Structural Biology
Prof. Saugata Hazra
Department of Biotechnology
Indian Institute of Technology – Roorkee

Lecture 01
Introduction: Why to Study Structural Biology

Hi everyone. Welcome to the course of structural biology. I am Doctor Saugata Hazra from the Department of Biotechnology, IIT, Roorkee. And I am your guide to take to this course. My idea to develop the Structural Biology course is very introductory to explain what is happening as a whole around structural biology, not only about structural Biology techniques. I am trying to give you a comprehensive idea of this journey, starting from how Biology is developed. It is a historical tour of how biotechnology is developing how the biotechnology research starts recorded, and further going through the biological macromolecules, and how they have to be readout, how that reading would be utilized towards developing structures; the structural Biology techniques and then how to read the 3D coordinates.

How to use them for visualization? How to look at this process the static structure which we are solving towards dynamics and then some applicative measures where you could see how we use Structural Biotechnology or Structural Biology techniques towards the development of science, towards applications.

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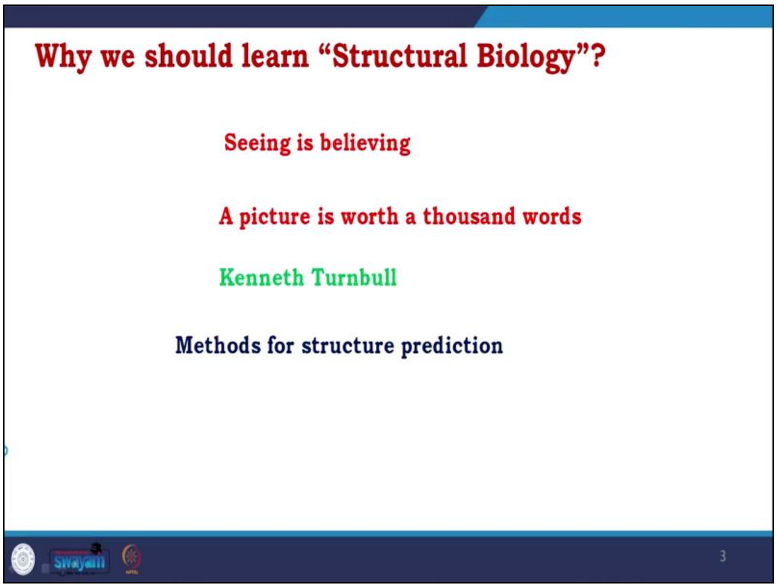

The one-line answer is seeing is believing, so, through Structural Biology, we could see the molecules, and as Kenneth Turnbull told, a picture is worth a thousand words. So we want to make a visualization. We want to take pictures to visualize what is happening in Biology.

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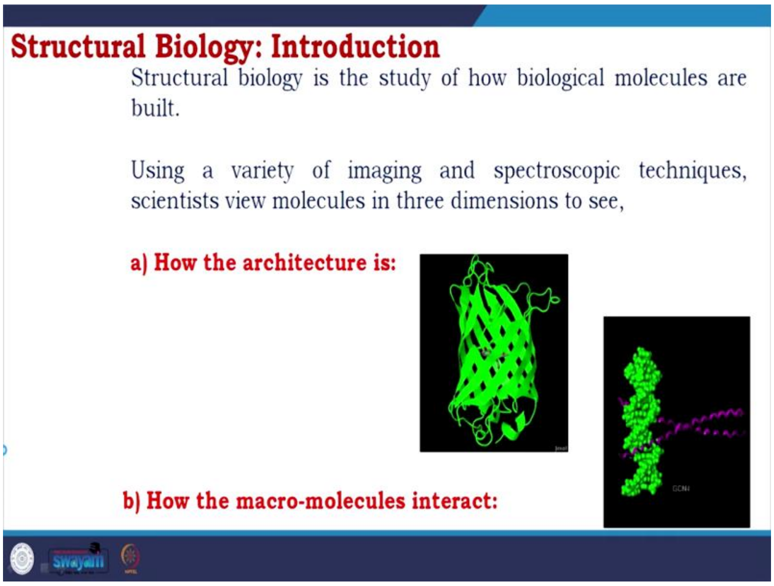

Structural Biology is the study of how biological macromolecules are built. Scientists use various imaging and spectroscopic techniques to view molecules in three dimensions to see different functions. One how the architecture is? How do the macromolecules interact? The interaction of macromolecules means communicating one of the most critical things in Biology. As you see here, there are different DNA protein interactions. There are different transcription factors, which have different motifs. We will talk about motifs.

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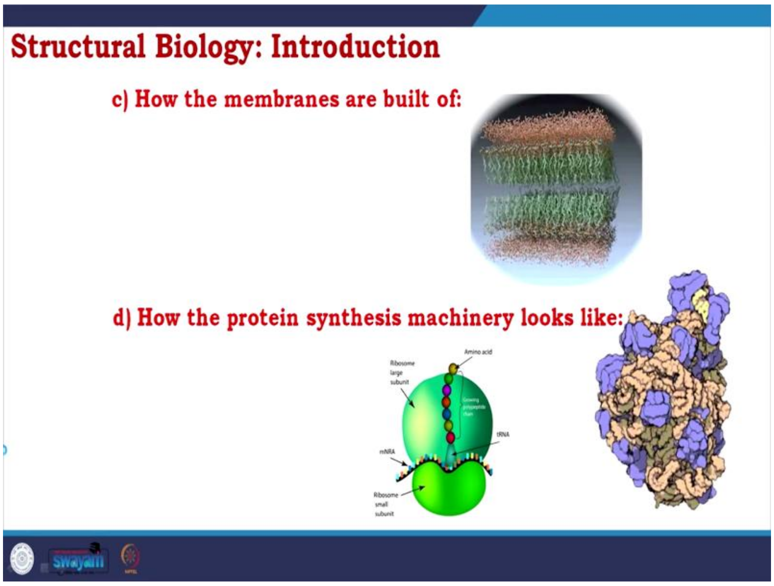

How are the membranes built? We know that the organelles in the cells are divided by membrane, and membranes have a critical role in making in and out for many molecules. So how the membranes are constructed, and how it makes differentiation, all these things would be part of structural Biology. What did the protein synthesis machinery look like?

