# BIOLOGY FOR ENGINEERS AND OTHER NON-BIOLOGISTS


**Prof. Suraishkumar &**
**Prof. Madhulika Dixit**

**Multidisciplinary**
Indian Institute of Technology, Madras

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**Biology for Engineers and other Non-Biologists**
**Professor: G.K.Suraishkumar**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Lecture Number 01**
**Introduction**

Welcome to this course "Biology for Engineers and other Non-Biologists". This is for anybody who has an interest in biology. We will go through some initial aspects of this course in the first lecture which is this.

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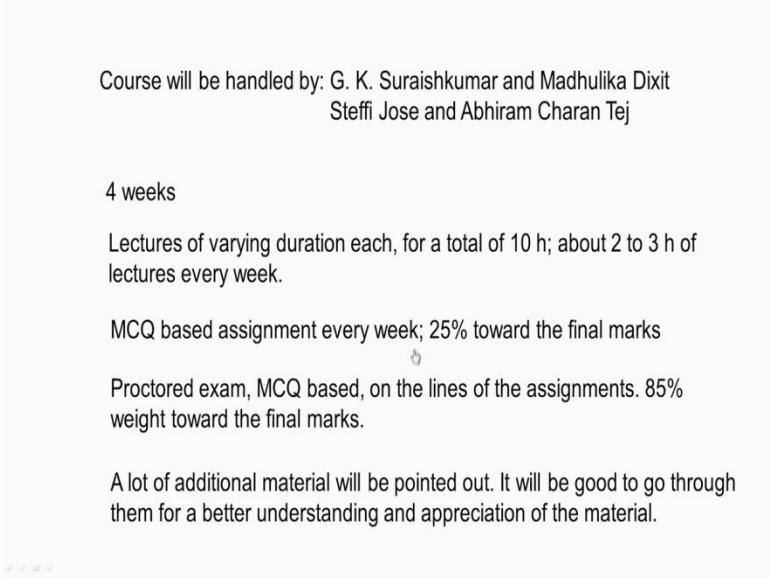


The course will be handled by me, G K Suraishkumar and my colleague, Dr. Madhulika Dixit. I am a biological engineer, Madhulika Dixit is a biologist. So this combination is expected to bring out the best of biology for non-biologist engineers and other non-biologists. You will be ably helped by Steffi Jose and Abhiram Charan Tej. They will interact heavily with you.

This is a four-week course or a ten-hour course. In other words, the lectures by us, Madhulika and I, would be about ten hours long, and they would be of much shorter duration. In other words, each lecture would be about anywhere between fifteen minutes to about forty minutes and all the lectures put together would be about a total of ten hours. And this would be given to you in four installments over four weeks. So that comes to about two to three hours of lectures each week, and we will work it out such that it is easy for you to assimilate, it'll be interesting for you to assimilate and so on.

This is for the evaluation. The learning is much more important than the evaluation is what we believe, but we also know that the evaluation is important for our audience. The evaluation would be two-fold, the first one is through assignments. There will be an assignment every week, typically about ten questions, and all of them or most of them would be multiple choice questions. All the four assignments together would carry twenty-five percent marks, twenty-five percent weightage toward the final marks. At the end of the course, there will be a proctored exam in different locations. This will be on the computer, again multiple choice questions based, and these, this exam would be on the lines of the assignments that are given. And this exam would carry eighty-five percent weight toward the final marks.

And as you would already know, the people who opt to take the exam are eligible for a certificate depending on their performance. I think the details are given in the NPTEL website, they keep changing from time to time. I think it's typically, till about a certain percentage, you get the participation certificate and then you get three other certificates depending on the level of performance. I think the final one has something to do with the gold star and so on. You can look at the details in the NPTEL website. These, questions would be designed such that they are all across the spectrum.

What we usually aim for is that about thirty percent of the questions are amenable to all people who are taking the course, all the participants of the course would be able to answer about thirty percent of the questions. Thirty to eighty depending on the level of engagement, skill and interest would be able to answer and one needs to be really good to get the last twenty percent. That's the way we typically design our assignments as well as the exams. A lot of additional material will be pointed out during the lectures as material that is available to you on the site and so on. It will be really good if you can go through them for a better understanding, and appreciation of the material.

Some of those would just be additional material, some of those we really feel, that means I really feel that you should see those videos and those figures and, (reading) and go through those reading assignments for a good appreciation of the course. Therefore, that would, I would say somewhat mandatory. We may even ask you questions from that; whereas the others that are clearly pointed out as additional material, it is upto you, you can go and read them up for better understanding and so on so forth.

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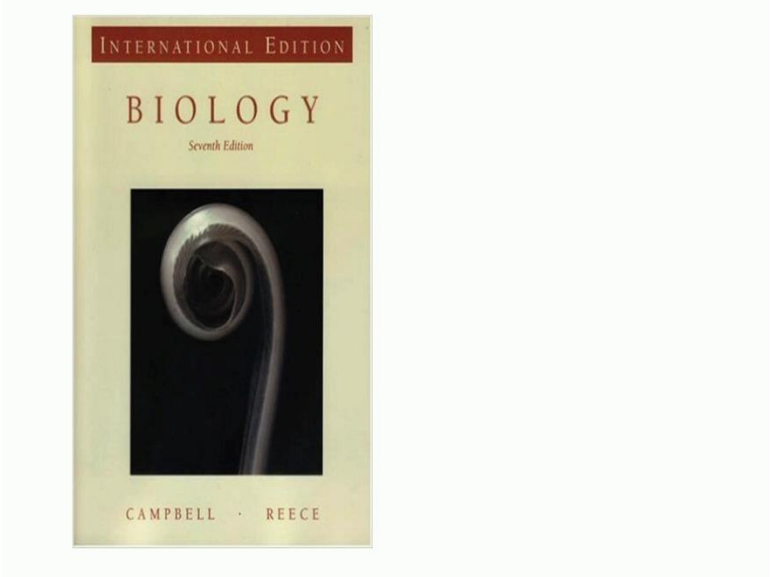


This is, the, cover of one of the reference books that will be used for this course. It's, “Biology” by Campbell and Reece, this is the seventh edition here, this is the international edition; the, country specific editions may look different. This is the seventh edition which came out a while ago. Now we are in the tenth edition. However, I’ve gone through the table of contents of seventh, eighth and the tenth editions. There are differences but those differences will not matter for an initial exposure to biology. Therefore you don’t have to worry about this.

This is another thick book, and any biology book would be a reasonably thick book because of the information that is available now. We will not be looking at all parts of it; we’ll be looking at some chapters and some sections of some chapters in this book. If somebody’s interested, we can point that out to them what chapters we are going to do in this particular book. These are reference books, so we won't be following them in the way they have addressed the subject and so on so forth. We’d be taking information from them.

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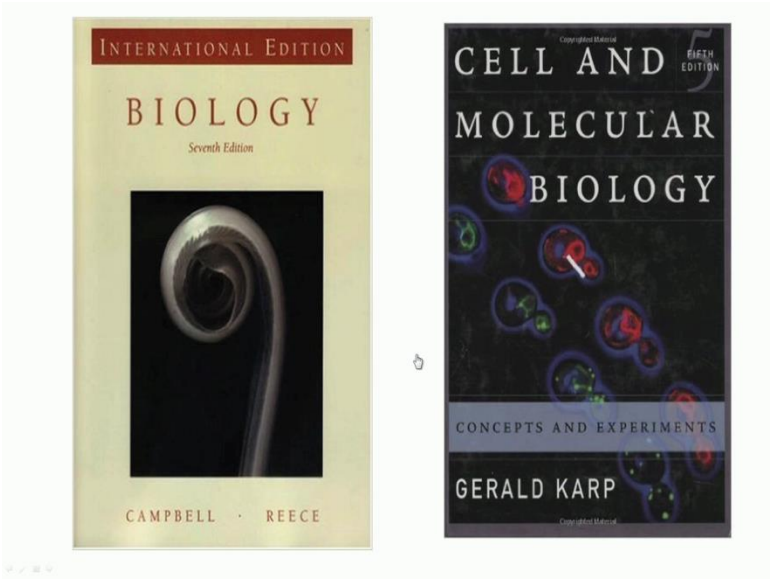


Another book that is also recommended as a reference book for this course is "Cell and Molecular Biology" by Gerald Karp. This is also a nice book; it has a subtitle 'Concepts and Experiments'. It's also a nice book. If you get your hands on these books, it might be good if you read them.

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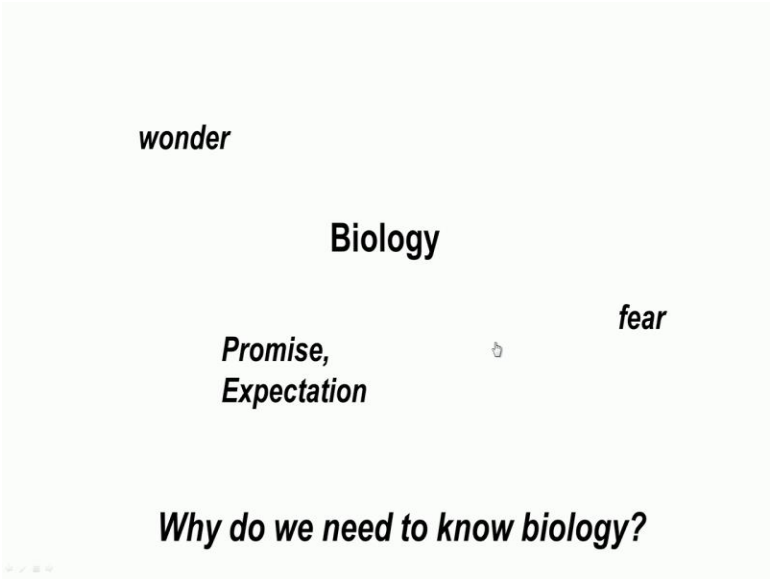


Now, let us start looking at "Biology", okay? When you think of 'Biology', what does it bring to your mind? I'm used to dealing with a certain homogenous set of students, I know what comes to their mind. I'll tell them, tell that to you in a little while. What comes to your mind?

Depending on your background, I think there is a certain aspect of wonder that comes to your mind when you think of biology. There's so much of things that you don't understand, we don't understand. We are fascinated about when we look closer at biology. There's so much to learn from biology and all these aspects are really wonderful. At the same time, if you ask my students, who are typically engineers, they have a fear for biology, mostly because of the way they have been exposed to biology in school, nothing else. So, we have an introductory course here for all engineers, typically in their third semester, now I think it's in slightly later semesters, where we work on getting them, asking, making them getting over the fear for biology.

I'll tell you the reason why. Most likely, engineers in this century may be interfacing with some aspect of biology in terms of the field that they'd be contributing to, and there's no point in fearing that field and it's nothing to fear about. That is another aspect that could come to mind. In any case, there's a lot of promise, there's a lot of expectation from biology, especially in this century.

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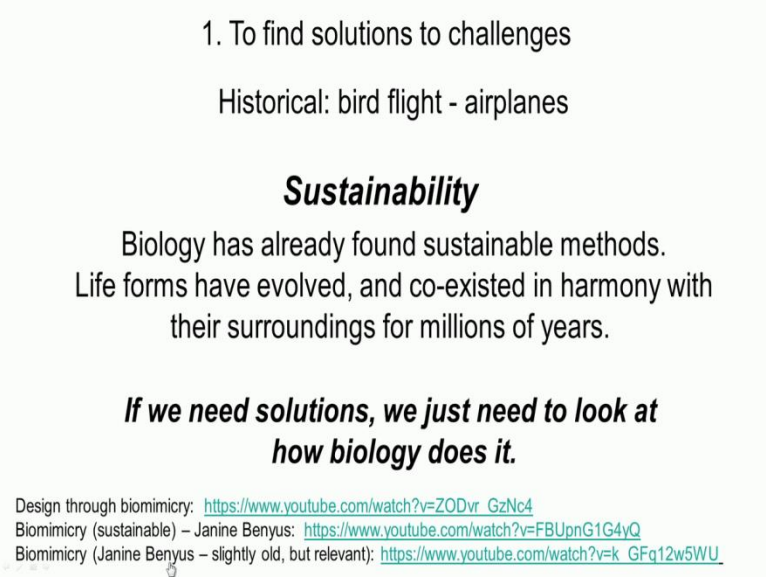


So, why do we need to know biology? First, to find solutions to challenges, that face mankind. You know, historically speaking, probably a century ago, airplanes became possible, atleast according to the history that we know. And, all of us know that the airplanes were inspired by a bird flying. Man saw a bird flying, he or she wanted to fly that way, and therefore made it

possible to fly that way using airplanes. It was not an easy task, but ultimately, mankind got there, and nowadays it's a very standard form of travel that we take for granted.

