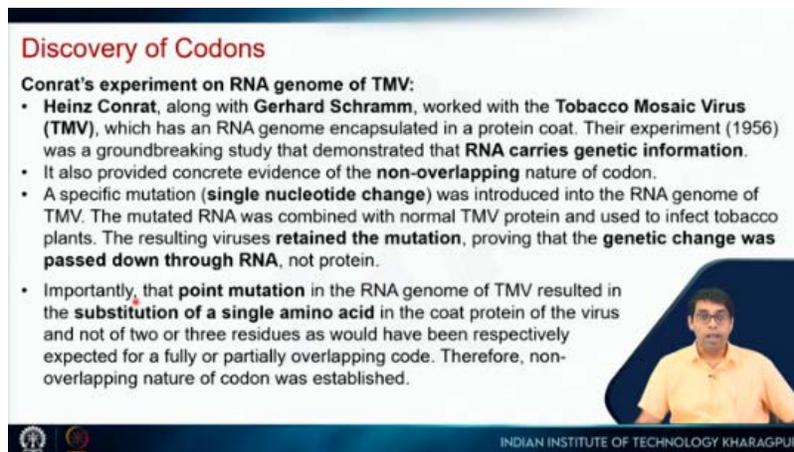
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So this was eventually proved to be wrong because now we know that multiple codons can code for a single amino acid. So there is degeneracy. But two important things that came out from their paper were that there are these codons which comprise three nucleotides, which proved to be right. The code is probably non-overlapping, which means that three nucleotides will code for one amino acid. Then the next three nucleotides will code for the next amino acid, and so on.

So, these codons will not overlap like this, one on top of the other. That is not permissible and eventually, this was proved by Conrat's experiment on the RNA genome of the tobacco mosaic virus.

So, Heinz Conrat and Gerhard Schramm worked on the tobacco mosaic virus, which has an RNA genome. So, their experiment in 1956 was groundbreaking in two ways because it showed that RNA carries genetic information. So, that was a big discovery, and the second outcome of their experiment was that these codons are non-overlapping, which was proposed by Crick and others in their paper. So, in this case, they produced single-nucleotide mutants of the RNA genome of the tobacco mosaic virus.

The mutated RNA was combined with normal tobacco mosaic protein and used to infect tobacco plants. So, in this case, the resulting virus retained the mutation, proving that the genetic change was passed down through RNA, not protein, and not DNA. So they were able to mutate, and then that mutation was passed on through several generations. So it proved that RNA is the genetic material. Also the point mutation in the RNA genome of TMV resulted in substitution of a single amino acid.



**Discovery of Codons**

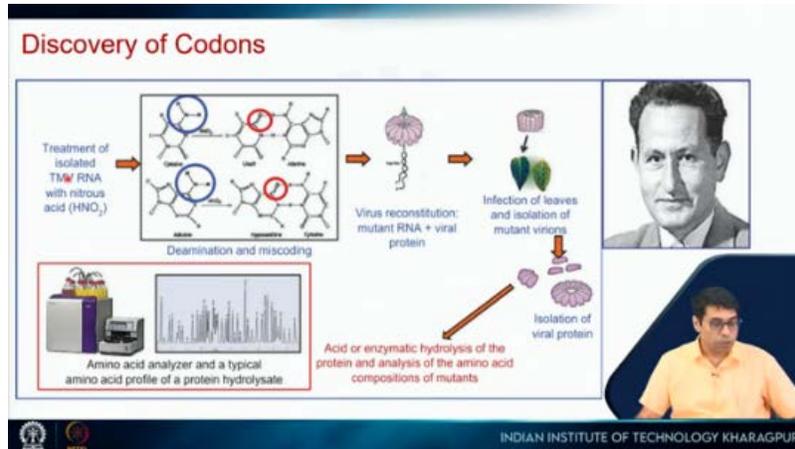
**Conrat's experiment on RNA genome of TMV:**