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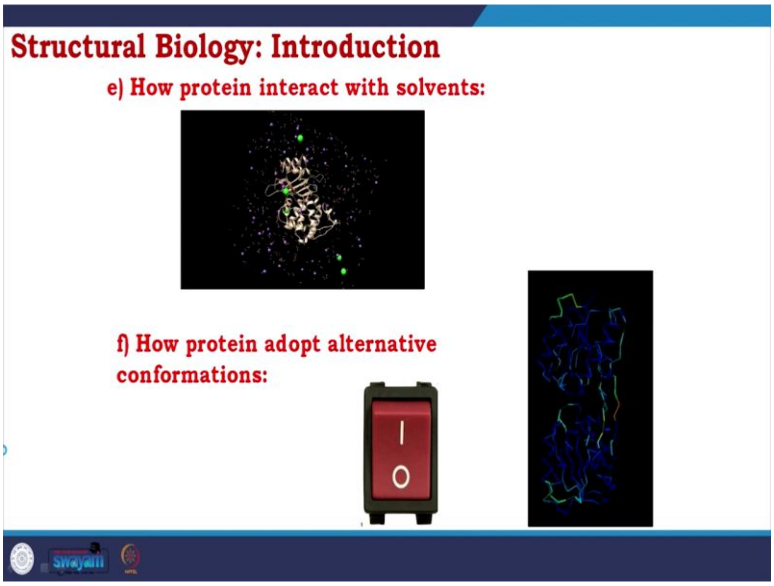

How do proteins interact with solvent? In the physiological system, proteins interact with water, and proteins interact with ions and all. So, their interaction takes an important role that also we are going to look at. How protein adopt alternative conformations, as we say in if you think about the cell or biological system, you will see that it works like a factory. The only difference is that no operator is standing there to switch on and off.

This type of conformational changes what you see in the protein here helps switch on and off and make the biological process go smoothly. As we all say in biology, enzyme work is good, especially for enzymes. Enzyme not work is not bad. But enzyme overwork is always bad. It could create a lot of diseases if the enzyme could not switch on and off in the proper time.

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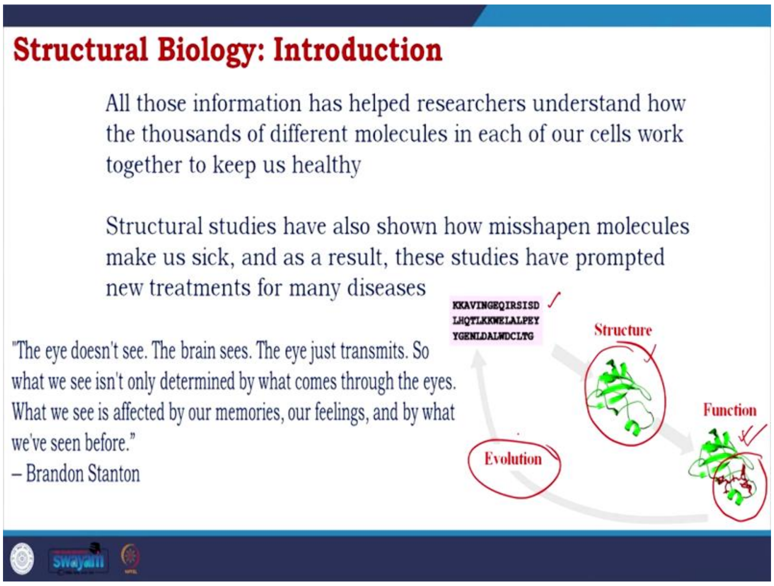

All those information has helped researchers understand how the thousands of different molecules in each of our cells work together to keep us healthy. Structural studies have also shown how miss happened molecule makes us sick, and as a result, the studies were prompted new treatments for many diseases. And by getting structure, we understand how it binds to the ligand and substrate. So the first and foremost application of Structural Biology is to utilize the sequences and help us get the function. But that is not the end. Structure helps us in many things like evolution. If you look at the changes of domain structure organization confirmation, help us track the evolution happening in this world.

So we started talking that seeing is believing, but there is something more than that. As Brandon Stanton told the eye does not see. The brain sees. The eye transmits. So what we see is not determined by what comes through the eyes. What we see is affected by our memories, feelings, and what we have seen before. So yes, structural Biology could be your eye, but it could be of the brain by taking it carefully.

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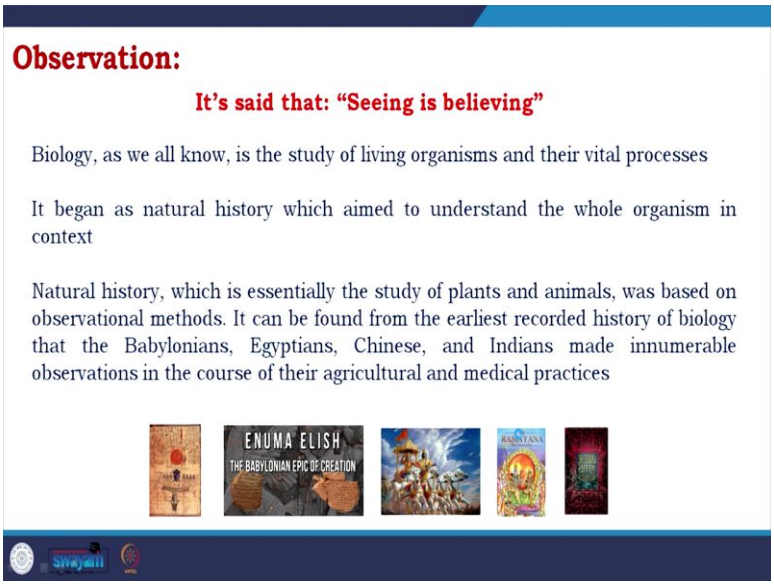

I will go to start with the observation; the first thing I talked about seeing is believing. As we all know, biology is the study of living organisms and their vital processes. And it began as natural history, which aims to understand the whole organism in context.

Natural history, which is essentially the study of plants and animals, was based on the observational method. It can be found from the earliest recorded history of Biology that the Babylonians, the Egyptians, Chinese and Indian made innumerable observations in the course of their agricultural and medical practices.

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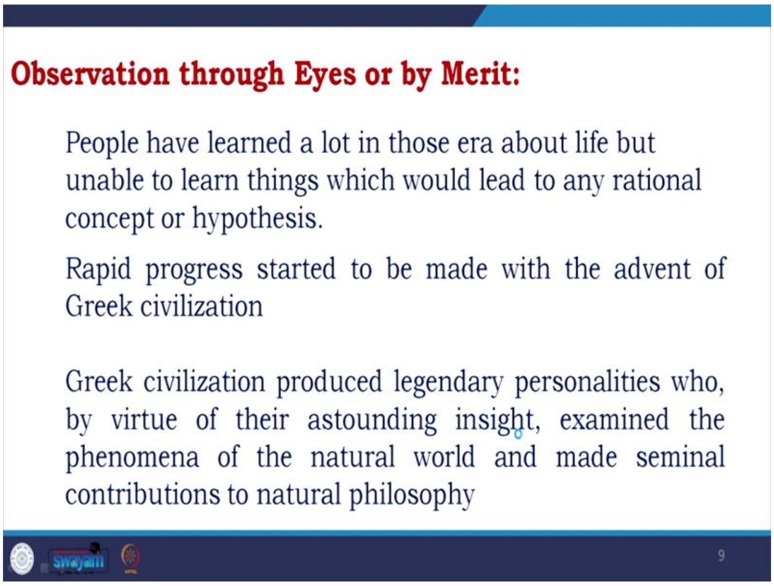

Observation through eyes or by merit: People have learned a lot in this era about life. But there are no learning things that would lead to any rational concept of hypothesis. Rapid progress started to be made with the advent of Greek civilization. Greek civilization produced legendary personalities who, by virtue of their astounding insight, examine the natural world's phenomenon and made a seminal contribution to natural philosophy.