Sustainability, it's a very big aspect nowadays. And, whatever we do, we like to do in a sustainable fashion, so that we don't spoil the our planet, and leave it for the next generations in the best state that we can. What we normally fail to recognize, is that biology has already found sustainable methods. This earth is probably 4.5 billion years old, primates developed about sixty-five million years ago, mankind, humans developed about fifty million years ago, round about that some million years ago. And, whereas earth itself has been around for billions of years, so about 4.5 billion years, and life evolved may be some billion years ago.

And, over time, biology has found methods to do things in a sustainable fashion. Life forms have evolved, co-existed in harmony with their surroundings for millions of years atleast, or even billions of years. So it's all, all there, we just have to look at it and learn from biology and adopt those practices to be able to lead a sustainable life, and sustainability is a very big challenge in front of us nowadays. So if you need solutions, just look at how biology does it, and it is there and we probably need to adapt to some of those. I would like you to look through these videos. There are three of them, let me list and then talk about them. Design through mimicry is this, there will be a file that is available to you as a pdf file, along with the course material. This would be a clickable link in that file, it will be available with every lecture.

You just need to go and click on these appropriately to go and view these videos or papers or figures and so on, they cannot be included here for obvious reasons. But they are very good videos, the first one is design through mimicry. This talks about design of things that we use, using principles that biology uses, okay? So bio-mimicry, we are trying to mimic biological aspects. So that's a nice small video, may be about three to four minutes long. Then bio-mimicry from a sustainable angle; Janine Benyus, who is a known person in bio-mimicry aspects. This is about again a short video, may be about three to for minutes long. The last one, bio-mimicry, is slightly old but very relevant, okay?

In this Janine Benyus talks about various examples, you know, the whale, she talks about this, one of the whales has a surface which does not allow bacteria to stick and grow on them, okay? And think about that. If we have some such surface, there is no coating, nothing like that, it's just

the nature of the surface, that does not allow bacteria which causes a lot of bad things as we know, infections, that does not allow bacteria to grow on it's surface. So think about it. If we can have a similar surface at the micro scale in our hospitals, then the problem of infection is obviated, right? So, the implications are big. There are beetles which use moisture in the air for their water requirements.

Their body structure or their, the structure of a part of the skeleton is designed such that to, in such a way to capture the moisture from the air. It lives in very arid places and the water that is collected directly goes into it's mouth and so on. And, some similar structures can be used to capture water from, like the humid air around us, especially in Chennai it's very humid. So, if that can be done probably the water shortage can be handled to a certain extent. And it so happens that there are companies doing this already, we may not have, we may not be very familiar with it, but I know of one of my students' fathers having a company which does exactly this, and so on and so forth.

So there are various ways. Let me give you one more example. We have been looking at the solar energy, right? This uses, chemical based solar cells to capture light. In other words, light falls on it, and electrons go out of it. And that's how you get electricity. Okay? Photosynthesis does exactly the same thing in a very efficient fashion, right? So can we get inspiration from the way photosynthesis is done in nature, and translate it to more efficient solar cells? This is something that is being worked on right now. Some level of success, it's a long way before it becomes completely viable and so on.

So there are various different things that you can, various different ideas, various different ways of doing things that we can learn from nature and use it for our own needs. So these three, videos and bio-mimicry would be able to give you more information on that.

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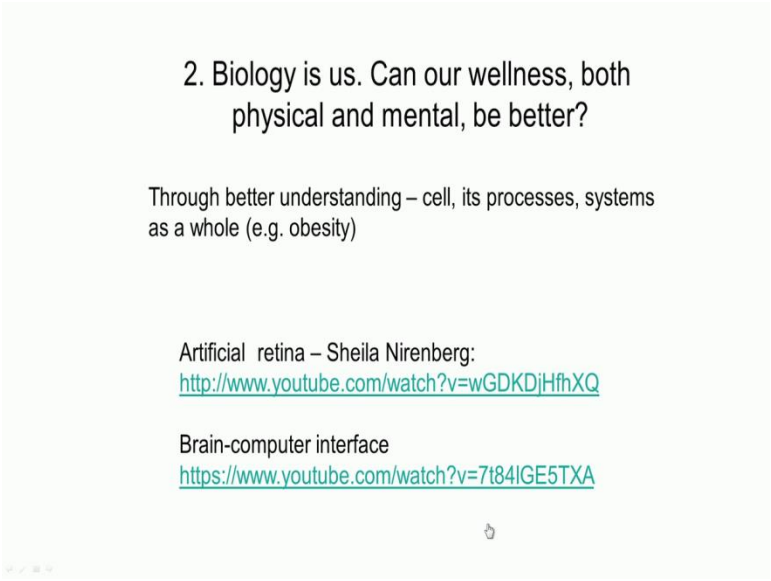


Second reason is, second reason for studying biology is that biology is us, we are all biological creatures. Can our wellness, both physical and mental be better? Surely. We all, all of us would like to feel good you know, be good without diseases, mentally be alert, mentally be mentally feel at peace and so on. Can that be done?

Through a better understanding of biology, the cell, it's processes, the systems as a whole, certainly yes. Even the simple things, such as obesity is not understood properly. You know, why people become obese? It's not a simple calorie counting kind of a situation here, and therefore can we understand that a lot better and so, so as to reproducibly get people to their appropriate weight without a lot of pain, as it is currently being done. There are a couple of videos that I'd like you to see and I say that these are essential videos to see, so were the first three.

The first one is a 2011 Ted talk by Sheila Nirenberg, professor Sheila Nirenberg. This is on artificial retina, okay? This is the link to that which will be available on the other clickable pdf that you'll have. When you watch through these videos, I would like you to see how your field, the aspects of your field are being used to solve huge problems, huge challenges, overcome huge challenges as the ones that are being given here. This is artificial retina which talks about a means by which people with damaged retina could be, would be able to see.

There's a good level of success already in this. This is 2016, a slightly old 2011, so there's a lot of development that has taken place after that, but the basic idea is all the same, the basic

concepts are all the same. If you're an engineer, you could look at what all aspects, is it computer science, electrical engineering, mechanical engineering, materials engineering and so on and so forth that go into making this challenge, or making or addressing, making it possible to address this challenge. You can look at that.

This is about ten minutes long, and the next video that I would recommend is on a brain computer interface. The link is this. This talks about the possibility of a quadriplegics walking again. Just by using the brain, just by using the electrical signals in the brain, whether they'll be able to walk again. There's a lot of development that has happened. There was a well known, or there was a publicity aspect that was also a part of the recent world cup, where a quadriplegic was supposed to kick the football using some such principles. So you may want to watch this, and while you are watching this, think of what all fields of yours are being used here to solve these problems.

And it is because of these, to make these possible, especially, by people like you who would be in several different fields that may not be directly related to biology as you see it. Getting those people to contribute to this, getting those people interested in this, is the purpose of this whole course, or one of the purposes of this whole course.

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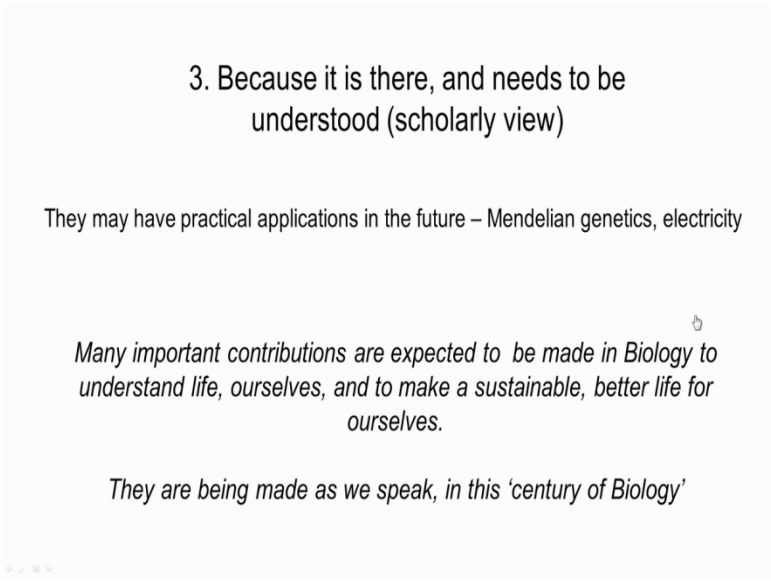


The third reason why we could, we would want to know biology, is because it is there, and needs to be understood. This is what is called a scholarly view, okay? Many somehow don't understand this view, that's okay. But, if you are oriented that way, you would already have a feel for it. You may not still accept it but you will certainly have a feel for it.

And with experience, you know that it is a very valid and a very high level kind of a view. Okay? Because it has significance over decades or probably even centuries. That is because you know we tend to appreciate something that has an immediate practical application. And such a view may not have an immediate practical application, you never know. Sometimes, something that seems very, scholarly, you know, you you want to understand it, you want to learn it just because it is there could have an application within a year as has happened in the past. And something like this, you know, we will be looking at Mendelian genetics. It's not a very standard or classic example of this kind, but Mendelian genetics is something that we would look at in a later lecture.

This was developed by Gregor Mendel, just because it was there. He was a monk, he wanted to study plants, and by studying plants, he came up with the essence of inheritance. How human beings inherit things from their parents, often their grandparents, and so on, the principles of that, and that has become huge now, may be a century later, (much) much more than a century later to be able to predict whether a disease would occur in a certain child, and so on. A classic example

is electricity. When electricity was found, people said all this is fine, but, is it really going to be useful?

And all the, all that the people could say who were involved with electricity is yes, may not be now, may be much later. And we all know what electricity is nowadays. We cannot even think of a life without support from electricity. So many important contributions are expected to be made in biology to understand life ourselves, and to make a sustainable better life for ourselves as well as our future generations, and those contributions are being made as we speak in this century for biology, or century of biology. Now let me, get a little deeper in this introduction itself.

I usually ask this question to our students, our engineering students, "How many of you have done biology in twelfth standard?" This is the first question I asked students, whenever I handled this course with my colleagues, and typically about, five percent, or less than five percent would have done biology in their twelfth standard. Even in our own department -biotechnology, about ten percent would have done biology in their twelfth standard, ninety percent would not have. And in the case of, all departments put together, all engineering departments put together, about may be ninety-eight, ninety-five to ninety-eight percent would not have done biology in their twelfth standard. Okay?

That is because for entrance into the bachelor's programmes here, they are tested on mathematics, physics, chemistry, and probably, yeah these are the main things that are tested, and therefore they don't need biology. Not just that, there are other social aspects that, kind of, straight-jacket students into some fields, which may not be really, desirable for the overall growth of the country. And, when, such students are addressed, there is usually a preference in them for certain subjects, which is certainly not biology.

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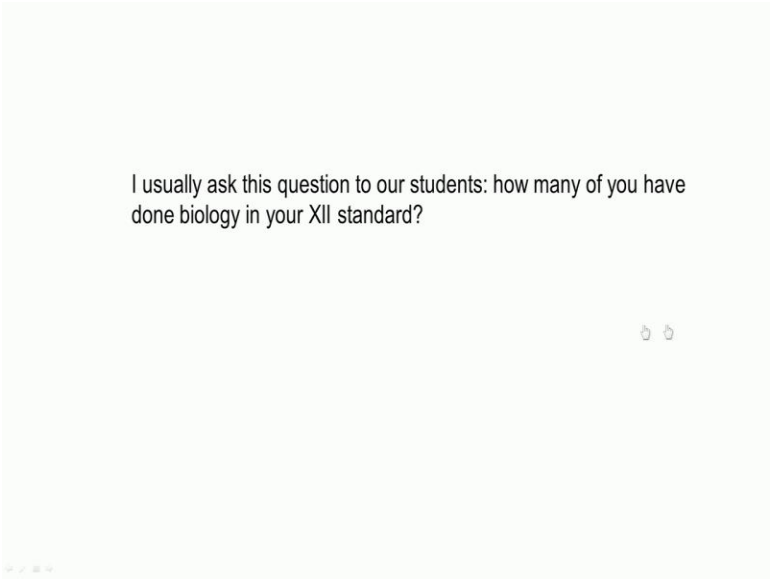


And many of them like mathematics a lot, irrespective of their own capability level in mathematics, they all like mathematics a lot. And, so, I asked them this question.