- **Heinz Conrat**, along with **Gerhard Schramm**, worked with the **Tobacco Mosaic Virus (TMV)**, which has an RNA genome encapsulated in a protein coat. Their experiment (1956) was a groundbreaking study that demonstrated that **RNA carries genetic information**.
- It also provided concrete evidence of the **non-overlapping** nature of codon.
- A specific mutation (**single nucleotide change**) was introduced into the RNA genome of TMV. The mutated RNA was combined with normal TMV protein and used to infect tobacco plants. The resulting viruses **retained the mutation**, proving that the **genetic change was passed down through RNA**, not protein.
- Importantly, that **point mutation** in the RNA genome of TMV resulted in the **substitution of a single amino acid** in the coat protein of the virus and not of two or three residues as would have been respectively expected for a fully or partially overlapping code. Therefore, non-overlapping nature of codon was established.

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So if there is a mutation, then it is present only in one codon and it affects only one amino acid. So it means that there is no overlapping of codons, which means that a single mutation is not affecting multiple amino acids. So since that is not happening, it means that these codons are non-overlapping. So this non-overlapping nature of codon was also established by this set of experiments that was done by Conrat and Schramm. So, the experiment is briefly shown here.

You have this TMV RNA. It was treated with nitrous acid,  $\text{HNO}_2$ , and the viral reconstitution was done. So, you have the mutant RNA, viral protein. So, you have the reconstituted virus which infects the tobacco leaves and then they will replicate.



So, now you isolate this viral protein and then you do this nucleic acid sequencing. What they found was that in the newly produced viruses, the mutation is present, which means that since we are mutating RNA and it gets carried over to the protein, RNA is carrying the genetic information and only one amino acid was mutated. So if you produce a single nucleotide mutation, only one amino acid is mutated, which means that there is no overlap of the codons. So RNA dependent RNA polymerase, RNA as a genetic material was established by them and also non-overlapping codons for RNA. Again around that time Crick produced another hypothesis. So this is something that is very important to note that to do good science you should have a hypothesis otherwise you cannot work on something.

So what I am trying to say is that one has to have some hypothesis that explains a certain observation and then you can design really clever experiments to test that hypothesis. Crick is called a theoretical biologist because he produced this really useful hypothesis that drove all the important experiments in molecular biology at that time. So again, another important hypothesis he came up with was that there must be an adapter molecule that links the genetic code in RNA to amino acids. So these three codons should get converted to one amino acid.

### Discovery of tRNA as Adaptor Molecules

**Crick's Adaptor Hypothesis**

- In 1955, Crick proposed that there must be an **"adaptor"** molecule that links the genetic code in **RNA to amino acids** during protein synthesis. He suggested that these adaptors would recognize specific codons in mRNA and bring the corresponding amino acid.
- **Robert Holley**, in 1965, **isolated the first tRNA molecule** (alanine tRNA) from yeast. He determined its structure, proving it was the **adaptor** that Crick predicted. The structure included the **anticodon (which pairs with mRNA) and an amino acid attachment site**.



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So there has to be some adapter molecule which will convert this or do this translation, and he said that there has to be some adapter molecule, and 10 years later, Robert Holley isolated the first tRNA molecule; it was coding for alanine from yeast. He determined its structure and proved that this adapter hypothesis is correct, and in the tRNA or the transfer RNA, the anticodon was found, which is complementary to the codon that will be present on the mRNA. So then Zamecnik and Hoagland effectively used an in vitro protein synthesis system to identify and characterize the tRNA and amino acid activating enzyme. So there has to be a tRNA, but then if once that is used, the tRNA will go back and the amino acid has to be recharged on that empty tRNA.

So you need activating enzymes for that. These were discovered by Zamecnik and Hoagland. They prepared cell extracts from rat liver containing ribosomes and other protein synthesizing components. So they added radioactive labeled amino acids, for example, carbon-14 leucine. Then they observed that before the amino acids were incorporated into proteins, they first bound to a small heat stable RNA molecule, which was the tRNA and that is how they demonstrated that amino acids must first attach to tRNA before being used in protein synthesis.