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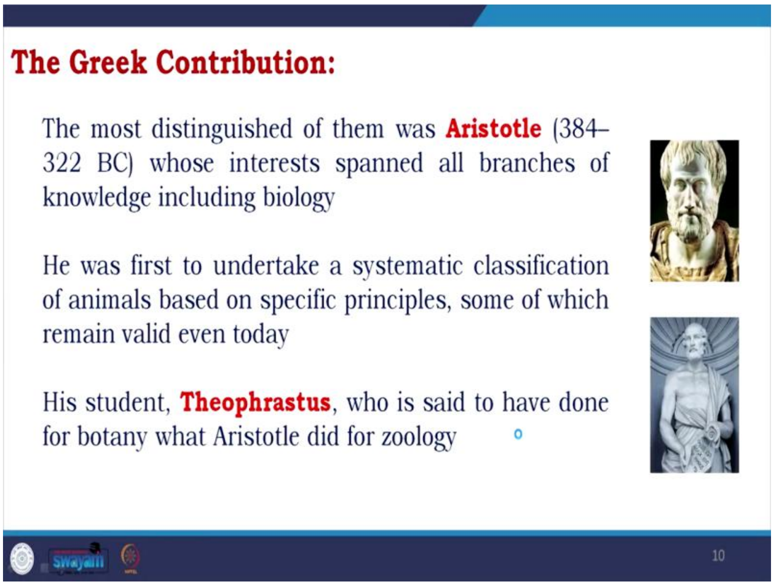

The most distinguished of them was Aristotle, whose interest spans all branches of knowledge, including Biology. He was first to undertake a systematic classification of animals based on specific principles that are even valued today. Theophrastus, his student, did that for plants.

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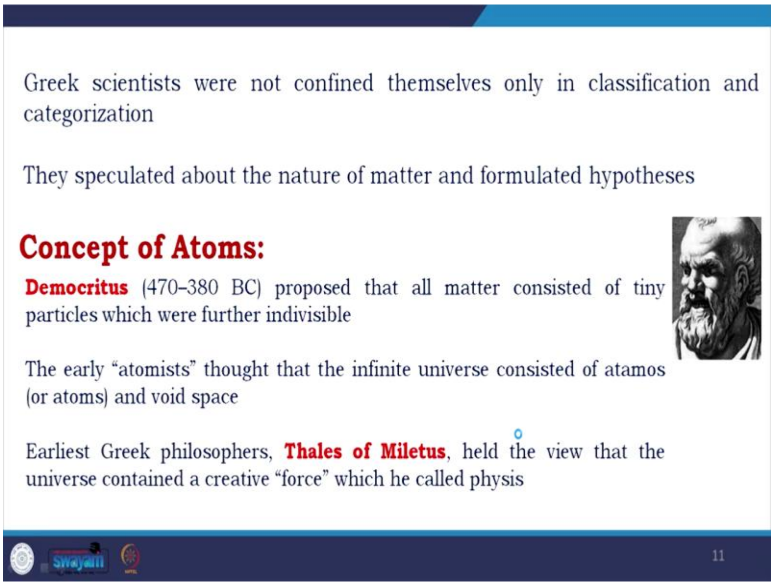

Greek scientists could not even find themselves only in the classification and categorization of animals and plants. They speculated about the nature of matter and formulated hypotheses. Democritus, another legendary scientist, proposed that all matter consisted of tiny particles which were further indivisible. So if you look at it with the modern eye, you can understand that you have already reached the concept of the atom. Democratic early atomists thought that the infinite universe consisted of the atom and void spaces. Early Greek philosopher Thales of Miletus believed that the universe contained a creative force, which he called Physis.

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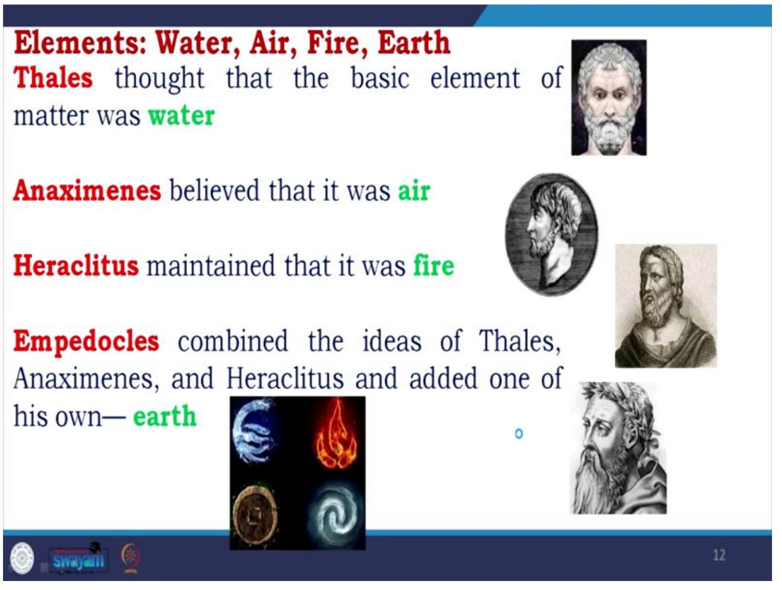

Thales thought that the basic element of material was water. Anaximenes believed that it was not water. It was air. Heraclitus maintained that it was the fire. Empedocles combines water, air and fire and improves his own Earth.