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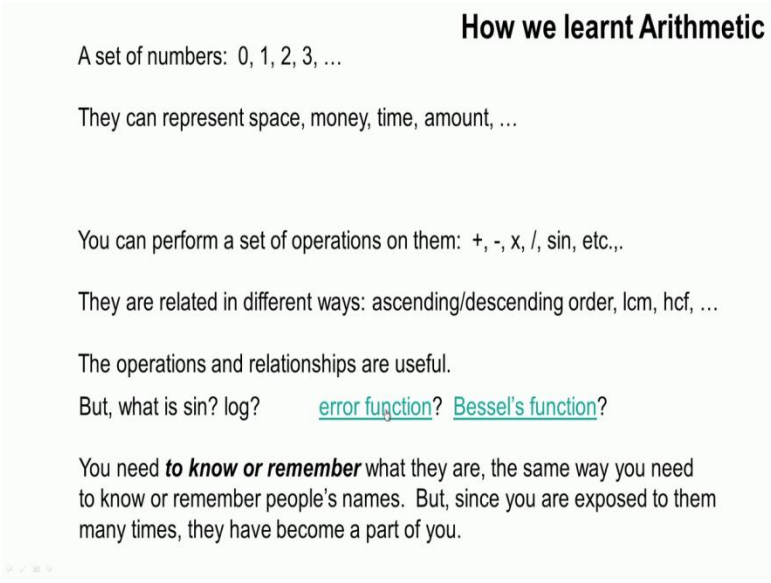


Let us think about how we learnt arithmetic, in mathematics. This is way back, may be in your, first standard, first grade, second grade and so on. There are a set of numbers, let's say, 0, 1, 2, 3 and so on. Initially when you think of numbers, you start from zero, one, two, three. They can represent many different things, they can represent, let's say the space that you live in, the area,

the volume and so on, the amount of money that one has, the amount of time that one has, the amounts of so many different things that are relevant to humans.

You can perform a set of operations on them, you can add two numbers together, you can subtract one number from another, you can multiply two numbers together, you can divide one number by another, you can; if after a certain while, you can take the sine of certain numbers, cosine of certain numbers, and so on so forth. You can perform a set of operations on them, and, you can also see that they are related in different ways, you could order them in an ascending descending order, if you have a set of numbers, there could be a least common multiple among them, there could be a highest common factor among them and so on.

And these operations, you know, these are the operations, these are the relationships, they are useful to us. For example, if you have five apples and four apples, together they make nine apples, right? And, if I have, let us say, hundred rupees, if somebody borrows twenty-five rupees, I have seventy-five rupees left. That would be subtraction. Similarly, multiplication, division sign when you have, cyclic occurrences and so on. And similarly, there are relationships that could be helpful, useful. But, what is even sine? This probably sine is learnt in your sixth, seventh, may be seventh, eighth standards, or may be ninth standard. And what is log? Which is again learnt sometime in high school.

When you start thinking about this, you start slightly doubting the level of comfort you have with numbers, okay? How did we kind of, look at this earlier, okay? Sine, log, probably ninth standard. Leave alone these. What is an error function? Okay. That's also mathematics. What's a Bessel's function? That's also mathematics, heavily used in engineering and so on so forth, okay? And when you think of these things, we deconstruct and deconstruct how we learnt arithmetic, then we come to realize something, you know? Whatever we feel so comfortable with, you know, performing a set of operations on numbers, and knowing the relationship between numbers and so on and so forth that we are so comfortable with, became comfortable because of repeated doing of the same thing with those numbers, nothing else, okay?

But, when, you are exposed to them for the first time, error function, Bessel's function and so on and so forth, you need to know or remember them, what they are, the same way that you need to

know or remember people's names, okay? There's no difference. But since you are exposed to them so many times, they become a part of you, okay? That's all. There's nothing else. Okay?

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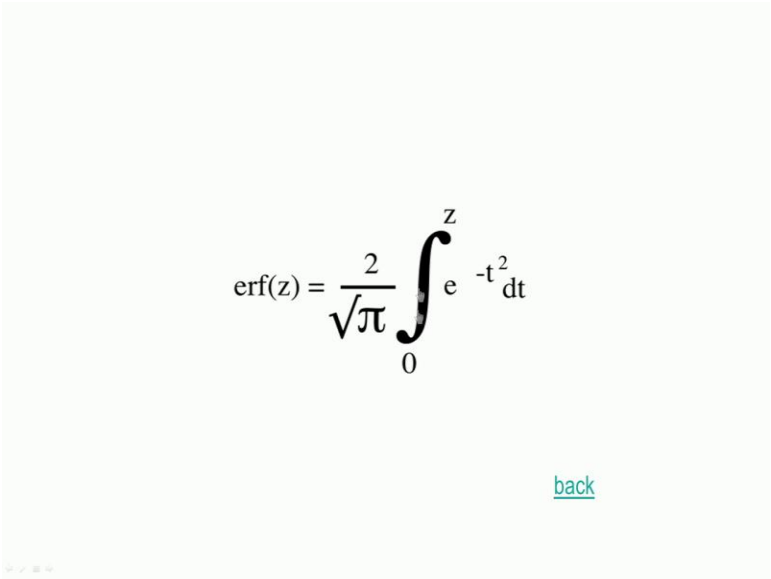


For people who have an interest in maths, you would like to know what an error function is. It is this, okay?

$$\mathrm{erf}(z) = \frac{2}{\sqrt{\pi}} \int_0^z e^{-t^2} dt$$

And, let me show you what a Bessel's function is,

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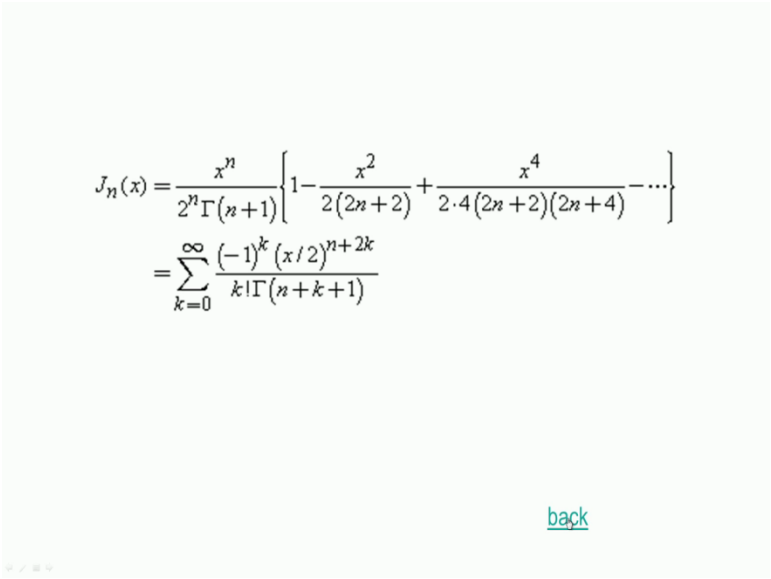


It is this. Okay? So you can look through the details of the Bessel's functions. There are Bessel's functions of many kinds and so on so forth. They are very useful in some aspects of engineering, including biological engineering. Right?

And therefore, knowing and remembering and recalling and so on so forth is no different from the way biology is done largely in schools, right? And arithmetic, when you started out, was done that way. It's just that you have done, repeatedly so many times, that you become very comfortable in that. Right? And so there is probably no basis in terms of learning, to place maths here, physics here, which people normally do, our students.

And then chemistry here, and biology hmmm, right? There is absolutely no basis to do that. And, biology, biological engineering, both have a lot of mats in them, as of now. A lot of quantification has come in, and there's a lot of math even if you like math, and you would be able to contribute a lot in biology even if you are a pure mathematician.

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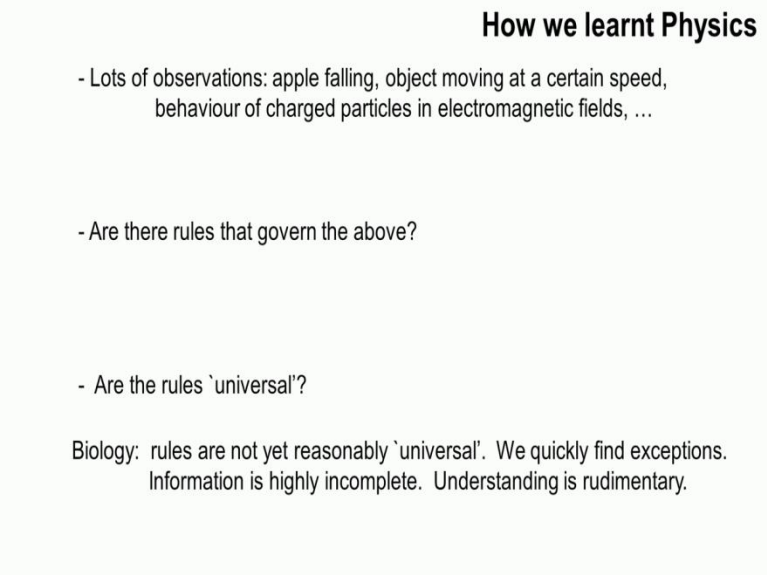


Let me go to physics now. How did we learn physics? Lot of observations, apple falling, object moving at a certain speed, behaviour of charged particles and electromagnetic fields, and so on. Are there rules that govern the above? Yes.

That's what physics is all about. Are the rules universal? Mostly yes, okay? There are a few exceptions, but mostly yes. But in biology, the rules are not yet reasonably universal, okay? We'll quickly find exceptions. Information is highly incomplete, understanding is rudimentary, and since it is a new science, there's a lot of information that one needs to rely on. It's changing as we speak. They'll all be, the information will be put into nicely understandable packets, and then it becomes much easier to manipulate them, right? So, you need to keep this in mind when you are looking at biology.

So what we thought we would do, is pick up some aspects of biology, that, one would need to know as basic information, as to how life formed, how life evolved, they are very interesting aspects which could have a relevance to some of the things that we're dealing with nowadays. And the very fundamentals of biology, the basic biomolecules, how they interact with each other to certain extent may be. Some genetics which are, which is helpful in, predicting diseases and some aspects of DNA, RNA, and so on so forth. Okay, we'll give this to you as a ten hour module, and that would equip you with some level of biology with which you can learn further with ease and also start applying with ease. Hope you enjoy the course, with Madhulika and I, we will alternate our lectures here, and see you soon. Bye.

**Biology for Engineers and other Non-Biologists**
**Professor: Madhulika Dixit**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Lecture Number 02**
**Origin of Life**

Hello and welcome to this course called “Biology for Engineers and other Non-Biologists”. My name is Madhulika Dixit and I am a faculty at Department of Biotechnology, and I am taking this course along with my colleague, Professor G K Suraish kumar. Now when this idea of taking this course for engineers and non-biologists was brought up to me, the idea was to essentially bring in biology, teaching biology at a level which will be easily taken up by the non-biologists. So for those students who have already taken some sort of a course in biology, a lot of things will sound very fundamental. However, the whole idea of this course is to essentially highlight the basic features of biological life.

Now, most of the times when I have taken these kind of courses with engineering students, I have always noticed an apprehension in terms of trying to learn a biological course, and the reasons that I have gotten more often from them is that it's a subject which requires a lot of memorizing. I would like to bring out to the students here that indeed there are lot of terms which seem very difficult, but I would also like you to understand that biology is at a very infant stage, in comparison to, for example physics or chemistry, where the logics of chemistry and physics are very well defined. For biology, we are still trying to understand those logics.

