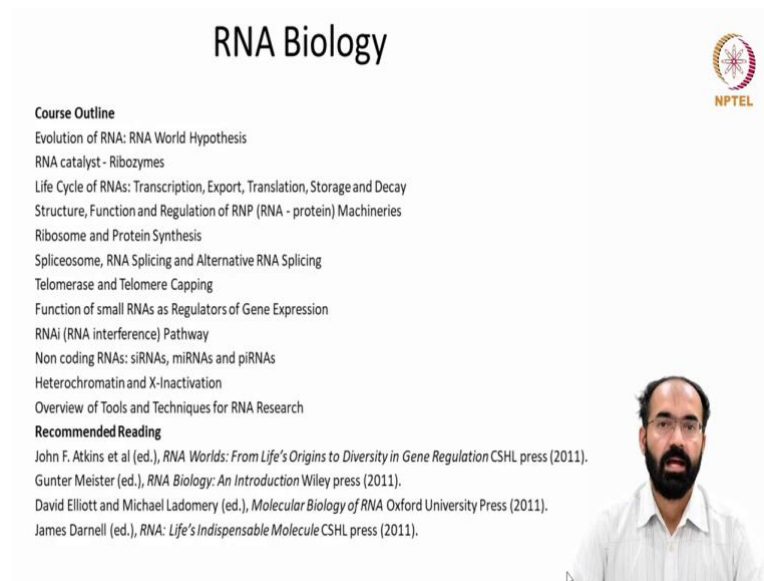



**RNA Biology**  
**Prof. Rajesh Ramachandran**  
**Department of Biological Sciences**  
**Indian Institute of Science Education and Research, Mohali**

**Lecture - 01**  
**Introduction to RNA Biology and RNA World-The Beginning**

(Refer Slide Time: 00:21)



**RNA Biology**


  
NPTEL

**Course Outline**

- Evolution of RNA: RNA World Hypothesis
- RNA catalyst - Ribozymes
- Life Cycle of RNAs: Transcription, Export, Translation, Storage and Decay
- Structure, Function and Regulation of RNP (RNA - protein) Machineries
- Ribosome and Protein Synthesis
- Spliceosome, RNA Splicing and Alternative RNA Splicing
- Telomerase and Telomere Capping
- Function of small RNAs as Regulators of Gene Expression
- RNAi (RNA interference) Pathway
- Non coding RNAs: siRNAs, miRNAs and piRNAs
- Heterochromatin and X-Inactivation
- Overview of Tools and Techniques for RNA Research

**Recommended Reading**

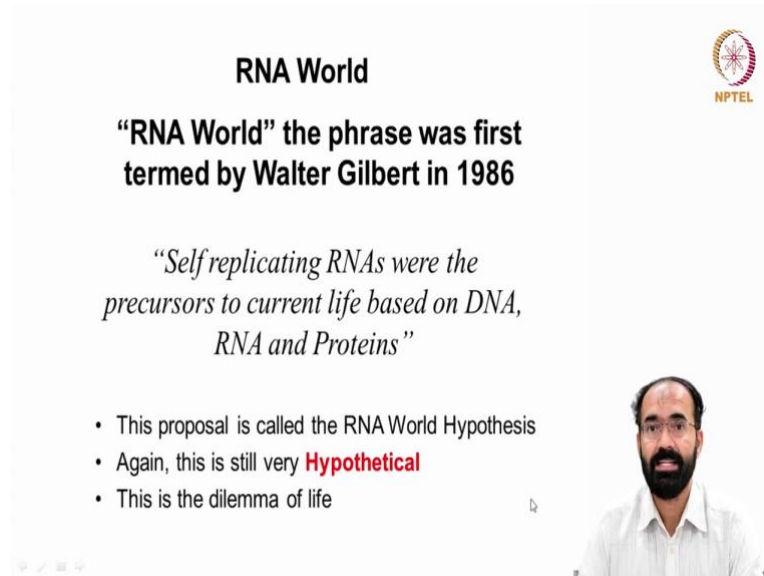
- John F. Atkins et al (ed.), *RNA Worlds: From Life's Origins to Diversity in Gene Regulation* CSHL press (2011).
- Gunter Meister (ed.), *RNA Biology: An Introduction* Wiley press (2011).
- David Elliott and Michael Ladomery (ed.), *Molecular Biology of RNA* Oxford University Press (2011).
- James Darnell (ed.), *RNA: Life's Indispensable Molecule* CSHL press (2011).



Hello everyone! Today, we are going to start the first lecture of the RNA Biology. and the course outline is present in this first slide itself. And we will go through each and every sections one by one within the stipulated time. And the recommended readings are listed below and I would be referring contents from various books and also published papers and research articles and reviews etc.