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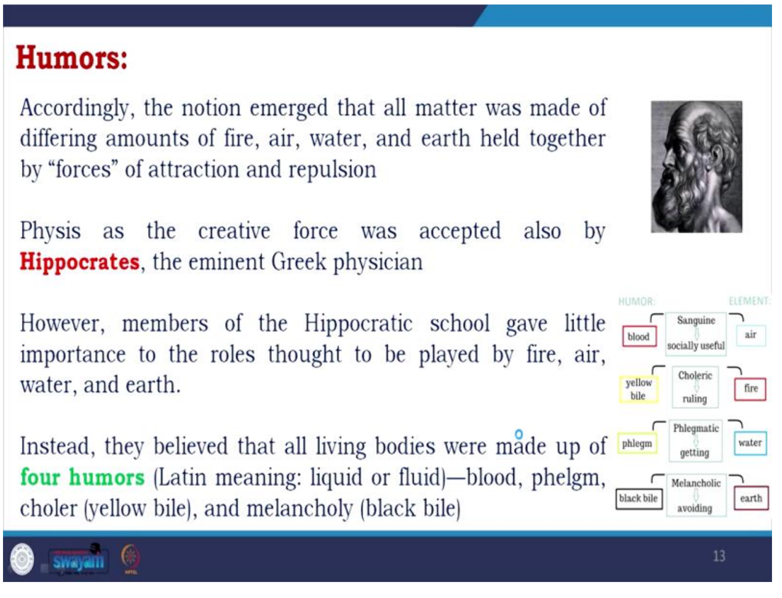

Accordingly, the notion emerged that all matter is made up of differing amounts of fire, air, water and earth held together by forces of attraction and repulsion. Physis as the creative force was also accepted by Hippocrates the eminent great physician, but the member of

Hippocratic school they do not give importance to roles of fire, air, water and Earth. But they believed that the living bodies are made up of four humors (liquid or fluid) blood, Phelgm, Choler (yellow bile), and melancholy (black bile).

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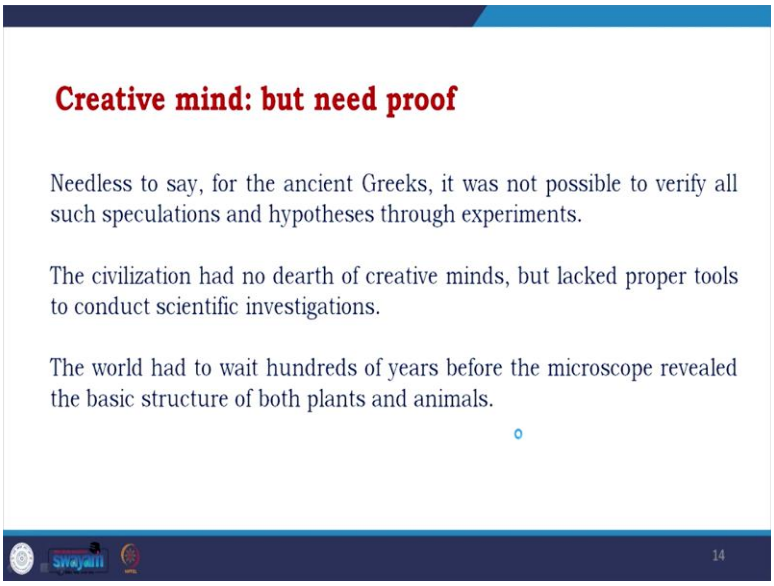

So they did a lot of initial observations made a hypothesis, and some of them, as I told still valid. But they couldn't verify all such speculation and hypothesis through experiments. The civilization had no dearth of creative minds, but lacked proper tools to conduct scientific investigations. The world had to wait hundreds of years before the microscope revealed the basic structure of both plants and animals.

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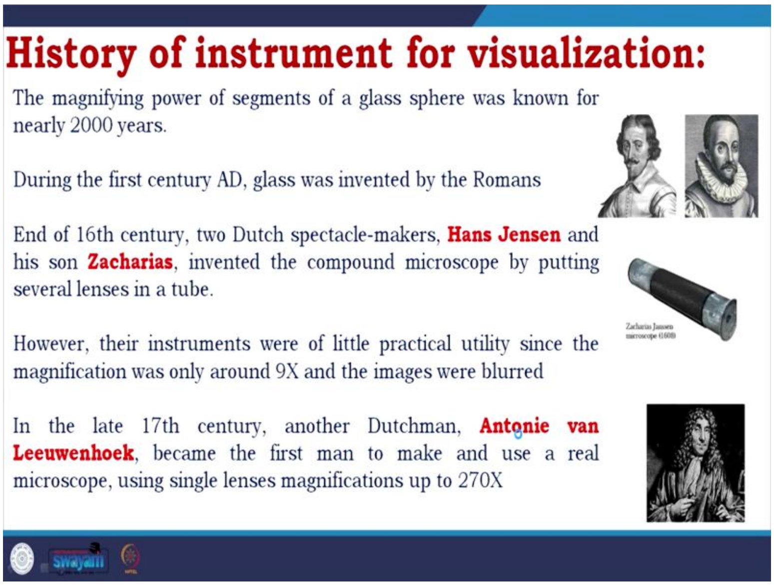

So let us come to the history of instruments for visualization. The magnifying power of a segment of a glass sphere was known for nearly 2,000 years. During the first century AD, glass was invented by the Romans. End of the 16th century, two Dutch spectacle makers Hans Jensen and his son Zacharias, invented the compound microscope by putting several lenses in a tube. However, that instrument was of little practical utility since the magnification was only around 9X, blurred images. The real breakthrough came true in the 17th century when another Dutchman Anthony Van leeuwenhoek became the first man to use a real microscope using single lenses magnification of 270X.

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When it came into that stage, the improvement of microscopy facilitated the introduction of a new concept in the internal structure of living organisms, which is called the cell. The cell's credit of the first description goes to Robert Hooke an English physicist and microscopist. Hooke found an air-filled compartment and introduced the term cell, published in 1665 in micrographia.

Chromatic aberration created problems with earlier microscopes, resulting in the compromise of the resolution for resolving power. The introduction of achromatic microscopes satisfactorily addressed the problem.

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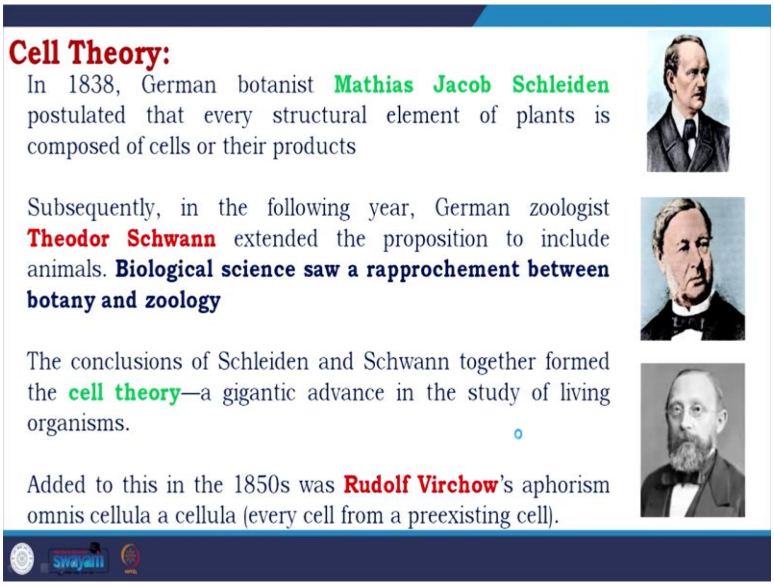

In 1838, German botanist Mathew Jacob Sheldon postulated that plants' structural elements are composed of cells or their product. Subsequently, the following year German zoologist Theodor Schwann extended the proposition to include animals. Biological science saw a rapprochement between botany and zoology when Aristotle first came up, which is prudent, and he had given the classification and categorization.