Hence, in this course, we’ll try to understand the logics of how a biological life is governed, and what is it in terms of reactions, in terms of physical entities, which help a life to survive and sustain. So let me start this course with my first lecture or rather first set of slides on origin of life. Now, life, or origin of life is a very intriguing topic because for a very long time, we still haven't been able to answer the question as to how life really evolved. But before I get into the actual topic of today, which is origin of life, it's important to understand what are the features of life. What is it that you call ‘life’ and how is it sets itself apart from non-living things.

Well, anything that you see around yourself which is living, you will notice that there are certain features which are fundamentally common across living world, whether you start from a bacteria, you go to a plant, you go to a fungal organism or you go all the way to human beings,

you find that there are certain features of life which remain constant, and the most important of them all is the ability to replicate and reproduce. Now this is one critical feature which sets the living world separate from the non-living world. But in order to do that, in order to sustain it's survival, in order to pass on it's characters to it's progeny or it's off-springs as we call it, an organism goes through a whole lot of processes, and these include it's ability to utilize food, it's ability to synthesize food if the need be, it's ability to digest the food, it's ability to throw out the waste material and keep the machine going.

So in other words, life or any biological system is a very highly dynamic system, and it has it's sole purpose of surviving and reproducing. So, this is one character of life which is very unique, and we are still puzzled and we still don't have a complete answer as to life, how a life really evolved on this earth. But, lot of studies done by geologists, by archaeologists, by molecular biologists, have noticed that there are enough proofs as of today, with which we can confidently say or at least speculate that the present form of life has evolved from non-living things. In other words, we have essentially evolved from chemical reactions.

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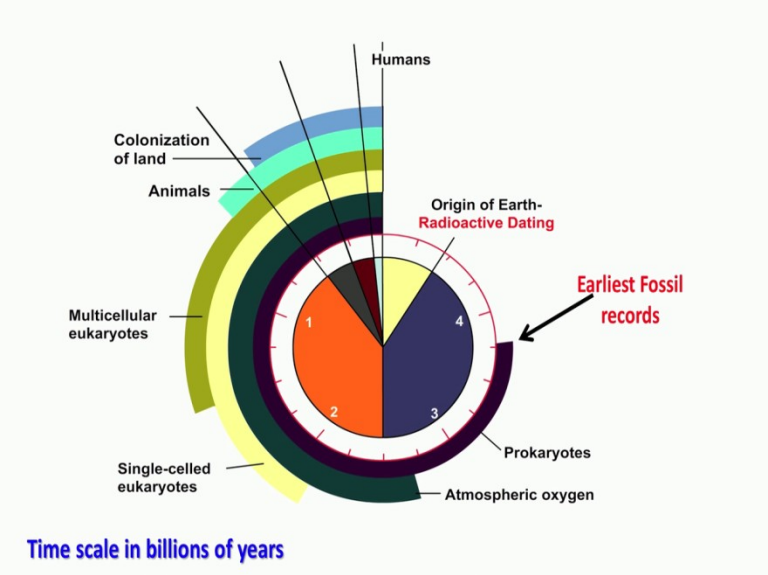


So, let's get to how the history of earth is, and how life really evolved during this history of earth. Now if you notice in the slide, if you were to look at the life of earth as a whole, and here I've drawn a clock and the time scale or in terms of billions of years, what you'll notice is that the radio active dating estimates that the origin of earth happened somewhere close to four point

eight billion years ago, and, the earliest fossil records which suggest existence of life forms is about a billion years later. So, it is possible for us to speculate and now you have enough proofs through fossil records that the life must have originated within seven hundred to eight million years of earth's existence.

So, how did the life evolve is something that we'll talk in this class today, but I want you to understand that a lot of this was initially a set of chemical reactions, and these set of chemical reactions were actually possible because the earth's atmosphere was highly conducive for such reactions to happen. And mind you, at that point of time in the early life of earth, the atmosphere of the earth was very different from what we see of today, which is essentially full of atmospheric oxygen. So in other words, the initial phase of earth's history, it had a highly reducing environment. And I'll come back to this when we talk about experiments which actually go on to prove how the initial biological molecules actually got synthesized.

So, the earth seems to be about 4.6 to 4.8 billion years old, the earliest fossil records are somewhere about 3.6 to 3.8 billion years old, and somewhere in between the life really evolved. And what we also observe is one of the earliest forms of lives are something which we even see them today and those are the bacterial forms. So it's interesting that something which evolved or which came into existence more than three billion years ago, has sustained it's survival for the last 3.5 to 4 billion years.

And in the process, the Prokaryotes have also evolved and has given rise, is what we think as of today to different forms of life. So we'll come back to this and see it in a step by step fashion.

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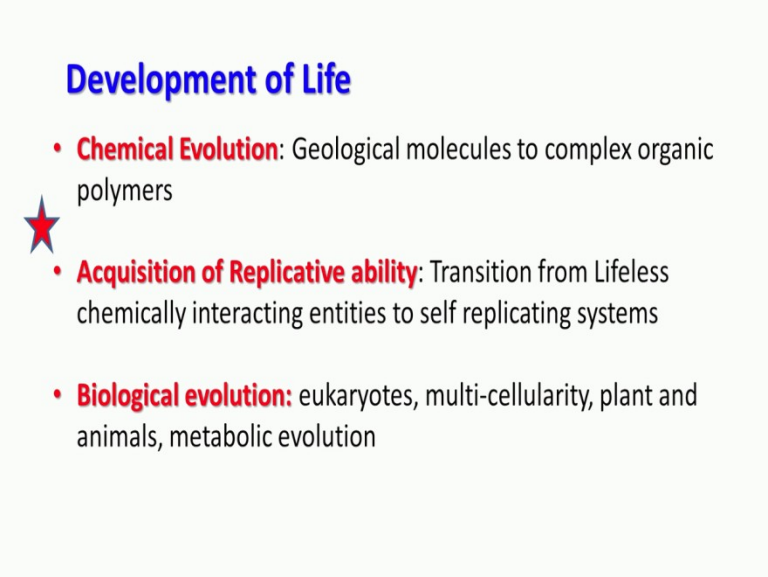


So, for simplicity, what we understand today or rather, based on the various evidences that we have from geological excavations, from chemical reactions, from molecular biology techniques, for simplicity's sake the biologists believe and have divided this very process of life and it's existence or origin into three different phases. The very first phase is of chemical evolution. Now this must be the phase which must have evolved during the very early stages of earth's life, when the earth's crust was very hot, the material has still not cooled down, the oceans, the springs, the hot pools were still boiling, the atmosphere was highly reducing.

And, it is during this phase that probably some sort of geological complexes or molecules would have interacted to form the initial and the early very building blocks of organic molecules. We'll come to this. Then came the most important feature which kind of sets apart life from chemistry, and that is the ability of these molecules, somewhere during the course of history, to develop into a property where it can replicate itself.

Now this is one of the most critical features, I would say, in terms of the origin of life, because it is at this stage you would find, that probably the system changed from just a set of chemical entities into developing a property where they could self-replicate.

And then, comes the last phase which would probably correspond to the rest of the phase all the way from here till what we see present today, is the evolution. And, what do we mean by evolution; it was just initially a set of chemical entities, all sitting together, these set of chemical

molecules must have evolved an ability to self-replicate, and then sooner or later, these entities would have enclosed themselves into organism-like entities, which were the initial prokaryotes, and then the prokaryotes would have evolved into much more complex forms of life, which is what you call as the eukaryotes, a single-celled organism to a multi-cellular organism to plants, to animals, and then, to the present day human beings.

So, in that sense, for simplicity, we kind of divide this development of life into three phases, chemical evolution, acquisition of the replicative ability by life, and the evolution of these early forms of life into what we see today as complex organisms and complex plants.

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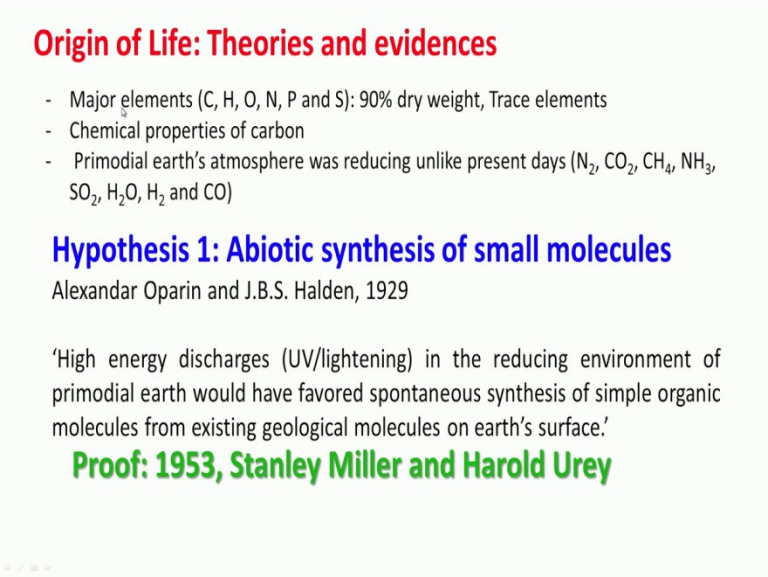


So let's go to what are the various theories and evidences for origin of life. But before I get into the theories and evidences of life, I want to highlight certain things, which are very important to understand how these theories were built up. If you were to look at any living organism as of today, we all know that our, seventy percent of our body weight is made up of water.

But if you were to just account for the dry weight, you'll find that the ninety percent of a dry weight of any living being essentially consists of these major elements – the most abundant being the carbon, hydrogen, oxygen, nitrogen, phosphorous and sulphur. In addition to these, you do have some trace elements like iron, copper, zinc; but in terms of bulk quantity, the bulkiest, or the one which is available in most quantity is the carbon, and that should not be a surprise looking at the chemical properties of carbon, because carbon has a versatile ability to form

covalent bonds, not just with itself, and thus lead to a infinite long chains of organic molecules, it can also form covalent bonds with other elements, like hydrogen and so on.

So in that sense, carbon seems to be a very nice element. And, it should also be noted that when geologists went on analyzing the chemical composition of the early rocks of earth and the meteorites, which continue to keep hitting us, they were found to be very rich in carbonaceous compounds. So clearly, the earliest building blocks were formed because of carbon. The other important thing to note is that the initial earth's atmosphere, which is what we call as the primordial earth's atmosphere, was highly reducing. As I mentioned earlier, it is in, in the initial seven hundred to eight hundred million years, you would find that the earth's atmosphere was highly reducing because of presence of nitrogen, ammonia, methane, carbon dioxide, and it actually did not have any atmospheric oxygen.

So, what was the first hypothesis? With this background in mind, the first hypothesis was put forth by Alexander Oparin and J.B.S Halden in 1929, and they said, that the very first molecules, or biological molecules would have arosen, because of the abiotic synthesis of small molecules. So the first set of sugars or amino acids would have been formed by a mere abiotic synthesis of these molecules. And how it would have been possible? According to them, in that initial reducing environment of the earth, any high energy discharge, either in the form of ultra-violet lights, or in the form of lightning would have favoured spontaneous synthesis of simple molecules from existing geological molecules on earth's surface.

Now this was just a hypothesis way back in nineteen twenty-nine and twenty-four years later, in 1953, it was actually demonstrated to be true by Stanley Miller and Harold Urey.

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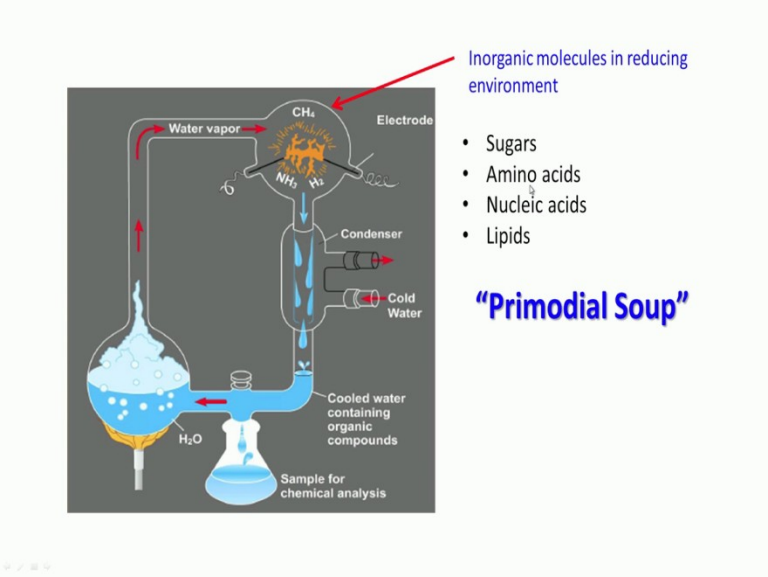


So what did Stanley Miller and Urey did is that they tried to create this kind of a primordial earth atmosphere, or the primordial soup, in this case I mean the early forms of oceans on earth which must have been at a very high boiling temperatures, and created that in a laboratory setup. So they had a primordial soup kind and they had an ocean kind of environment at a very high temperature, which is the boiling water.