And I will be giving reference for those work, every time, when I go through a given content.

(Refer Slide Time: 01:05)



The slide features the title "RNA World" at the top center. To the right is the NPTEL logo. Below the title is a quote: "“RNA World” the phrase was first termed by Walter Gilbert in 1986". Underneath the quote is another quote: "“Self replicating RNAs were the precursors to current life based on DNA, RNA and Proteins”". At the bottom left, there are three bullet points: "• This proposal is called the RNA World Hypothesis", "• Again, this is still very **Hypothetical**", and "• This is the dilemma of life". On the right side of the slide, there is a small video inset showing a man with a beard and glasses, wearing a white shirt, looking towards the camera.

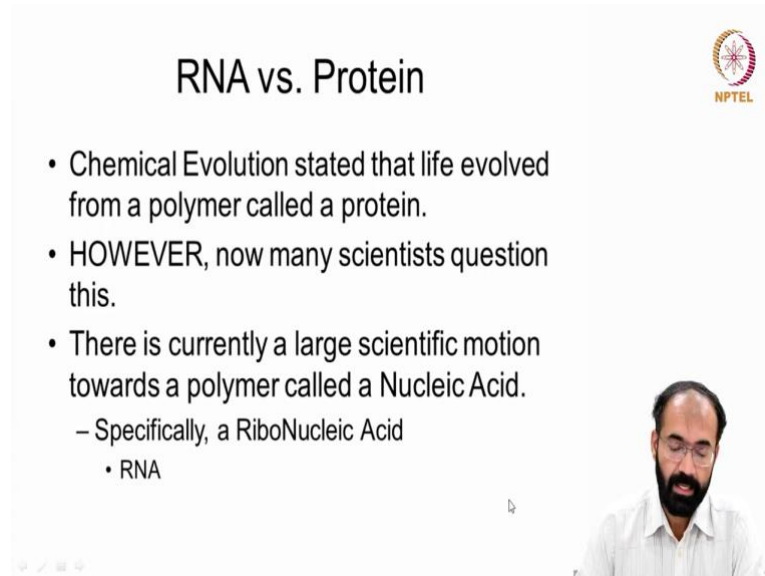
So, the first topic is about the ‘RNA World’. So, the word itself indicates what an “RNA world” the terminology stands for. ‘RNA world’ is a coin termed initially by the famous scientist “Walter Gilbert” in 1986 although this idea is much older than that. However, “Walter Gilbert” was the one first to point this term ‘RNA world’.

So, it basically means there was a time before the life originated on this planet that there was a time the life the so-called activities related to life was carried out through RNA molecules. And so, what it indicates there is a self-replicating RNA that were acting as precursors of current life forms that is purely based on the DNA.

Of course, I am not denying the fact that there are many living forms such as viruses they have RNA as their genome even today. But eventually, majority of the life forms have DNA as their genome which transcribes into RNA and later into proteins, which we will see them one by one; however, having said that this so-called “RNA world” has its own dilemma which we will be addressing one by one.


So, this proposal is basically called as the ‘RNA World’ Hypothesis means the life had a stage in this planet before the organisms started appearing that stage was preceded by a RNA world. And we should understand this whole idea is quite hypothetical and reason being very simple because it is very hard for anyone to replicate the lifeless planet in that large scale. So, we have to look for the evidences and also look for is there any options to prove it.


(Refer Slide Time: 03:16)



## RNA vs. Protein

- Chemical Evolution stated that life evolved from a polymer called a protein.
- HOWEVER, now many scientists question this.
- There is currently a large scientific motion towards a polymer called a Nucleic Acid.
  - Specifically, a RiboNucleic Acid
    - RNA





So, we should understand the current biological functions in every living organisms starting from bacteria to human are carried out by proteins mainly the workforces or proteins. So, is there any difference or is there any similarity between the RNA and protein? Because this is very important to have clarity to start with.

Simply because, one may wonder if RNA world existed why it shifted to DNA? and then protein molecules and their dependency in the later on living organisms. So, the ‘chemical evolution’ because there is parallel hypothesis that existed that talks about you know evolution of life forms through a “chemical evolution”, which basically means that various molecules chemical molecules.

Of course, RNA itself is a chemical molecule, but chemical evolution basically says the congregation of various chemicals would have contributed to the origin of life. So, the chemical evolution basically started when the life evolved from a polymer called protein and it exists even today in all living organisms the workforce is the proteins or proteins in a way proteins basically means the structural proteins and functional proteins they can be called as the workforce of life in any living organism.

So, several questions have arisen based on the RNA world. And one of the major constraints of this RNA world hypothesis is that we do not have an RNA world the way we define in this modern world. And there are many questions have arisen against the

RNA world hypothesis and we have to look how can we prove it or disprove it we will see them one by one.