The conclusion of Schleiden and Schwann together formed the cell theory, a gigantic advance in the study of living organisms. In the 1850, Rudolf Virchow's aphorism Omnis cellula a cellula (every cell from a pre-existing cell).

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So in cell theory, they have made some postulates. All living organisms are made of cells. Cells are the basic unit of life. Cells arise from pre-existing cells. Hereditary information is passed from cells. All cells have a basic chemical composition. Energy flow occurs within the cell.

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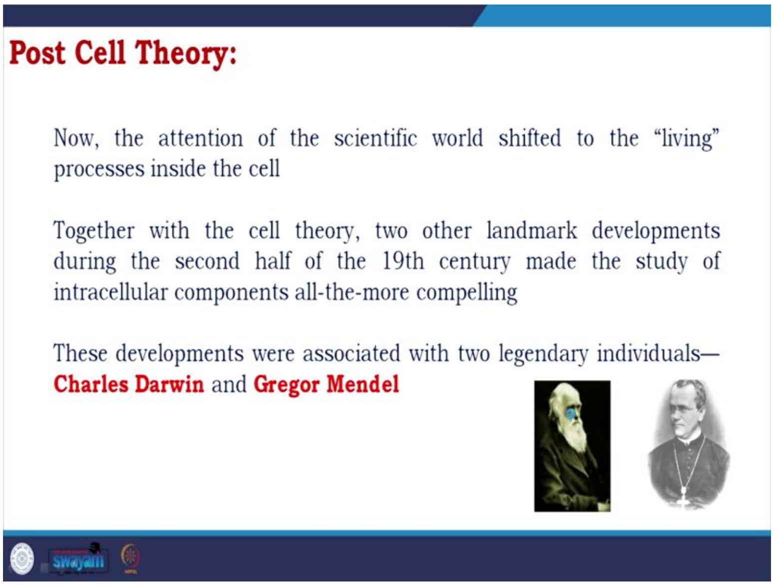

Now, when preliminary cell work was established, the scientific world's attention shifted to the living process inside the cell. The cell theory to other landmark development during the second half of the 19th Century, makes the study of intracellular components all the more compelling. This development was associated with two legendary individuals, Charles Darwin and Gregor Mendel.

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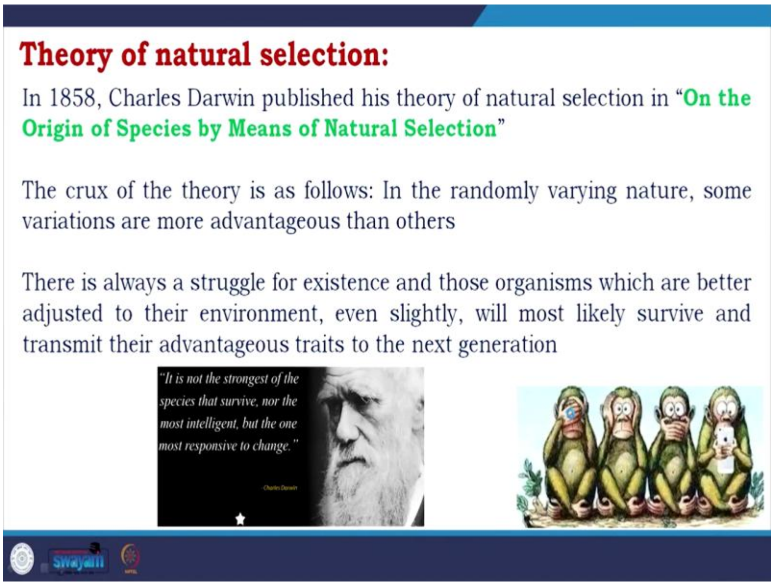

Theory of natural selection by Charles Darwin in 1858: Charles Darwin published the theory of natural selection on the origin of species through natural selection. The Crux of the theory was as follows. In the randomly varying nature, some variations are more advantageous than others. There is always a struggle for existence. Those organisms better adjusted to the environment even slightly will most likely survive and transmit their advantageous traits to the next generation.

So, according to his; own language. It is not the strongest species that survive nor the most intelligent but the most responsive to change. This is called adaptation

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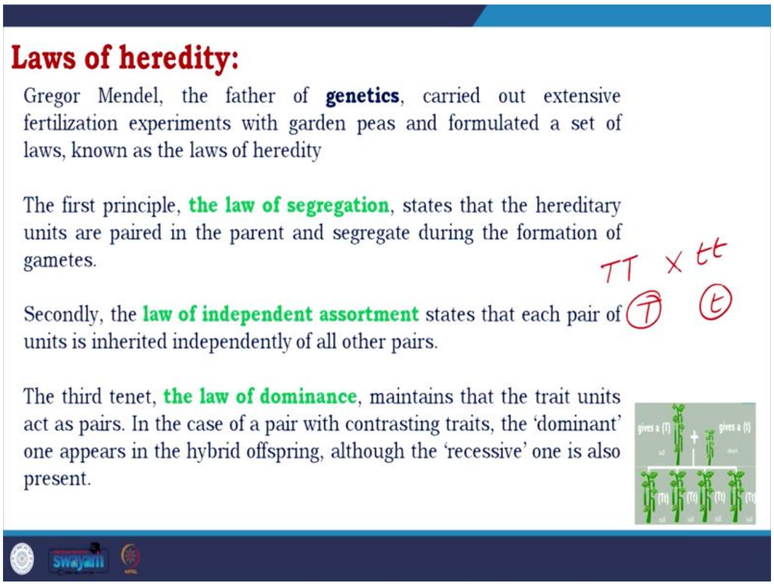

In two laws of heredity, Gregor Mendel, the father of genetics, carried out extensive fertilization experiments with garden peas and formulated a set of laws known as laws of heredity. The first principle, the law of segregation, states that the hereditary units appeared in the parent and were segregated during the formation of gametes. So their pair, suppose there TT and tt when it cross becomes T from here and t from here.

The law of independent assortment states that each pair of units is inherited independently of all other pairs there is no relation in that way. It is independent. And the third one, the most important law of dominance, maintains that the units of the trait act as pairs. In the case of a pair with contrasting traits, the dominant one that appears in the hybrid offspring of the recessive one is also present. Like if you see here, there is a great trait big T, and there is a small t, so they give Tt; all these are tall, so the dominant species is working here.