So, these were all very important steps that are present in protein synthesis, which we now take for granted, but they were all discovered by these really carefully designed experiments by different biochemists. So, these also confirmed Crick's adapter hypothesis. Now there is this adapter, but you have to discover the codon table that shows which three triplets of nucleotides code for which amino acid. Now remember that Crick had originally

proposed that the genetic code would be non-degenerate, so only one codon would code for one amino acid, but later when people tried to look into that they found that this is not entirely correct.

### Discovery of tRNA as Adaptor Molecules

**Zamecnik & Hoagland's contribution**

- By effectively using the **in vitro protein synthesis system**, Paul Zamecnik and Mahlon Hoagland identified and characterized in the 1950s **tRNA and the amino acid activating enzymes**.
- They prepared **cell extracts from rat liver** containing **ribosomes** and other protein-synthesizing components. They added **radioactively labeled amino acids** (e.g., carbon-14-labeled leucine). They observed that before amino acids were incorporated into proteins, they first bound to a small, heat-stable RNA molecule (later identified as tRNA). This demonstrated that amino acids must first **attach to tRNA** before being used in protein synthesis.
- This confirmed **Francis Crick's "Adaptor Hypothesis"**.



Paul Zamecnik  
1912-2009



Mahlon Hoagland  
1921-2009



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Nirenberg used this cell free system that was I describe in the previous slide to see what type of RNA is producing which type of protein. So, he used poly-U RNA. So, all the nucleotides are uracil and he found that the protein that is made is made up entirely of phenylalanine. It means that UUU codes for phenylalanine.

Similarly, when he used poly C, polycytosine, so all the nucleotides are C, he found that it produces polyproline. So, this way, he was able to decode 54 of the amino acid codons by 1966. Parallely, another scientist, Har Gobind Khorana, was working at Harvard on the same problem, and he also used different types of synthesized RNA molecules to see what amino acids were being synthesized.

### Deciphering the Genetic Code

**Nirenberg's contribution**

- Marshall Nirenberg (1961-1965) was the first to crack the genetic code, providing experimental proof that **mRNA codons specify amino acids**.
- He used a **cell-free system** (*E. coli* extract) that could synthesize proteins in vitro. He added synthetic **poly-U RNA (UUUUUU... – only uracil bases)** and Found that this RNA directed the synthesis of a protein made entirely of **phenylalanine**.
- This proved that the codon **UUU codes for phenylalanine**.
- In analogous experiments with other synthetic RNAs, they found that **poly-C** directed synthesis of **polyproline**.
- Nirenberg's group eventually **decoded 54 of the amino acid codons** by 1966.



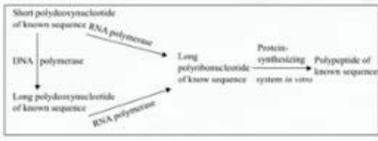
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So, he was able to confirm that AUG is the start codon and that there are three stop codons: UAA, UAG, and UGA.

### Deciphering the Genetic Code

**Khorana's contribution**

- Har Gobind **completed the genetic code**.
- He (1965-1966) synthesized defined RNA sequences (e.g., repeating units like UCUCUC or AUUAU) and showed that specific sequences directed the incorporation of specific amino acids in cell-free protein synthesis system.
- He helped **confirm the full 64-codon genetic code**, including start (AUG) and stop codons (UAA, UAG, UGA).





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So, together, Khorana and Nirenberg were able to produce and show that there are actually multiple codons which can code for certain amino acids, and they built the entire genetic codon table, which looks like this. This data was also curated by Francis Crick, so they would send their data, and Crick would tabulate all of them in a form like this to prepare the genetic code table. The 1968 Nobel Prize was awarded to Robert Holley, Har Gobind Khorana, and Marshall Nirenberg for their interpretation of the genetic code and its function in protein synthesis.