There is currently a large scientific notion that is existing towards a polymer called Nucleic Acid. And nucleic acid as the name itself indicates they are broadly classified into deoxyribonucleic acid and ribonucleic acid, but what we are talking here is about the ribonucleic acid which acted as a workforce in the prebiotic world even before the life originated on this planet. And the short form of ribonucleic acid is called RNA and it is the abbreviation.

(Refer Slide Time: 05:48)

The slide is titled "What is Life?" in blue text at the top center. Below the title, there is a sub-heading "What Is Life?". The main text is divided into two columns. The left column starts with a large "T" and discusses the search for extraterrestrial life and functional definitions. The right column discusses biochemical definitions and a personal definition. At the bottom left, there is a citation: "Carl Sagan (1994) *Sci. Am.* Oct. 1994: 92-99." In the top right corner, there is an NPTEL logo. In the bottom right corner, there is a video inset showing a man with a beard and glasses speaking.

**What is Life?**

**What Is Life?**

The search for extraterrestrial life must begin with the question of what we mean by life. "I'll know it when I see it" is an insufficient answer. Some functional definitions are inadequate: one might identify life as anything that ingests, metabolizes and excretes, but this description applies to my car or to a candle flame. Some more sophisticated definitions—for example, life as recognizable by its departure from thermodynamic equilibrium—fall afoul of the circumstance that much of nature (such as lightning and the ozone layer) is out of equilibrium.

Biochemical definitions—for example, defining life in terms of nucleic acids, proteins and other molecules—are clearly chauvinistic. Would we declare an organism that can do everything a bacterium can do if it was made of very different molecules? The definition that I like best—life is any system capable of reproduction, mutation and reproduction of its mutations—is impractical to apply when we set down a spacecraft on another world: reproduction may not be done in public, and mutations might be comparatively infrequent.

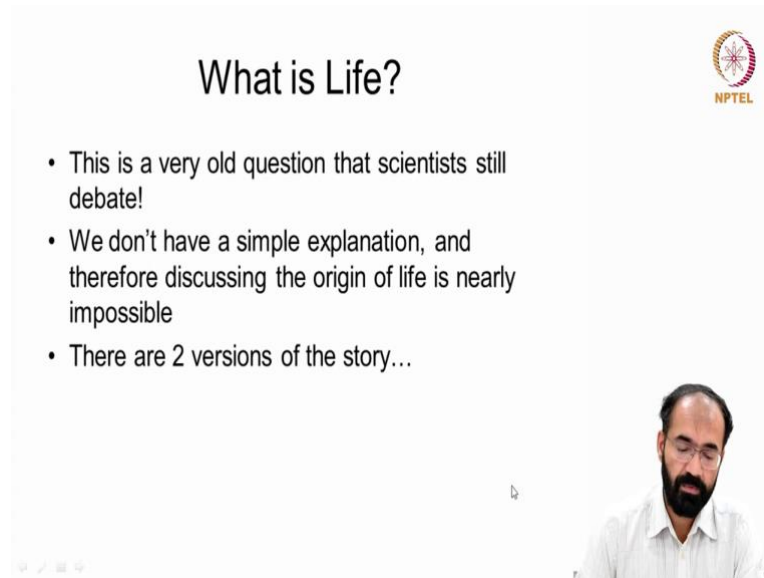
Carl Sagan (1994) *Sci. Am.* Oct. 1994: 92-99.

So, let us look into what is life? Because it is a very broad question and this particular citation is written by the famous physics scientist Carl Sagan and when he published in Scientific American. So, life can be defined in various ways; however, we should understand what is the most fundamental or the most simplistic form that covers all aspects of life.

So, one could assume life is a system that is capable of reproduction and mutation. We will come to that what is mutation. Mutation simply understand that a change in the normalcy if something is happening 1 plus 2 is equal to 3 and now you are saying 1 plus 2 is not equal to 3. So, that is called a change that is a mutation. So, in life form any system that is capable of reproduction and change it in some way or the other a mutation and the reproduction of those mutation.

That means a change is there, now are you able to reproduce this change? Then that has to be tested through the time whether or not this change or the so-called mutation is winning the test of time and that is the fundamental definition of life that one can simply put in.

(Refer Slide Time: 07:16)



**What is Life?**

- This is a very old question that scientists still debate!
- We don't have a simple explanation, and therefore discussing the origin of life is nearly impossible
- There are 2 versions of the story...