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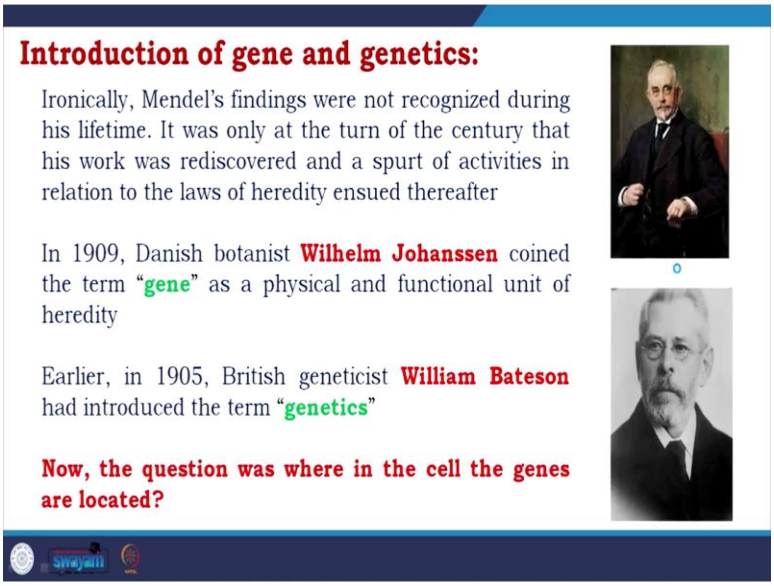

Ironically Mendel's findings were not recognized during his lifetime. He has done very notable work, but nobody understands that time. It was only at the turn of the century that his work was rediscovered in this part of activities about the laws of heredity ensure. In 1909 Danish botanist Wilhelm Johanssen coined the term gene as a physical and functional unit of heredity. Earlier in 1945, British geneticist William Bateson had introduced the term genetics. So now, when we know about genes and genetics, the question is where the genes are located in the cell.

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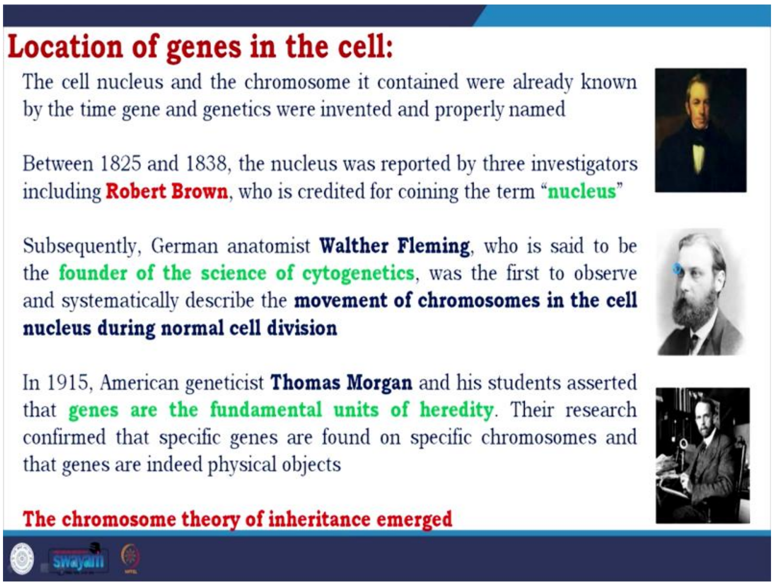

The cell nucleus and the chromosome the time gene and genetics already knew its content invented and properly named. Between 1825 and 1838, the nucleus was reported by three investigators, including Robert Brown, who is credited for coining the term nucleus. Subsequently, German anatomist Walter Fleming, who is the founder of science of cytogenetics, was the first to observe and systematically describe the movement of chromosomes in the cell nucleus during normal cell division.

In 1915 American Geneticist Thomas Morgan and students asserted that genes are the fundamental units of heredity. Their research confirms that specific genes are found on specific chromosomes and that genes are the physical object.

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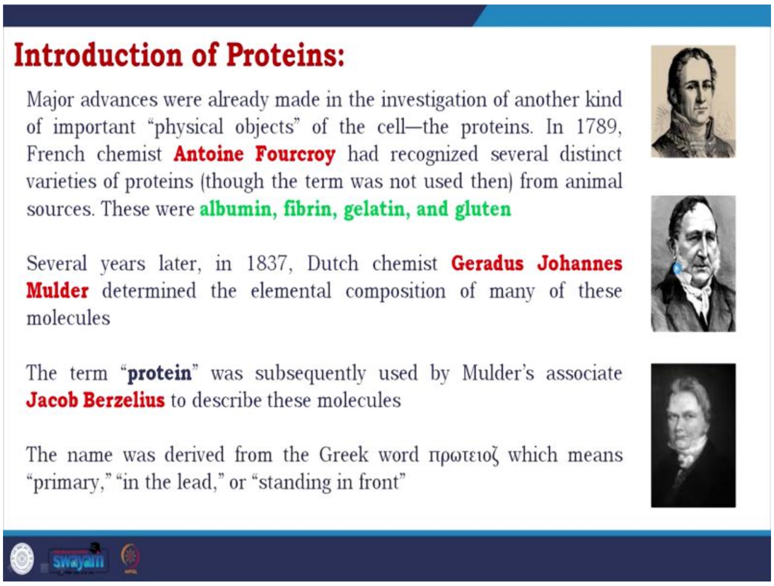

And when they were focused on genes, the protein was not even neglected. And major advances were already made in investigating another important physical object of the cell, the proteins. In 1789 French chemist Antonie Fourecoy recognized several distinct protein varieties though the term was not used then from animal sources. They have purified studied albumin, fibrin, gelatin, and gluten.

Several years later, in 1937, Dutch chemist Geradus Johannes Mulder determined the elemental composition of many of these protein molecules. Mulder's associate Jacob Berzelius subsequently used the term protein to describe this molecule. The name was derived from the Greek word which means primary in the lead or standing in front.

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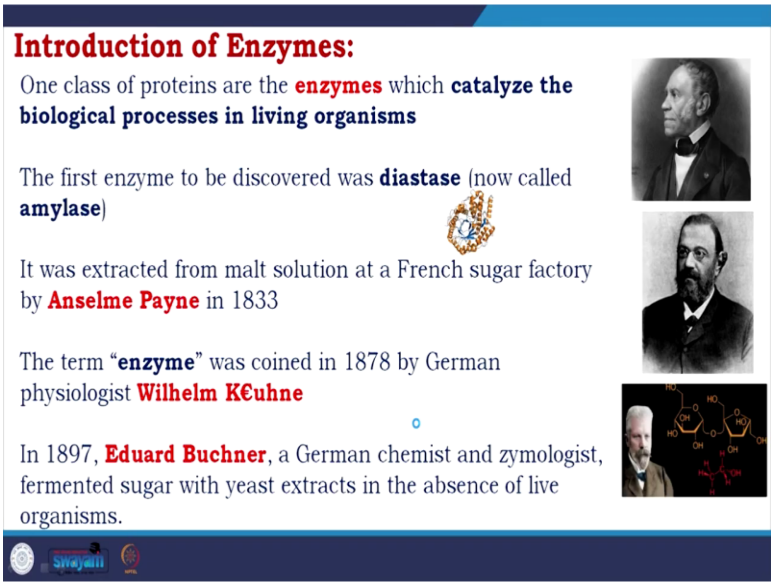

One class of proteins that enzymes catalyze the biological process in living organisms. The first enzyme discovered was diastase, which is now known as amylase and is still a very industrially relief in protein. This structure was later extracted from malt solution at a French sugar factory by Anselme Payne in 1833. The term enzyme was coined in 1878 by German physiologist Wilhelm Kuhn.