### Deciphering the Genetic Code

		Second letter				
		U	C	A	G	
First letter	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA } UCG }	UAU } Tyr UAC } UAA Stop UAG Stop	UGU } Cys UGC } UGA Stop UGG Trp	U C A G
	C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U C A G
	A	AUU } AUC } Ile AUA } AUG Met	ACU } ACC } Thr ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G
	G	GUU } GUC } Val GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }	U C A G

The Nobel Prize in Physiology or Medicine 1968 was awarded jointly to Robert W. Holley, Har Gobind Khorana and Marshall W. Nirenberg "for their interpretation of the genetic code and its function in protein synthesis"  
- Nobel Prize Committee

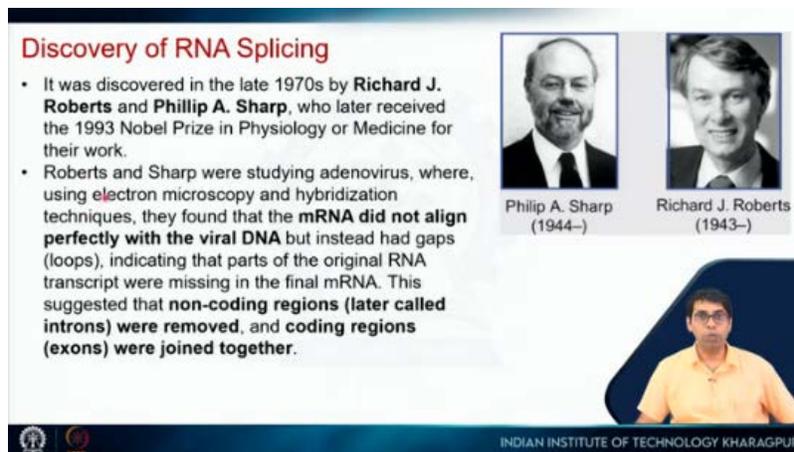


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Now we know that this part required the work of many scientists, and their names are listed here. So, what we show as just one slide is actually the contribution of some really smart people working in tandem and figuring out all these different details by conducting some very clever experiments. One more topic present in this is RNA splicing. It was discovered

in the 1970s by Richard Roberts and Philip Sharp, who later received the Nobel Prize in 1993 for their work.

What they showed was that they were studying adenovirus and, using electron microscopy, they were hybridizing the messenger RNA and the DNA. RNA is synthesized from DNA, so they hybridized this messenger RNA with the DNA and found that it was not a perfect match because there were a lot of regions in the DNA that did not match with the RNA, forming these large loops like this. So in red you have the messenger RNA and in blue you have the DNA. The DNA codes for the same information, which is transcribed into messenger RNA, but when you hybridize them, you see that the DNA comes like this, then there is a big loop because there is nothing that matches it, and then it matches, forming a double strand. Then there is another loop, and again it forms the complementary strand, then there is another loop, and so on.



**Discovery of RNA Splicing**

- It was discovered in the late 1970s by **Richard J. Roberts** and **Phillip A. Sharp**, who later received the 1993 Nobel Prize in Physiology or Medicine for their work.
- Roberts and Sharp were studying adenovirus, where, using **electron microscopy** and hybridization techniques, they found that the **mRNA did not align perfectly with the viral DNA** but instead had gaps (loops), indicating that parts of the original RNA transcript were missing in the final mRNA. This suggested that **non-coding regions (later called introns) were removed, and coding regions (exons) were joined together.**

Philip A. Sharp (1944–)      Richard J. Roberts (1943–)

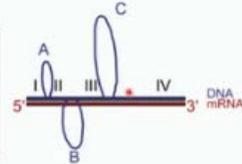
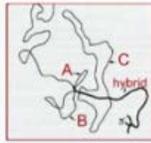
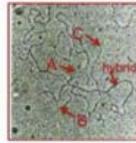
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So, these loops are what they call the regions that they refer to as the introns and the exons. They hypothesized that it is these introns which are spliced out, and the exons are joined together to form the mature messenger RNA. So this is your mRNA splicing. Later on, Tom Cech showed that this mRNA splicing can be catalyzed by the messenger RNA itself, the RNA itself. So it is very clear that RNA can also have catalytic activity and he shared the Nobel Prize with them in 1993.