So, there is a lot of you know arguments debates etcetera have existed based on the simple question, what is life? And there are different groups of researchers and scientists who debate still on this. But we cannot simply have a easier explanation and therefore, the discussing of origin of life is nearly impossible and for the simple reason that none of us were completely aware of the situation before the life existed on this planet and we cannot comprehend the actual depth of life as of now.

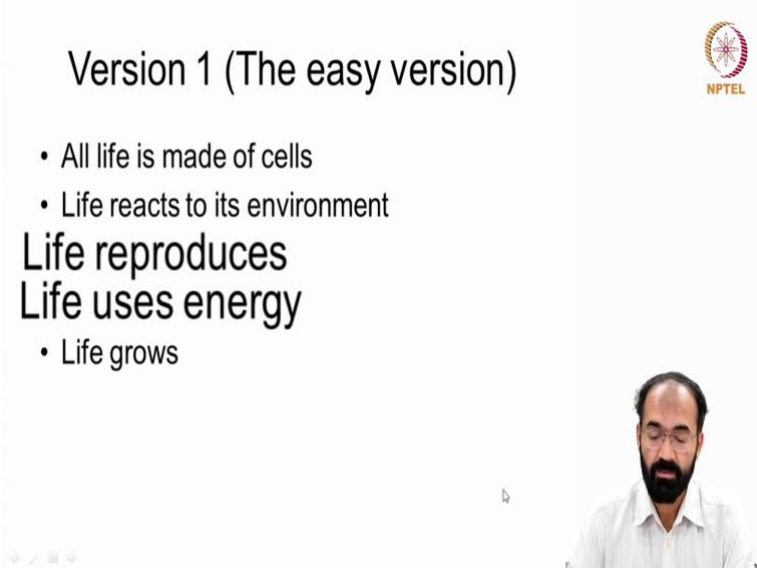
Because simple example I can tell you many scientists say virus is a non-living entity, but many people say virus is a living entity, both are correct. So, you may wonder how this is possible because virus when it is not inside a host it is impossible to propagate itself it is impossible to divide itself and of course, there are many bacteria also existing they need a host in order to propagate.

Now, the question of reproduction falls apart it cannot reproduce it need a host, but then one can argue the host itself is part of its atmosphere or part of its surrounding or part of its habitat then it becomes a part of definition. So now, you have to introduce a topic is it

self sufficient? and of course, viruses and some bacteria are not self sufficient unless a host organism is provided.

So, that is why the definition of life becomes debated. So, there are 2 versions of the story can be put forward and we will see them what are these 2 versions of story that is so-called life.

(Refer Slide Time: 09:16)



Version 1 (The easy version)

- All life is made of cells
- Life reacts to its environment

Life reproduces  
Life uses energy

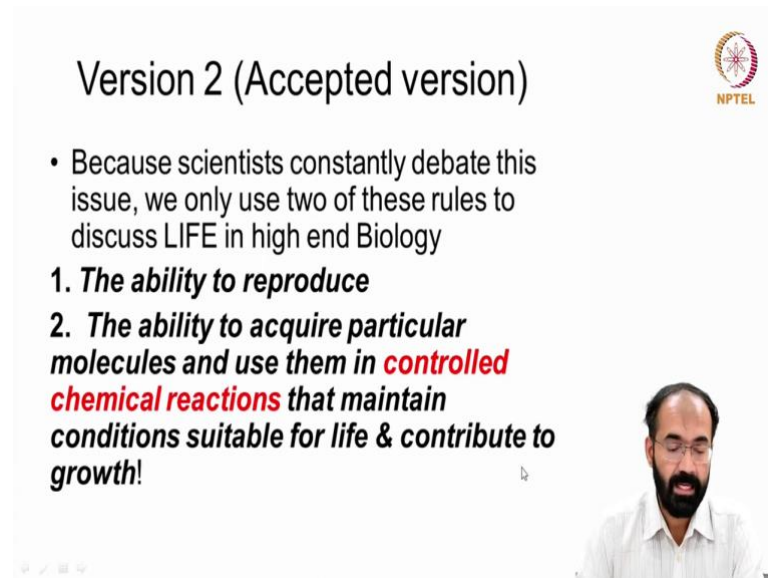
- Life grows

The version one, which is basically an easier version and which is easy for us to understand because all life forms are made of cells point number 1. And point number 2, life reacts to its environment which is also very valid. And then life reproduces point number 3, life reproduces.

Then life uses energy because any forms of life need energy source can be chemical energy or you know light energy it need some forms of energy to sustain itself. And then life grows because life do not exist it will be any organism whether it is a bacteria, virus human, they keep growing it may not grow in physical size after some stage, but they are replenishing the lost part.

And so, these are easier points which we can consider for the version one of the definition of life. But life reproduces is something which you should keep in mind and life uses energy is something we should which we should keep in mind because they are going to be most important for the survival of an organism.