In 1897 Eduard Buchner, a German chemist and zymologist, fermented sugar with yeast extract in the absence of live organisms. This also shows that the enzyme could work without a living organism, so another breakthrough innovation simultaneously.

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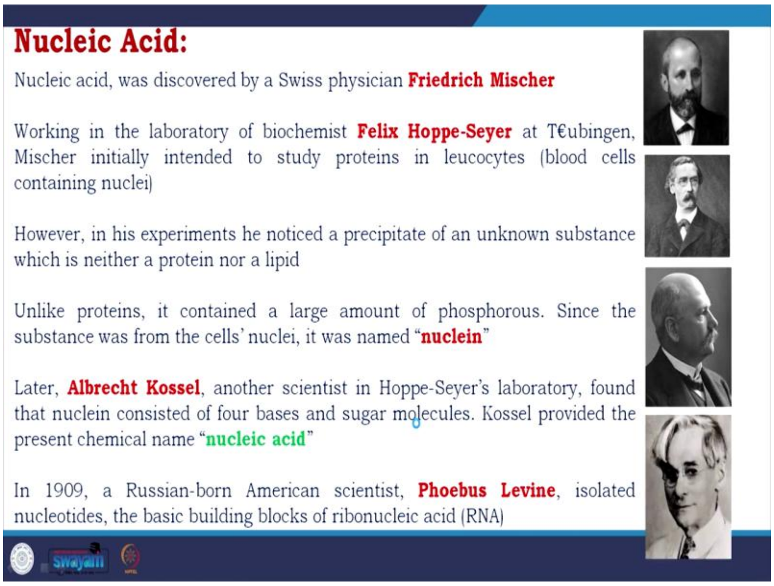

Swiss physician Frederick Mischer discovered nucleic acid. Frederick was working in the laboratory of Felix Hoppe Sayer, a very famous laboratory of that time in Tuebingen. Mischer initially intended to study protein in leukocytes, a blood cell containing nuclei.

However, in Frederick's experiment, he noticed a precipitate of an unknown substance and established a test showing that this is neither a protein nor a lipid.

Unlike protein, it contained a large amount of phosphorus since the substance they got from the nucleus. They name it nuclein. Later, Albrecht Kossel, working with Frederick Mischer at the same lab of Felix Hoppe-Sayer, found that nuclein consists of four bases and sugar molecules. Kossel provided the present chemical name nucleic acid. In 1909 a Russian-born American scientist, Phobus Levine, isolated nucleotides, the basic building block of ribonucleic acid.

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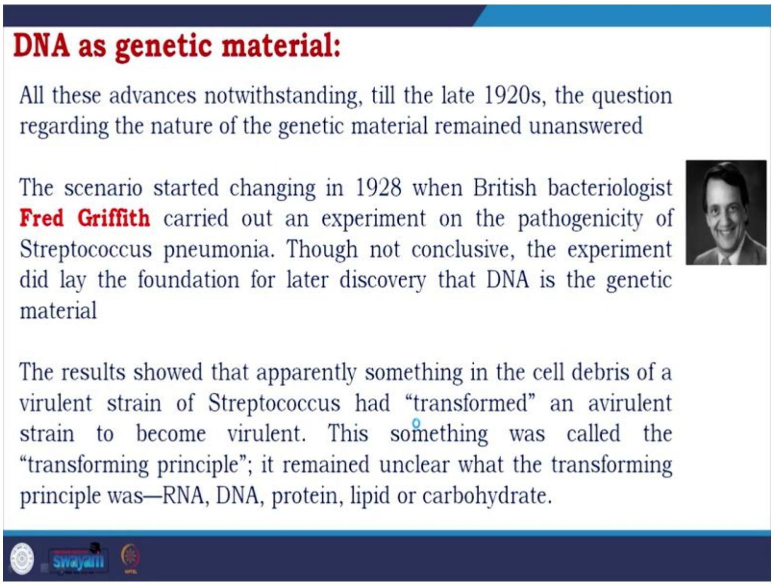

DNA as genetic material, all these advances not making impact till the late 1920's the question regarding the nature of genetic material remain unanswered. The scenario splattered, changing in 1928 when British bacteriologist Fred Griffith experimented on the pathogenesis of Streptococcus pneumonia. Though not conclusive, the experiment lay the foundation of the later discovery that DNA is the genetic material.

The results show that apparently, something in the cell debris is of a virulent strain of Streptococcus had transformed an avirulent strain to become virulent. This something was called the transforming principle at that time. The transforming principle remains unclear: RNA, DNA, protein carbohydrates biological macromolecules.

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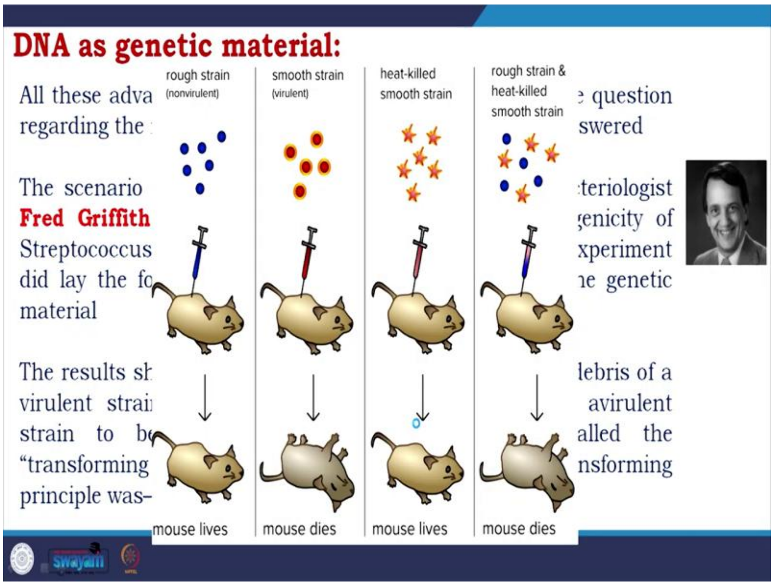

This is the description of the experiment. They choose a rough strain that is non-virulent and inject it into the mice. Then they take a smooth strain of Streptococcus which is virulent, and when injected into the mouse, the mouse dies. Then they use heat kills smooth strain the mouse is now not killed. But when the mix-up that heat kills smooth strain with the rough strain, they see that the combination kills the mouse effectively.