They collected the water vapour under a very reduced environment consisting of methane, ammonia and hydrogen. Now in this reduced environment, when the energy was supplied, to mimic ultra-violet radiations or lightnings through electrodes, whatever was being spontaneously generated was then eventually condensed by the means of a condenser, and then collected at the collecting pole, or the collecting conical flask. And when they analyze the composition of this cooled water, they found to their amazement that it consisted of a lot of amino-acids that we even see today, such as alinine and glycine, sugars, nucleic acid, and nucleic acid bases like adenine and lipids. So this was one of the earliest proofs to the hypothesis which was put forth in nineteen twenty-nine, that the early forms of biological molecules must have had an abiotic synthesis.

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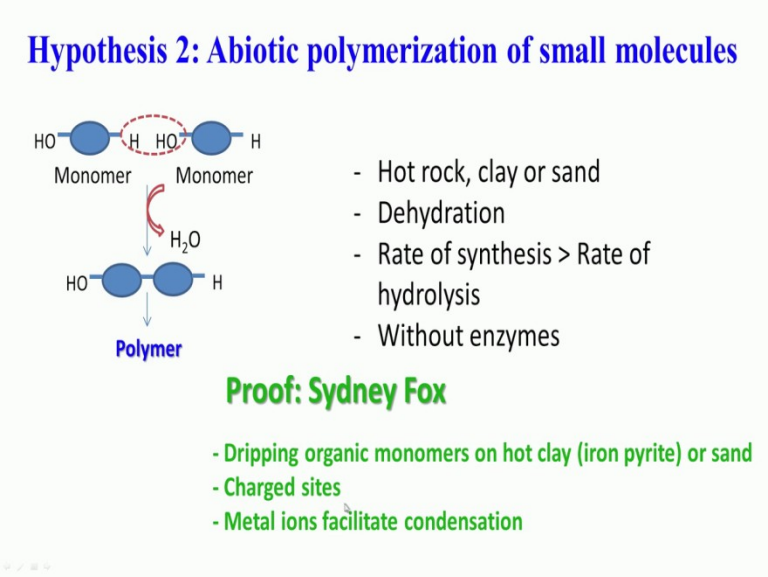


But then, just having small molecules is not enough, because if we were to look at the present day life, you find that you have macromolecules, and not just smaller molecules. For example, if you look at the plant cell, the outer covering of the plant cell is the cell wall, and we'll come to it when we're talking about cell structure and cell function is actually a polymer of glucose, which is made up of, which is what you call as cellulose, and these are huge polymeric molecules.

So how is it that these abiotic molecules eventually went on to polymerize and form higher order molecules? So the second hypothesis is that during the early phase of earth, when the earth's crust was still very hot, you find that there were hot rocks, clay or sand, and under such hot conditions, it was very easy for one monomer to interact with another monomer, through the process of dehydration. And since there were sufficient carbonaceous material available in the earth's crust, the rate of synthesis was much much higher than the rate of hydrolysis. So the postulation is that after the abiotic synthesis of monomeric units, there must have been spontaneous polymerization due to very high rate of dehydration and that is simply because of the presence of hot rock, clay or sand which will promote this chemical reaction.

And the proof of this was then supplied by Sydney Fox where he went about dripping actual organic monomers onto a hot clay. To be specific, on iron pyrite or on sand, which do have these charged sites, and he found that these monomeric molecules would eventually join together to form polymeric molecules, and in the process it is the metal ions which are helping or facilitating

the process of condensation. So, we do know, or we do now believe that yes, the initial set of biological molecules were from abiotic synthesis, then you ended up having polymerization of these molecules, and then the question is, 'When did the replicative ability begin'? Because all this while, we are just talking about chemical reactions which are happening in a primordial earth, which has got a highly reducing environment, still doesn't have atmospheric oxygen, and it still has a very hot surface.

But, as I told you in the initial part of my presentation, what sets apart life from the non-living things is the ability to replicate. At this time, we don't really have the clear proof as to how this change happened from a non-replicative to the replicative form and life, but, if one were to look at which set of molecules would have been the ideal initiator of this process, we now have sufficient proof or suggestions which suggest that the earliest genetic material must have been RNA.

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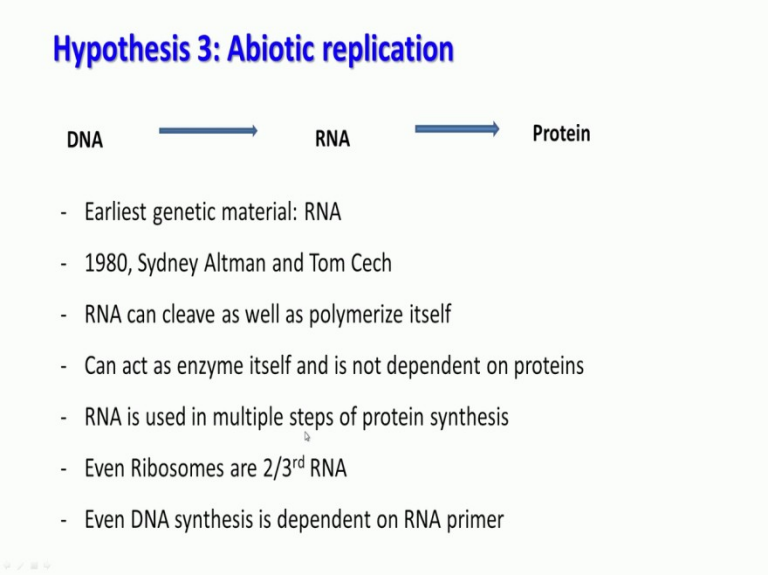


Now before I get to why we think it would have been RNA, it's important to understand the flow of information in a living world. And we all know, and that in our system, any organism for that matter, most of the information is encoded into what you call as the DNA, as of today. And this DNA is then transcribed and this process is called as transcription into RNA, followed by the actual work horses of your body or a cell, which is the protein. But, if one were to look at RNA.

so we will discuss DNA to RNA, the process of transcription, RNA to protein, the process of translation, in subsequent videos.

But for today, we'll focus our attention on RNA. So, there are enough proofs which suggest that RNA might have been the earliest genetic material, and this was also supported by the recent findings in nineteen eighties, where Sydney Altman and team, that RNA can not only polymerize, it is also has the ability to cleave itself. So, it not only acts as a molecule which can polymerize itself, it also can act as an enzyme, and this catalytic activity of RNA is not dependent on proteins.

So it's a very versatile nucleic acid molecule in that sense, that it not only can polymerize itself if the need be, it can cleave, and if the need be, it can also act as an enzyme. What is even more interesting and intriguing is to note that as we go into the details of protein synthesis, multiple steps in protein synthesis are dependent on RNA. For example, the actual machinery which puts this entire chain of amino acids into a protein which you call as the ribosomes, is chemically made up of RNA. Two third of ribosomes is made up of RNA. And not just that, even in today's world, where DNA, the so-called our genetic material which has the entire coded information.

The DNA needs to replicate, it does depend on RNA, and I'll talk about this, when we talk about the process of DNA replication. So we still don't have concrete proof, but all these properties of RNA make it a very interesting molecule for it to be probably the earliest molecule capable of acquiring the ability to replicate.

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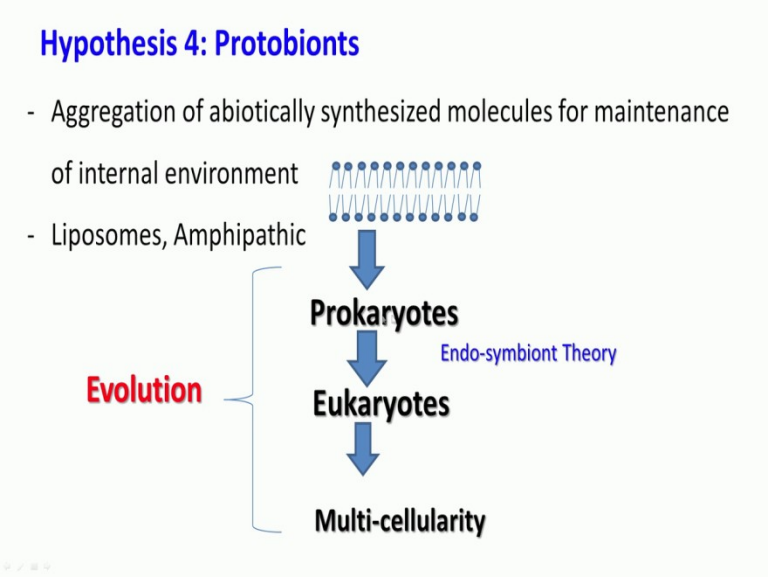


Then the fourth theory is, ‘how did cells come into existence’? I mean all this while we are only talking about chemical synthesis of monomers, their polymerization to larger forms, hopefully acquisition of replicative ability. But then, how do you get to cells? And, this is where we have to consider that slowly, as the time went past, chances are the sources became skewed, lesser and lesser.

There was far more entity for these cluster of molecules to kind of guard and maintain their properties, and in the process what would have happened is that lipid molecules would have assembled like liposomes. These are amphiphatic, in the sense that they do have a polar head, and a non-polar tail, and these, it's like you take a soap solution and when you drop it in water, these lipid molecules will end up forming bubbles. So these initial lipid liposomes would have somehow enclosed these abiotically synthesized self-replicating biological molecules to form the first and the most earliest form of life, which is what you call as the protobionts.

And somewhere down the line, the protobionts would have then evolved into prokaryotes. But mind you, this time scale of change from abiotic synthesis all the way to prokaryotes has not happened overnight, it has happened over millions of years, and then, the earliest form of prokaryotes would have evolved into what we know as eukaryotes, we’ll talk about this when we talk about the cell biology, and would have finally evolved into what we see today as multi-

cellular highly evolved organisms. So this journey, all the way from protobionts to the present day organisms, we'll cover them in the, in the topic of evolution.

But for now, so you find that there have been four hypotheses, abiotic synthesis of small molecules, polymerization of small molecules, ability of the small molecules to replicate, and then, the enclosure of these biological soups, or these biological molecules by means of lipid molecules, and probably the earliest forms of plasma membrane. But then, there has been a very important and one of the most crucial turn of events in history of earth, and that has been the oxygen revolution.

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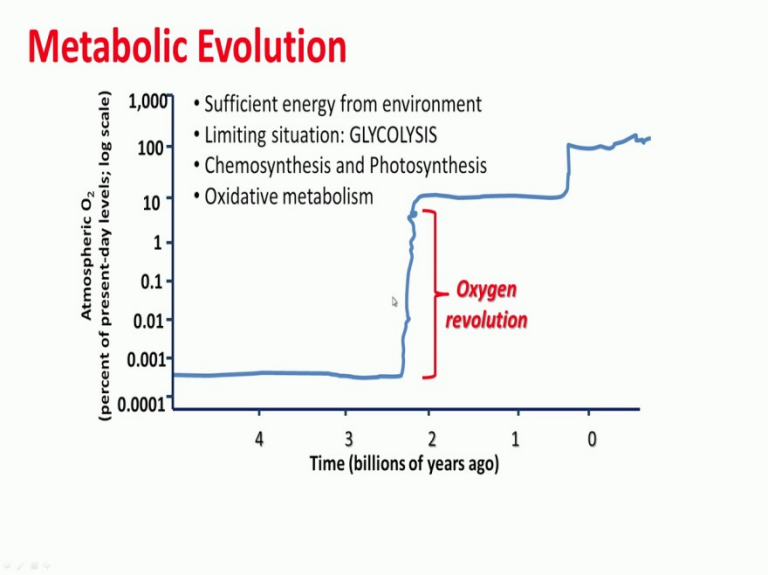


So if you see this curve where I have plotted atmospheric oxygen at the present day, right? You find that the earlier earth had very low oxygen. Almost for the first two and a half billion years, you find that there was hardly any oxygen in the atmosphere.