(Refer Slide Time: 10:36)



Version 2 (Accepted version)

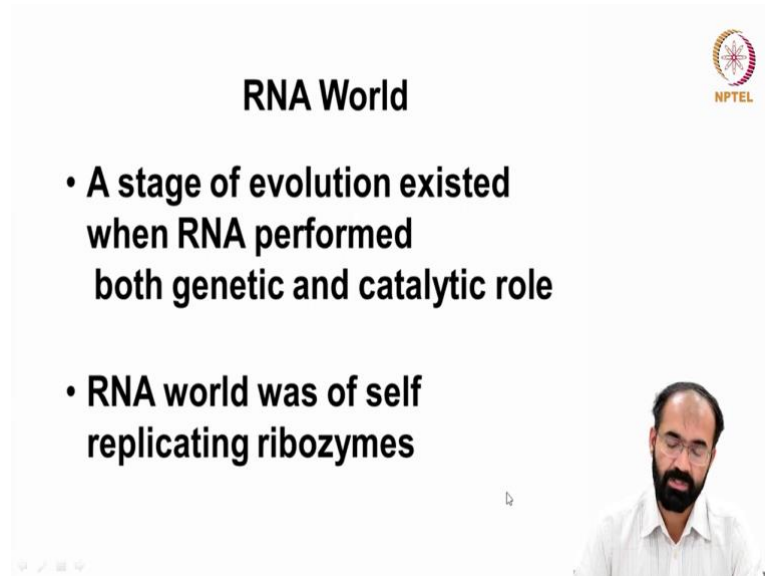
- Because scientists constantly debate this issue, we only use two of these rules to discuss LIFE in high end Biology

1. **The ability to reproduce**
2. **The ability to acquire particular molecules and use them in *controlled chemical reactions* that maintain conditions suitable for life & contribute to growth!**

And the version 2 is the most accepted version and we will see why this is so? Many scientists constantly debate as I already told you this issue on the definition of life because we only use two of these rules to discuss life in high end biology; that means, life reproduces and life uses energy. So, the ability to reproduce is one of the fundamental requirement of life. Reproduction basically means - any new character or any maintenance of existing character it has to have the ability to make a copy of itself.

And then the ability to acquire a particular molecules and use them in controlled chemical reactions that maintain conditions suitable for life and contribute to growth. So, this is another important point that has to be addressed by every life form without which it becomes impossible for the sustenance of life. So, we can say the version two is the most accepted version of life.

(Refer Slide Time: 11:55)



**RNA World**

- **A stage of evolution existed when RNA performed both genetic and catalytic role**
- **RNA world was of self replicating ribozymes**

NPTEL

*(A small video inset in the bottom right corner shows a man with a beard and glasses speaking.)*

So, coming back to RNA world because we already defined the life and now, we have to see the RNA world with regard to these definitions because in a prebiotic world we had only RNA molecule as the initiators of life on this planet. So, a stage of evolution existed when the RNA perform both genetic and catalytic role. So, evolution is a new term which we are introducing.

Evolution basically means that you have something say for example, you can think in any form, see you have designed a very beautiful pen or you have designed a very beautiful mobile phone. Now the question is are you able to make a replica of it? So, sometimes you know many art painting, many artists will make a beautiful painting, but based on the technology which they have used they may or may not be able to reproduce it.

Say you made a art work by say by random approach, then it becomes very difficult even for the same artist to reproduce it; however, if you have made anything through a defined process then it becomes rather easy for you to reproduce. Just like in a manufacturing or assembly line of a car or a mobile phone or a pen or anything. So, it is very important that you are able to make a copy of itself.

Now, the evolution term will be much easier. Now you will may have seen in the older time the mobile phones were very simple which is a miniature version of your landline phone. You have a buttons and you can simply dial a phone a number. Now you slowly



started getting a camera, a FM radio or maybe you are able to play songs etcetera. So, this is called evolution.

That means you are introducing newer and newer and newer features on to an existing thing and now it is getting adapted. See introducing is something whether it is getting adapted to, is another thing. many a times if a mobile phone is having a feature which is absolutely no use to anyone. Then what will happen? People do not prefer that phone or people do not want to have that feature as a requirement in their phone.