So then they understand that there is something in this virulent and non-virulent strain that helped the mouse kill.

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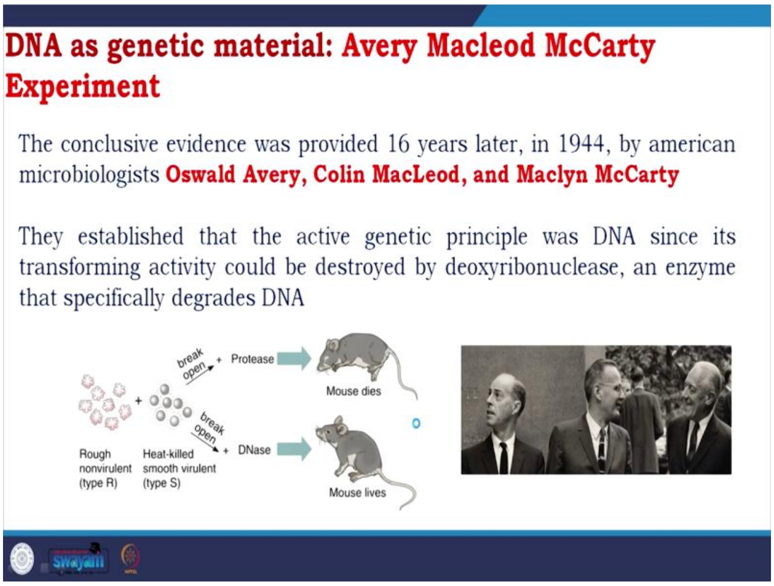

Avery Macleod McCarty experiment of DNA as genetic material. The conclusive evidence is provided 16 years later, in 1944, by American microbiologist Oswald Avery, Colin Macleod, and Maclyn McCarty. These three people established that the active genetic principle was

DNA since its transforming activity could be destroyed by deoxyribonuclease, an enzyme that specifically degrades DNA.

They take the rough nonviolent, and heat kills smooth virulent, which already had taken in the earlier experiment by Fred Griffith. They combine them, and add protease to one and DNAase to another. When they add protease, the mouse dies, they could not kill, the killing ability for killing power of the mixture. Whereas when they add DNAase, the mouse is now survived, which means DNA's make the factor involved in killing ineffective.

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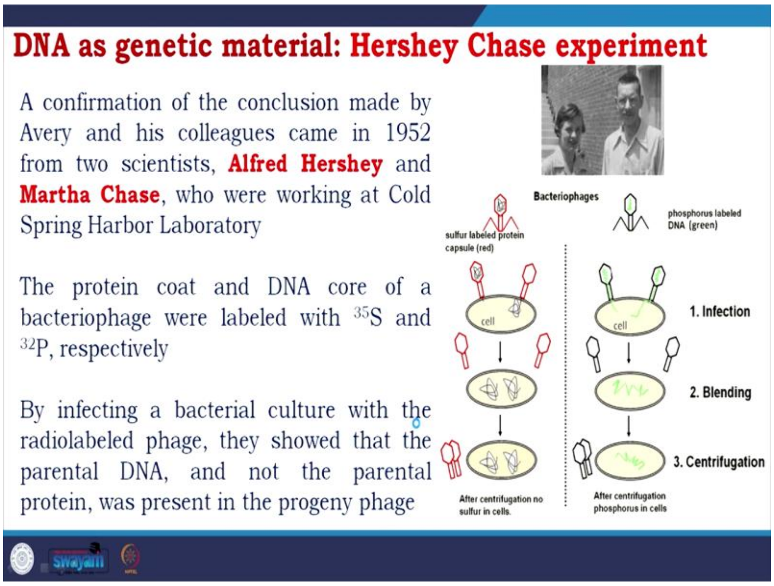

Another very significant experiment was called the Hershey Chase experiment. A confirmation of the conclusion made by Avery and his colleagues came in 1952 from two scientists, Alfred Hershey and Martha Chase, who are working at Cold Spring Harbour laboratory.

A bacteriophage is an organism (virus) that generally kills bacteria. It was used to label with S35 and 32P, respectively. The radioactive Sulphur and radioactive phosphorus. A bacterial culture with the radiolabeled phage showed that the parental DNA, not the parental protein, was present in the progeny phage. So they made this experiment sulfur labeled protein is called Red they go in the cell, and after centrifugation, no sulfur goes inside the cell.

So, it is not the proteins responsible, whereas when they labeled the DNA, which is labeled as green, then make the infection and isolated, they found the radiolabeled DNA in the progeny virus.

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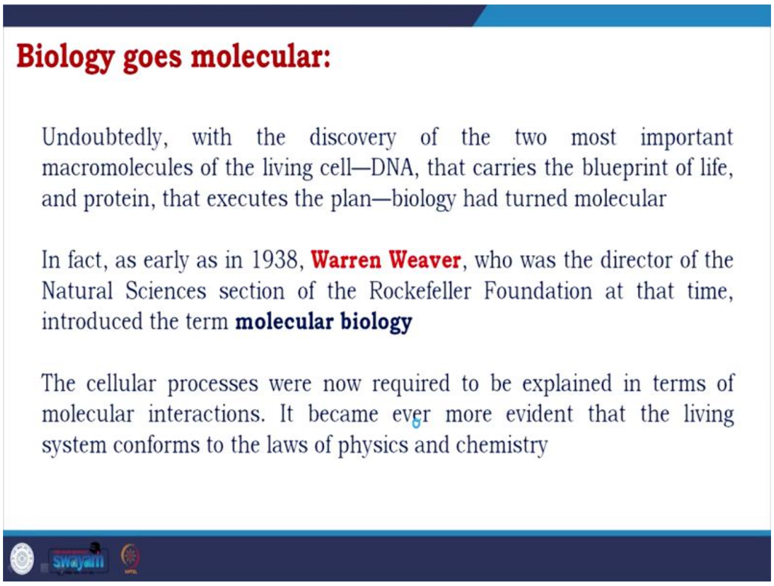

Undoubtedly the discovery of the two most important macromolecule of the living cell DNA that carries the blueprint of life and protein that executes the plan biology had turned molecular. In fact, in 1938, Warren Weaver was the director of the national science section of the Rockefeller Foundation. At that time introduced the term Molecular Biology. The cellular processes were now required to be explained in molecular interactions.

They want to go deeper than the behavior of DNA or a protein. They want to go for individualistic characterization. It became even more evident that the living system conforms to the laws of Physics and Chemistry. So there is a scenario created before people have done separate things, but now an interdisciplinary platform will be developed.

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