Something must have happened at this turn, which would have led to the sudden burst, and if you noticed, within a few million years, from hardly any oxygen to reach a point where the atmosphere becomes highly oxidized. And it's at this stage, we believe in earth's life that the plants or the photosynthetic organisms must have evolved. And the reason must have been pretty evident, the reason being that by this time, by the time of two and a half billion years, resources must have become lesser, it would have compelled the organisms to survive as I said, one property of life is to survive.

So if the resources are getting lower and lower, all that pool of carbonaceous inner compounds, energy rich phosphates, if they are getting exhausted, the organism, since it needs to survive, has to find alternate means of obtaining this energy, and one possible way by which it would have done that would have been to use the reducing power which it did, as per our understanding in the initial phases using hydrogen sulphide. But then even hydrogen sulphide would have got exhausted and then, the organism must have developed a much smarter strategy of actually utilizing the unlimited pool of energy from solar energy or from the sun and hence the process of photosynthesis evolved.

And in the process of this, oxygen became a by-product and as a result the atmosphere becomes highly oxidized. Now, if the atmosphere has become highly oxidized, there are still ways by which the organism has to derive energy by breaking down it's larger molecules into smaller entities. Earlier it was fairly easy. But now in this oxidized environment, there had to be a specialized structure possible to efficiently do breakdown of these polymers.

The release of energy, and that is where it is postulated that the mechanism of what you call today as respiration must have evolved. So in other words, we started the initial life on earth in a highly reducing environment, and somewhere around I would say close to about two point six billion years ago, the transition must have happened where the organisms became smarter, started utilizing the solar energy, and the chemical energy from other reducing compounds like hydrogen sulphide, and in the process, they started generating oxygen as a by-product, because of which now the atmosphere became highly oxidized.

So the new set of chemical reactions had to evolve which under these conditions, oxidizing conditions could still breakdown polymers into monomers for release of energy, and that is somewhere here probably, that the evolution of mitochondria would have happened.

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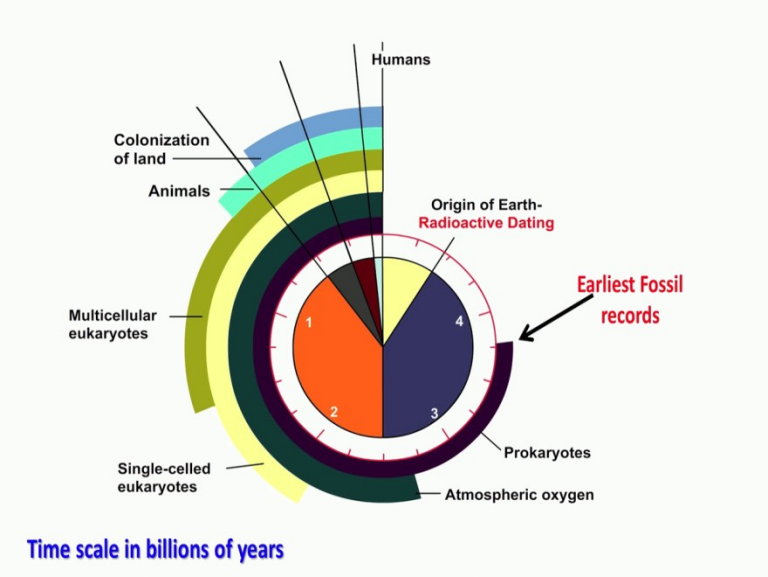


So let's come back to the slide which I was talking about. So this is where we think the origin of earth happened. This was the phase, I would say all the way up to here, you find that the earth's atmosphere must have been highly reducing, and then, you have evidences of early life, somewhere here, which says about seven hundred million years after the earth's origin, the earliest protobionts must have evolved somewhere here, later transitioned into what you call as the prokaryotes and then due to lack of sufficient high-energy compounds available and the resources becoming lesser and lesser, a point in evolution would have happened where the organisms would have developed a property of synthesizing their own food, using solar energy and in the process, went on generating oxygen as a by-product.

So it is at this point somewhere in earth's life that the earth's atmosphere became highly oxidate. And as a process evolved, the prokaryotes ended up becoming well formed, which is what we call today as eukaryotes, we'll talk about this, and then somewhere, as it's life became more and more complex, these single-cell eukaryotes went on to become multi-cellular eukaryotes, plants, animals, and then finally, the humans.

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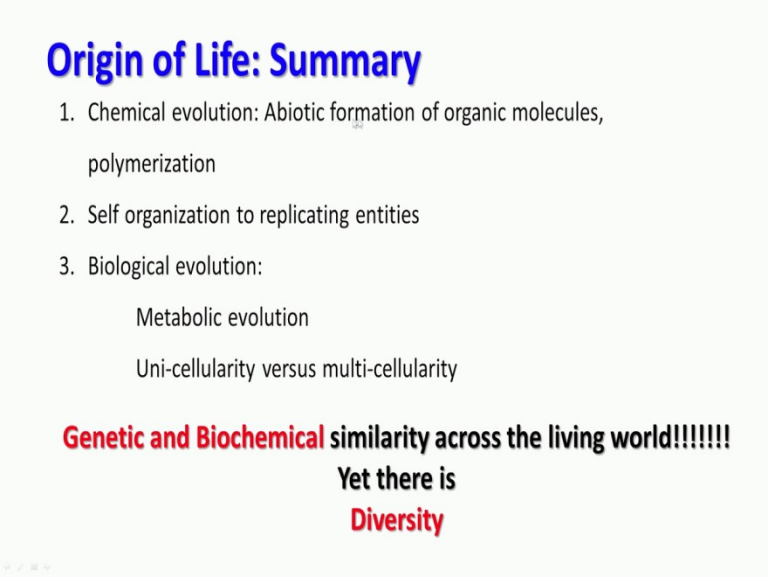


So, to summarize, what we understand today, is that the origin of life essentially is divided into three stages; the first stage is the stage of chemical evolution, which is abiotic formation of organic molecules and it's polymerization; the second is the self-organization, and the ability of these polymers to somewhere develop the property of replication and formed the early protobionts.

And then came the actual process from where the life went on evolving again over billions of years, and these evolutions were in terms of evolutions in metabolic reactions, what I mean by metabolic reactions are the chemical reactions which happen inside a living organism, is how we call as metabolism. These metabolic reactions to allow an organism to now synthesize food, and having synthesized food, also have the ability to break down the food if it needs to have some energy, and eventually transition from a single-celled organism to a multi-cellular organism.

One point which still puzzles, and is still very intriguing in life, and study of life is, that despite the varied forms of life that we see today, what we really find interesting is that when we look at some of the fundamental chemical reactions in biology, what you call as metabolic reactions, or the way the information is coded in our DNA, genetic information, you find that right from prokaryotes all the way till humans, a lot of these informations and the processes are conserved.

In other words, the codes by which the messages are stored in DNA, the information is stored in DNA, you find that that language which is used for coding, or information has remained more or

less uniform all across life, starting from bacteria all the way from human beings, and yet, they are highly diverse when you look at our forms and our phenotypes; phenotypes are our physical appearances, and we'll try to answer some of these questions in our next class which is on evolution.

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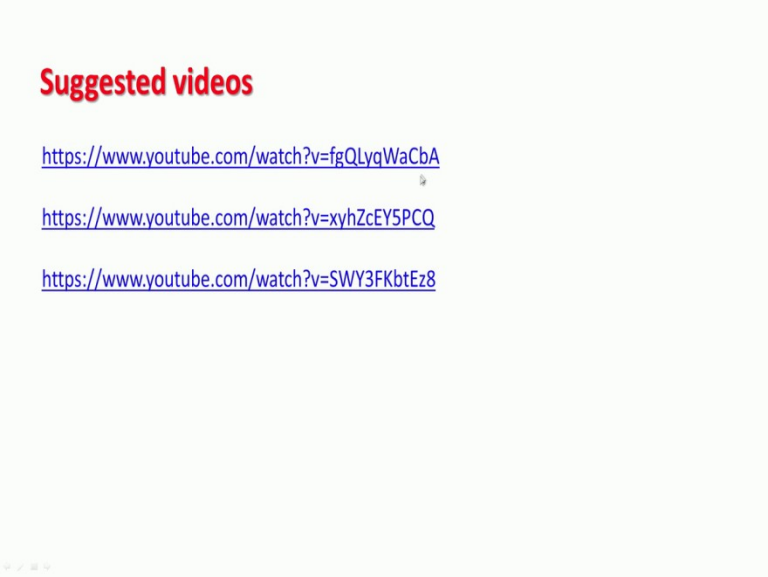


So with that, we'll end origin of life, I would urge you to go through some of the very informative videos, which very explicitly and beautifully through animation also try to explain you how life must have evolved on earth. Thank you.

**Biology for Engineers and other Non-Biologists**
**Professor Madhulika Dixit**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Lecture Number 03**
**Evolution-2**

So welcome back to this course on "Biology for Engineers and Non-biologists". In the last video we spoke about origin of life and how life evolved from non-living things, essentially through chemical reactions, and then the ability of these early synthesized molecules to self-replicate and eventually formation of protobionts and then the early form of life. In today's video, what we're going to talk about is that how from this early form of life, the life evolved to the present day organisms and there's a huge array of them; it's not just one just bacteria, you see that the life expresses itself in multiple forms.

So what is it that caused this variation, this distribution in different life forms, and that is evolution. Now, evolution is a very important subject and topic to study, and I would like to start this particular class by quoting Theodosius Dobzhansky, that "Nothing in biology makes sense except in the light of evolution". What you'll appreciate hopefully by the end of this class is that though we have these different forms of life, its evolution which has played the key role. And mind you, this evolution has not happened overnight, this evolution has happened over billions of years. So coming to evolution, and what is evolution?

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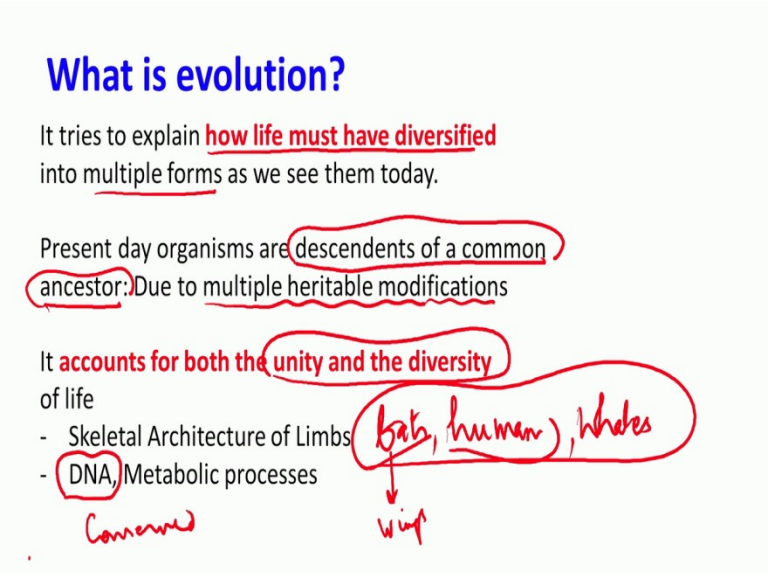

Evolution essentially tries to explain, how life must have diversified into multiple forms as we see them today. And these present forms are believed to be the descendants of a common ancestor. Now how does this happen, how did these descendants came from a common ancestor. We believe, during the course of history of earth's life, a lot of changes happened in organisms which went on, being passed on to subsequent generations.

In other words, multiple heritable modifications from one generation to the next, and it is this evolution which essentially accounts for what you call as the unity and the diversity of life. Let me give you an example. If one were to look at the skeletal architecture of, let's say, bats, human forelimbs; so if you were to look at the wings of bats, compare that with our forehands or with the flipper of the whales. One thing which unites these three is the basic architecture of the skeleton, and that is unified, whether you look at the wrist architecture, the finger architecture.