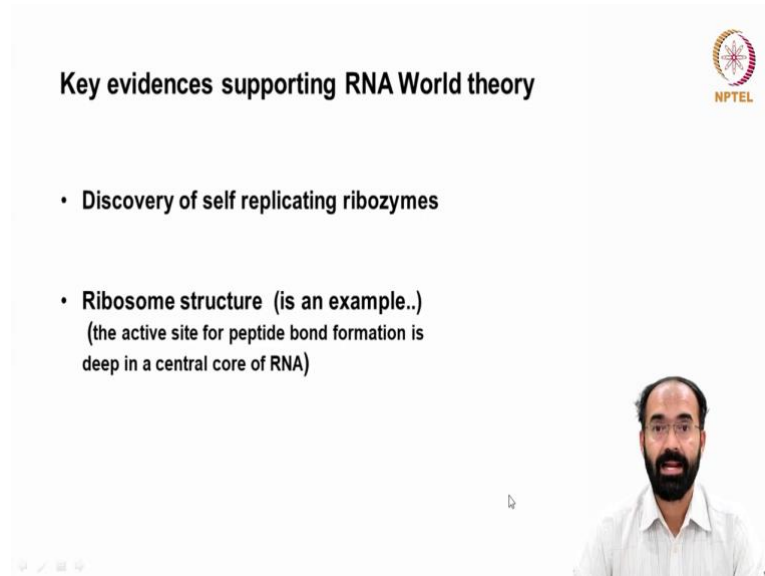
So, naturally that particular phone version will get outdated. On the other hand, you introduced a feature which is very important people were just waiting for it looking for it and if you get that phone then that will be propagated and more and more manufacturers will start introducing it. So, this is evolution; that means, any change that is introduced has to win the test of time. In the case of mobile phone, it is the customers.

So, that is what evolution means basically in the simplistic form. And RNA world has a basically a molecular world which is capable of replicating its own molecule; that means, we can say that an RNA world existed where a lot of RNA molecules were there and many of them were able to make a copy of themselves through their catalytic role.

‘Catalytic’ is a new word which I am introducing. Basically, mean it is capable of carrying out some of the important functions like if I give you a pen and a paper and you are able to write it is a function. Paper and pen will not write you are the one who is writing; that means, you are the catalyst. So, here if there is no you existing only paper is there or pen is there. In that situation either paper should have the ability to use the pen and write it, or pen should have the ability to write automatically.

Then that can be called as a function or a catalyst. So, RNA molecules if it has to make a copy of itself either it need a third party or it itself should be able to make a copy of itself. So, RNA world was found by a group of self-replicating RNA molecules called ribozymes. We will see more in detail about ribozymes in the subsequent classes.

(Refer Slide Time: 16:16)



The slide features a title 'Key evidences supporting RNA World theory' in bold black text. In the top right corner is the NPTEL logo, which consists of a circular emblem with a stylized 'N' and 'P' and the text 'NPTEL' below it. The main content is a bulleted list with two items. The first item is 'Discovery of self replicating ribozymes'. The second item is 'Ribosome structure (is an example..)' followed by a sub-point in parentheses: '(the active site for peptide bond formation is deep in a central core of RNA)'. In the bottom right corner of the slide, there is a small video inset showing a man with a beard and glasses, wearing a light-colored shirt, looking towards the camera. At the bottom left of the slide, there are small navigation icons for a presentation.

**Key evidences supporting RNA World theory**

- **Discovery of self replicating ribozymes**
- **Ribosome structure (is an example..)**  
(the active site for peptide bond formation is deep in a central core of RNA)


So, let us think are there any evidence we are talking about RNA world etcetera etcetera. Are there any evidence, are there any supporting theory for the RNA world hypothesis. So, we have evidences to prove that there are plenty of molecules RNA molecules exist even in modern world which have the ability to replicate itself and we call them ribozyme.

And another example is ribosome it is different from ribozyme. Ribosome indicates a molecule that is present from bacteria to human inside a cell which is essential for the production of protein in a cell. So, it consists of protein and RNA molecule; however, the biological function of say peptide bond formation during protein synthesis is exclusively carried out by the RNA component.

So, in principle we can call ribosome also a ribozyme. So, we have two examples of course, there are many examples that will come as we go on with the class.


(Refer Slide Time: 17:26)

**Some Milestones in Origin-of-Life Science-1**



- ~ 2,000 yrs ago: *The Bible* states God created humans & higher organisms.
- < mid 1800' s: Creationism + insects, frogs & other small creatures observed to arise spontaneously from mud & rot.
- mid 1800' s: (1) **Pasteur** demonstrated bacteria & other microorganisms arise from parents resembling themselves. *Spontaneous generation is dead.*
- (2) **Darwin** proposes natural selection, the theory that environmental pressure results in the perpetuation of certain adaptations. Evolution of complex organisms therefore possible, & all current life forms could have evolved from a single (last) common ancestor.
- **Darwin** suggested *life could have arisen from chemistry*: “in some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity, etc., present.”

Adapted from Orgel (1994) *Sci. Am.*, Oct. 1994, 77-83.