## Discovery of RNA Splicing

- Further studies revealed that RNA is first transcribed as a **precursor messenger RNA (pre-mRNA)**, which contains both introns and exons. **Introns are then spliced out**, and exons are joined together to form the mature mRNA that gets translated into proteins.

(A)



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So in conclusion, landmark experiments in molecular biology have fundamentally shaped our understanding of life, which is the molecules of life. From the discovery of DNA as genetic material to the double helix, all of these things have paved the way for all the modern molecular biology and genetics that we do today. So, they form the fundamental basis. So, we have seen how Nirenberg and Khorana discovered the genetic codon.

So, all of these things that we have seen in this lecture. The discovery of RNA splicing by Roberts and Sharp. So together, these breakthroughs have provided a comprehensive view of molecular biology, from genetic information storage to its expression as functional proteins, which do all the work in our bodies. So these foundational discoveries continue to drive advancements in biotechnology, medicine, and genetic engineering that we do today.

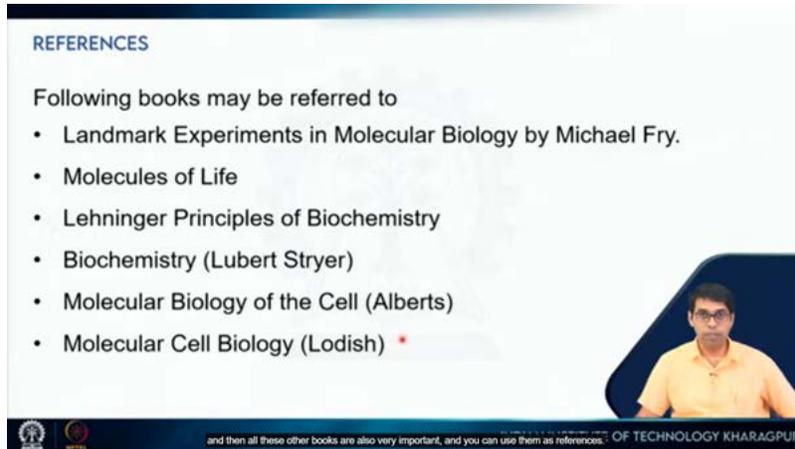
## CONCLUSIONS

- Landmark experiments in molecular biology have fundamentally shaped our understanding of life at the molecular level. From the discovery of **DNA as the genetic material** in the Hershey-Chase experiment to the revelation of its **double-helix structure** by Watson and Crick, each breakthrough has paved the way for modern genetics and biotechnology.
- The **discovery of the genetic code** by Nirenberg and Khorana demonstrated how nucleotide sequences encode amino acids, leading to the identification of codons, the three-letter sequences specifying each amino acid. The **role of transfer RNA (tRNA)** in translating this code was uncovered through the work of Hoagland and Zamecnik, revealing tRNA's essential function in linking amino acids to the corresponding codons during protein synthesis.
- The **discovery of RNA splicing** by Roberts and Sharp further revolutionized our understanding by showing that genes are not always continuous and that introns must be removed for proper gene expression.
- Together, these breakthroughs have provided a comprehensive view of molecular biology, from genetic information storage to its expression as functional proteins. These foundational discoveries continue to drive advancements in biotechnology, medicine, and genetic engineering.



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So if you are interested in reading about these landmark experiments, you can follow this book called Landmark Experiments in Molecular Biology by Michael Fry and then all these other books are also very important, and you can use them as references. Thank you.



**REFERENCES**

Following books may be referred to

- Landmark Experiments in Molecular Biology by Michael Fry.
- Molecules of Life
- Lehninger Principles of Biochemistry
- Biochemistry (Lubert Stryer)
- Molecular Biology of the Cell (Alberts)
- Molecular Cell Biology (Lodish) \*

and then all these other books are also very important, and you can use them as references. OF TECHNOLOGY KHARAGPUR