Yet, you find that in bats, you have these forelimbs modified into wings of bats, which allows the bats to flipper and fly. So, the basic origin was the same, yet the functionalities are different despite having similar architecture. Similarly, if we were to look at the DNA, as I mentioned last time also, as far as the language which codes the information in DNA has been fairly conserved. Yet, the DNA which is found in humans is slightly different from the DNA which you find in earlier organisms, not in terms of its chemical entity, but in terms of further architectural development. So, evolution essentially talks about these changes which have been acquired by various life processes over the course of earth's history.

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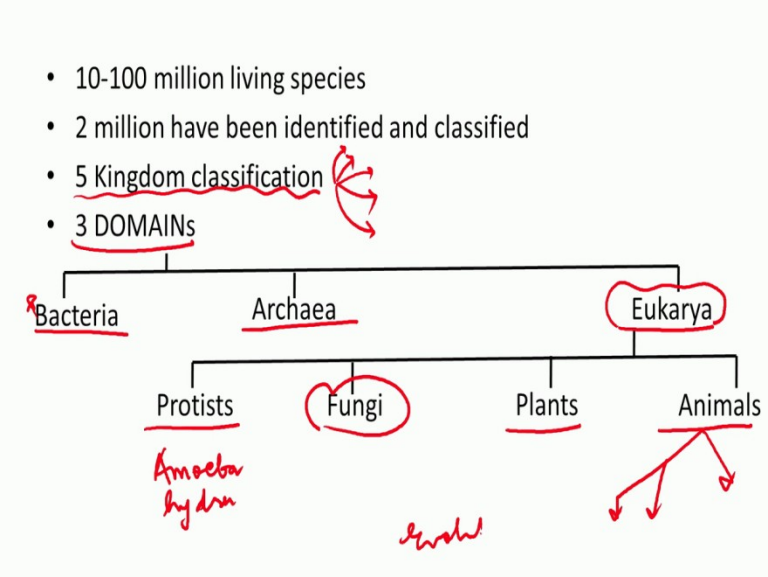


So let's look at what all is available and how many different forms of life are available on today's earth. You find that there are close to about ten to hundred million species, living species living on earth as of today, of which about two million living species have been already identified and classified. Now those of you who would have taken a classical biology class in their high school, they would have been taught that the all the living forms are classified into five major kingdoms, which is, the bacteria, the protista, the fungi, the plant, and the animal kingdom.

But as of today, we have classified the living organisms, and this is how is normally followed these days, are into three major domains; the bacteria, the archaea and the eukarya. Now we'll come to the differences which we observe in bacteria and archaea vis-à-vis eukarya in our next class, but eukarya is the most evolved of these living entities, and it itself is divided into four different groups; the protists, this is where you'll come across an amoeba, or the hydra, then the fungi, where you come across your regular mushrooms, the plants and the animals. So, there is diversity, starting all the way from bacteria to what you see among animals, whether it is the whales, the bats, or the human beings.

There are certain things which are unifying, yet there's a huge diversity. So how do you explain this diversity, and that is essentially explained through evolution.

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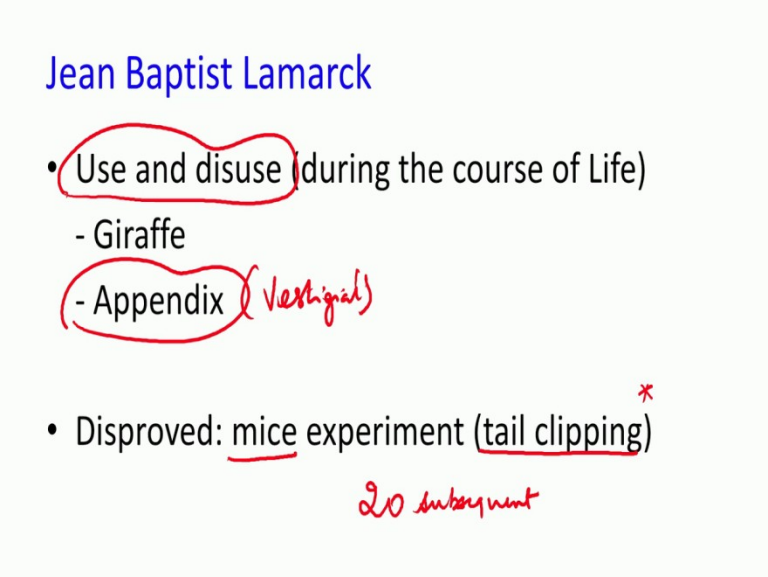


Now one of the earliest scientist who brought in the concept of evolution was Lamarck, and though his theories were disproved later, he still was one of the earliest scientist to bring in that very concept of evolution. According to him, it is the, a particular organ, or a particular feature in an organism develops during the lifetime of that organism based on the usage of that organism. According to him, if we look at the long necks of giraffe, it would have developed because the giraffe would have stretched its neck to reach to the food at tall trees and it's constant stretching would have led to the elongation of the neck and he believed that whatever features were acquired by a giraffe in its lifetime would be passed on to his progeny.

And, he also reasoned that those organs which are not being used by the organisms will eventually become useless and that is what we call as the vestigial organs, and one of the examples is the appendix that we see in our body. But, his ideas were later disproved through multiple experiments and one classic experiment was the experiment which was done on mice, where for about twenty subsequent generations, the tail of the mice were clipped, in the hope that if the tail is being clipped every generation, and it's of no use, then, by the twenty-first generation in mice, the mice which will be born in the twenty-first generation should be without tails, but that essentially didn't happen.

So in a sense, his theory based on use or disuse of a particular part was not the reason for evolution, though he did bring in the concept of evolution during the course of life, and in the course of earth's life.

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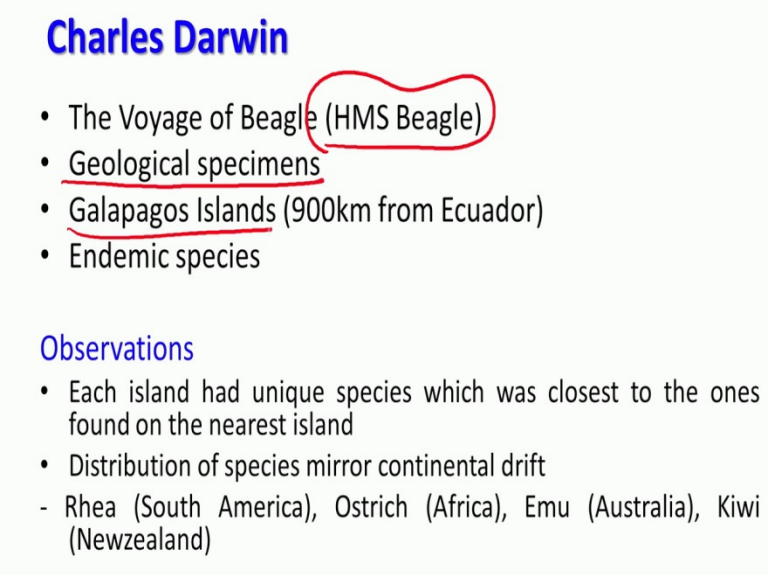


Then came the remarkable observations done by Charles Darwin. In terms of biology, even today, a lot of our discoveries, we owe it to the observations of Charles Darwin. Had it not been for Charles Darwin's observations, we would not understand biology the way we understand it today. And, it all started with this interesting trip which Charles Darwin took on HMS Beagle. He went on a voyage along the coast of South Africa, South America through the Galapagos Islands, which are about nine hundred kilometers west of Ecuador, and then all the way to Australia and back.

He essentially joined this voyage as a geologist who wanted to collect geological specimens and study them. And in the process of studying these geological specimens, he did observe a lot of fossils of not just high end animals, but even molluscs, or shelled, shelled organisms. And, the most interesting observations that he made were in the Galapagos Islands. And these are a group of islands which are spread across a few kilometers, very close to each other, but about nine hundred kilometers from Ecuador, and what we observed is that in each island, there were unique birds, species and tortoises and no two islands had the same kind of species. Yet, a bird or a

tortoise seen in one island was very similar, though not exactly the same, was very similar to the birds or the tortoises found in the nearest island.

So that was one observation that he made on the Galapagos Islands, the other observation that he made during his voyage across these coastlines of Africa, Southern America, and Australia, was that he observed that the distribution of these species was mirroring how the continental drift actually happened in the earth's history. For example, he observed that giant flightless birds like Rhea which is found in South America, Ostrich which is found in Africa to Emu which is found in Australia, they all look similar, and they all are flightless, yet they are so much spaced apart in terms of the geography.

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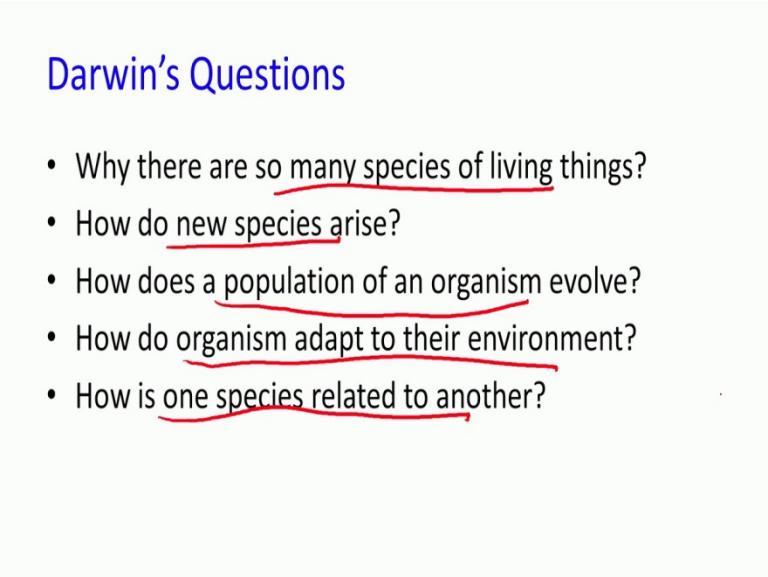


So he felt that this distribution of species has got something to do with the continental drift of the earth itself. So he asked a few questions. His first question was why there are so many species of living things, how do these new species come into existence, within a population, how does an organism evolve, and how does an organism really adapt itself to the changing environment. And how is one species actually related to another. So for example, if we look at ourselves, we would find within the population, some people are tall, some people are shorter, does it provide a survival advantage? Some people have a better muscle activity than the other, does it provide a survival advantage?

And how is it that some people are taller, while some people are shorter? And how is it, even if you look at from the evolution perspective, how is it from one generation of humans, to the next generation of humans, the features are changing? Is it because it is changing because they are trying to adapt to their environment? These are the kind of questions that Darwin was asking at that point of time.

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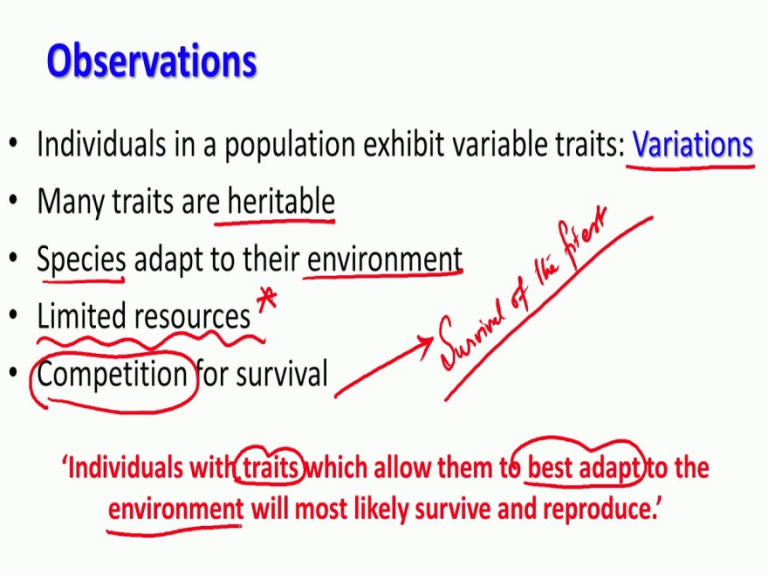


So he made a few observations. What he observed is that, in a given population itself, for a given species, so if we talk about humans for example, among ourselves and in our own population, there will be individuals with different features. Somebody will be taller, somebody would have brown hair, somebody would have black hair, and these differences, within the same species is what you call as variations, and many of these changes are actually heritable, which means, some of these changes can be passed on from the parents to the offspring.