So, let us see some key milestones of RNA world hypothesis that is origin of life based on scientific support or scientific evidences. Around 2000 years ago, the Bible stated that ‘God created humans and higher organism’ that was the understanding around 2000 years ago. By around mid 1800 the so-called creationism of insects, frogs and other small creatures known to arise spontaneously from you know rotten meat and rotten dirt etcetera.

And people started believing you know spontaneously living organisms can come from dirt. And late 1800s Louis Pasteur the famous French scientist and he demonstrated that the bacteria and other microorganisms arise from parents resembling themselves means he proved that spontaneously nothing can form it has to have some pre-existing bacteria or any other living organism to make.

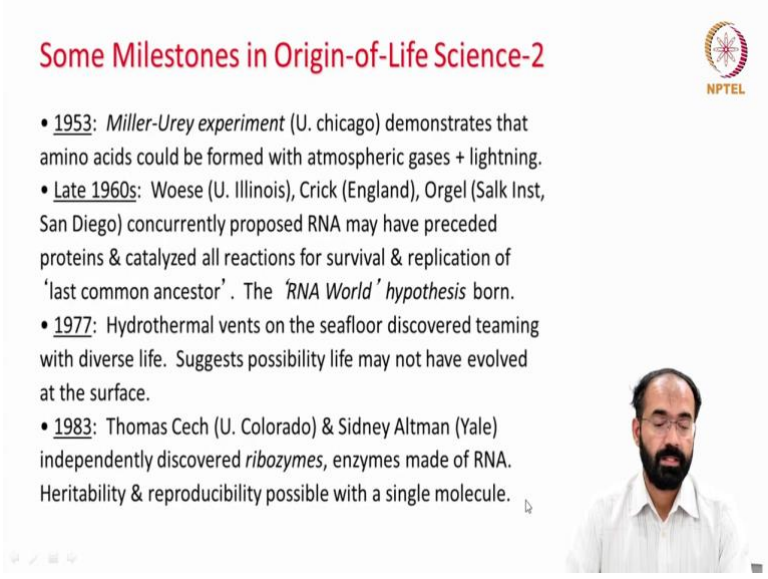
So, this spontaneous generation is closed; it is no more valid. Later on Darwin, Charles Darwin, came up the proposal of natural selection. So, basically means the environment contributes a significant extent for the exerting pressure on various kind on to the living forms and allow them to evolve in a differential manner; that means, a now diverts into b and c.

That means it has the characters of a, but b have unique characters different from c. So, now, you have two lineage that is one is b line another is a c line and like that that is the origin of theory from Charles Darwin on evolution. And he also proposed that ever y

living organisms in this modern world have originated from one common ancestor that is one unique common ancestor for all living life forms on this planet.

And Darwin also suggested that life could have arisen from chemistry; that means, chemical molecules in some warm little pond with all sorts of ammonia, phosphoric salts, light, heat, electricity, etcetera etcetera.


(Refer Slide Time: 19:53)



**Some Milestones in Origin-of-Life Science-2**

- 1953: *Miller-Urey experiment* (U. Chicago) demonstrates that amino acids could be formed with atmospheric gases + lightning.
- Late 1960s: Woese (U. Illinois), Crick (England), Orgel (Salk Inst, San Diego) concurrently proposed RNA may have preceded proteins & catalyzed all reactions for survival & replication of 'last common ancestor'. The *'RNA World' hypothesis* born.
- 1977: Hydrothermal vents on the seafloor discovered teeming with diverse life. Suggests possibility life may not have evolved at the surface.
- 1983: Thomas Cech (U. Colorado) & Sidney Altman (Yale) independently discovered *ribozymes*, enzymes made of RNA. Heritability & reproducibility possible with a single molecule.

NPTEL



So, some other milestones of life on the origin of life. In 1953, Urey and Miller they are called Miller and Urey experiment at the university of Chicago. They demonstrated for the first time that the amino acids which is the building blocks of proteins could be formed from atmospheric gases and lightning that is purely inorganic substance and physical molecule there is no organic material involved, but amino acid is a organic molecule that can be formed from inorganic substances.

And in late 1960s famous scientist Woese at university of Illinois and Crick at England and Orgel at Salk institute Salk institute at San Diego he concurrently proposed that the RNA may have preceded proteins and catalyzed RNA and also various other biological functions and they could have also given rise to the last common ancestor which Darwin has proposed and in short form it is called last unique common ancestor it is abbreviated as LUCA L U C A.

And from that time onwards the RNA hypothesis is born. by around 1977, a lot of hydrothermal vents were put into study and hydrothermal vents are seen usually in the bottom of the ocean where the due to the tectonic activity because you know the below the surface of planet earth there is lot of heat being generated and magma comes out and this can give rise to lot of heat in the bottom of the ocean.


And they discovered that a lot of diverse forms of life existed besides the hydrothermal vents and these events are created by the inorganic molecules and also the heat. And by 1983, Thomas Cech at university of Colorado and Sidney Altman at Yale University independently discovered the molecules, the protein independent molecule that is ribozymes.

They do not have any connection with proteins, they are RNA molecules that is capable of doing some function and we call it ribozymes and they are enzymes made up of exclusively RNA. No dependency on whatsoever other molecule. Of course, there are some supporting molecules like some metal ions can come into picture, but there is no organic molecule support except the RNA itself.



And these molecules have the heritability and reproducibility possible within a single molecule; that means, ribozymes a single RNA molecule can form its own copies and also able to perform some functions.

(Refer Slide Time: 22:55)

**Some Milestones in Origin-of-Life Science-3**



- 1988**: Günter Wächtershäuser theorizes that **Fe & Ni sulfide minerals** at hydrothermal vent systems provided the *template & catalyst* for formation of biological molecules.
- 1997**: Jay Brandes (Carnegie Inst.) demonstrates that  $N_2$  is converted to  $NH_3$  in the presence of  $H_2$  & magnetite ( $Fe_3O_4$ ), at typical of hydrothermal vents. *Mineral surfaces and HT vent environments can produce biologically-useful form of N.*
- 2000**: Cody et al. demonstrate synthesis of **pyruvate** using mineral catalysis under hydrothermal conditions. **Pyruvate is branch point for many extant biosynthetic pathways.**



So, now, some other milestones of origin of life on earth on scientific evidence. In 1988, Gunter theorized that iron and nickel sulfide minerals at the hydrothermal vent systems they provided the template and catalyst for the formation of the initial biological molecules.

And we will see them one by one what are they. In late in 1997 Jay Brandes demonstrated that nitrogen is converted into ammonia in presence of hydrogen and magnetite, magnetite is a ore of iron at typical hydrothermal vent temperature conditions. And several mineral surfaces at the hydrothermal vent environments can produce biologically useful form of nitrogen.

So, these are all contributing factors for the formation of life on earth. By around 2000, Cody and colleagues have demonstrated that a pyruvate, pyruvate is one of the major component of Krebs cycle which produces ATP. pyruvate can be produced spontaneously using mineral catalysis in hydrothermal vent condition; that means, the conditions of hydrothermal vent is good enough to form a purely organic molecule called pyruvate. Pyruvate is a branch point for many biosynthetic reactions including Krebs cycle.



(Refer Slide Time: 24:28)

**Murchison Meteorite**

Sept 28 1969, A carbonaceous chondrite type meteorite fell in Murchison town in Victoria Australia

Evidence for Extraterrestrial Amino-acids and Hydrocarbons in the Murchison Meteorite *Nature* (1970) **228**, 923 - 926

Compound class	Concentration (ppm)
Amino acids	17-60
Aliphatic hydrocarbons	>35
Aromatic hydrocarbons	3319
Fullerenes	>100
Carboxylic acids	>300
Hydrocarboxylic acids	15
Purines and Pyrimidines	1.3
Alcohols	11
Sulphonic acids	68
Phosphonic acids	2



So, now coming back to another important evidence that is called the Murchison meteorite. Murchison meteorite all of you know what is a meteorite. Meteorite are the molecules that is coming from extraterrestrial planetary orbits. They form you know one

of the meteorite collisions would have been proposed to have caused extinction of dinosaurs roughly around 65 million years ago.

So, one such example is the Murchison meteorite which in 1969 in September month it landed in Victoria Australia and the place is called Murchison town. And they could get usually the meteorites will burn out in through the friction from the atmosphere, but they could able to recover some leftover of this meteorite and they did some chemical analysis.

And what they found? They found plenty of biological materials present in them, such as amino acid, aliphatic hydrocarbon, aromatic hydrocarbon, fullerenes, carboxylic acid, hydro carboxylic acid, purines and pyrimidines, alcohols, sulfonic acid and phosphoric phosphonic acid. So, you can see their percentage in ppm that is parts per million the level is very low.

But we should understand a meteorite at high very high temperature cannot hold any of these molecules on its own unless it carried from the extra-terrestrial environment itself. What we should understand that meteorite using a lot of the inorganic molecules present in itself through this high temperature and this harsh condition is able to convert into various organic molecules as listed here.

So, what we should understand that meteorites composition can give some important clues on to how and when and where life could have originated, okay. So, we will conclude the session now and we will continue in the subsequent classes with more and more evidences regarding the life and the supporting factors of life and we also get into the Urey-Miller experiments and.

Thank you.