The other thing that he observed as he found in the Galapagos Island, is that though the species were different in different islands, based on the environment which was being provided by the island, the species could adapt to that environment. For example, in those islands where the vegetations were found at a much lower level, the neck of the tortoises were smaller, while in those islands where the vegetations were slightly at a higher level at a higher height, you found, or he found rather that the tortoises had a longer neck.

So he suggested that the species can adapt to the changing environment. The other observation that he made is that, as a population grows in size, eventually, the resources become limited. Now this is a crucial point. If the sources are going to become limited, there is going to be a time, where there will be a competition among the individuals of a population for the resource, and this is what led to what we all routinely talk about and classically called as 'The survival of the fittest'. So, with his observations, what he observed and rather postulated is that individuals with traits, or rather variations which allow them to best adapt to the environment are most likely to survive and reproduce, and not only just survive and reproduce, but pass on these favourable characters to the next generation.

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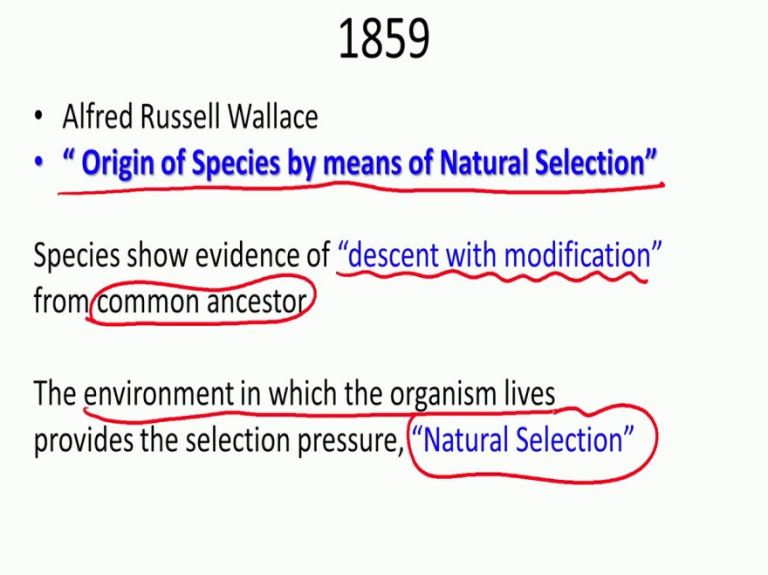


So while he was formulating all these ideas, there was another naturalist, Alfred Russell Wallace, who wrote to Darwin bit similar ideas and the two of them then decided to present their ideas to the London Philosophical Society in eighteen fifty-nine, and eventually Darwin published his most famous work, which is called as 'The origin of species by means of natural selection'. Now what does this talk about? Essentially it talks about two things. It says, any species always shows an evidence of descent from a previous a species with modifications. So, species show evidence of descent with modification, and they all will have common ancestor.

The second he said, the pressure which brings about these modifications, or the variations, is the environment in which the organism lives. So, if the environment is changing and that changing

environment demands the organism to change, some of them, not all of them in a given population, certain individuals will adapt better because they have acquired these modifications. This pressure, which is provided by the environment is what is called as the 'natural selection'. So, in other words, let me reiterate, what Darwin said was that a species arises from a common ancestors because certain individuals in that species would have developed modifications which are favourable, which allow that organism to survive better in the changing environment, and these favourable traits get passed on to subsequent generations, allowing eventually, over a time scale of a few million years for evolution of a newer species with better characteristics.

And many a times, these species may kind of get segregated if there are continental drifts and changes happening on earth. But this was just a theory in 1859 and later on, within a very short span of about fifty years, and this has been the classic example of natural selection, has been around the time of industrial revolution, and this has been the generation of the peppered Moth.

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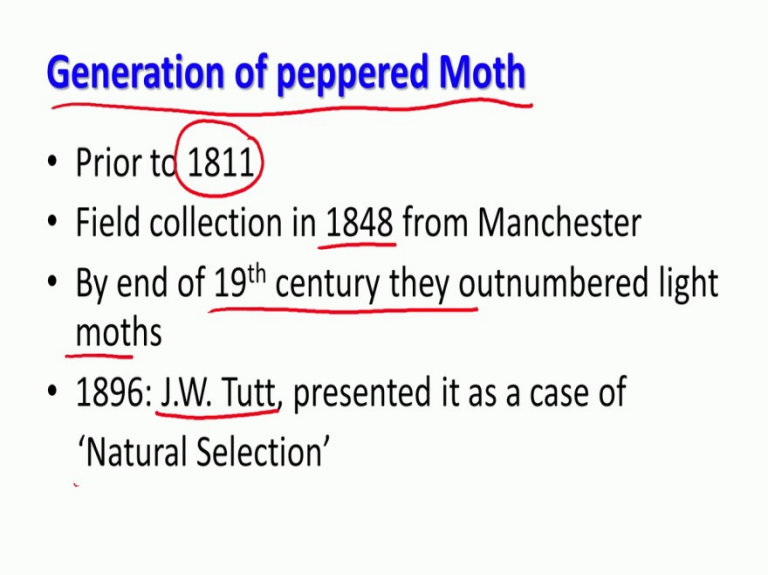


Now this was an interesting observation, which was made by J.W. Tutt and what was known then is that way back in 1811, most of the moths which were found in England , mainly in England were light colored. But a field trial, or a field collection in 1848, around the time when the industrial revolution was taking place in Manchester, a lot of peppered and black coloured moths were observed, and by the end of that nineteenth century, in a period of about ninety years, the light coloured moths was totally outnumbered by these dry, dark coloured moths. Now what

could be the reason? The reason was, that during industrial revolution, because of a polluted environment, in order to survive the prey, that is the bird which will be eating on these moths, the light coloured moths will become extinct because they will become easily visible and will easily be spotted by the preying bird.

But a dark coloured moth will, being able to in the background of the soot being generated, thanks to the industrial revolution, would be able to camouflage itself to such an extent, that it will not be noticed by the predatory bird, and it would have given it a survival advantage. So this was then presented by Tutt as a case of natural selection. So here is an example where changing environmental conditions due to, in this case, human activity in industrial revolution, actually led to the generation of a peppered moth. So what are these different kinds of selections that we are talking about?

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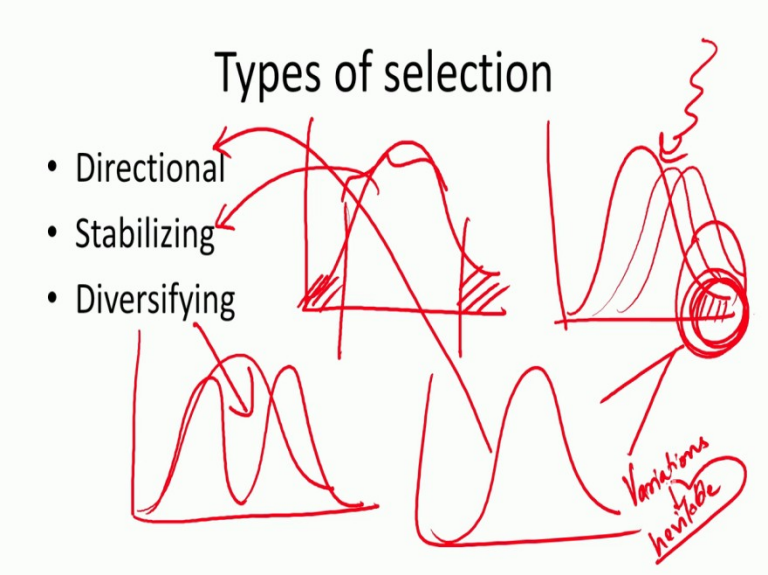


Well, as I mentioned earlier, the selection pressure is essentially provided by the environment, and how does the organism adapt to that environment. So, assume that you do have a population trait, you have a trait for which there is a normal distribution and you find that if the environment is conducive and the conditions are fine, there's no need for the outer extreme of the organisms to grow. I mean they become redundant. Now such kind of a selection where the central most acquired characters are retained is a stabilizing selection. But then, you can have a situation where you still have a population with these kind of display where you have some extreme

variations and suddenly, the environmental conditions force in such a fashion that it is this set of extreme outliers which have a survival advantage.

As a result, what'll happen in due course of time is that, this set of population will tend to move towards the favourable acquired characteristics of this set of organisms and it is this set of organisms which then eventually will form a new species. Now this is an example of directional selection. There are also examples of diversifying selection wherein you have originally your population, and then either because of a continental drift, or some other kind of catastrophe, this gets split into two populations with different characteristics and that is called as the diversifying selection.

But, thing which remains common and unifying across all this are two; one, it's the environment, which provides that pressure, the selection pressure, and two, it is the ability of a certain individuals in a given population to adapt to that change, and the reason they are able to adapt to that change is because they manage to acquire new characteristics which are favourable, and these characteristics is what you classically call as variations. If these changes are heritable, you will find that over successive generations, the variation, this originally acquired variation which eventually become a characteristic of that particular group of organisms.

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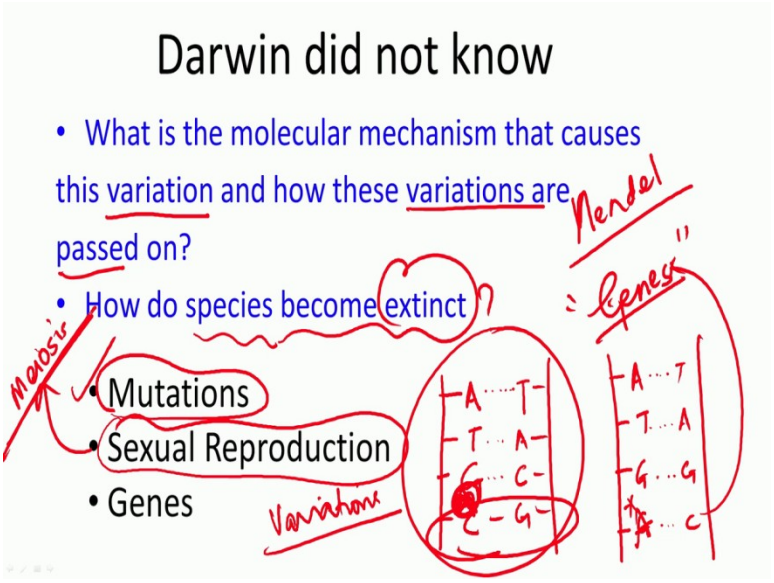


But, Darwin did not know certain things. He did not know what is the molecular mechanism which actually causes this variation. He knew that the changes are happening, but what is

causing it? What is the molecular mechanism which is causing these variations? And how are these variations are being passed on? And this is where the role of Mendelism and Mendel's genetics becomes very important, which will be covered by my colleague, Professor G.K. Suraishkumar. And this is where the role of genes, each gene in our DNA codes for a certain character. It is the genes which carry these variations, but, what is it and how are these variations introduced into these genes? We'll talk about that a little later from now.

But, Darwin did not know the mechanism. He also did not understand why certain species for which he had collected the fossil samples become extinct. What is the cause of extinction? For example, for dinosaurs, and answer was not available then. So how are these variations caused? There are multiple ways, and the most classic way is the process of mutation. Now, we all know that our genetic material is encoded in DNA, so let me just draw a strand of DNA with a certain sequence. So the complimentary strand, we'll cover this again when we talk about DNA replication, but for the simplicity sake, each strand of DNA mirrors it through a complimentary strand. Right?

This is a DNA. Now, if this molecule of DNA were to undergo replication, and for some strange reason, there error happens because of which the sequence in the DNA, let's say, changes, and the errors are a part of life, I mean they do happen pretty common, and now, you'll have a new DNA molecule which has a mutation where this C has now been replaced by a G, or a let's say an A. So now, this mutation has happened and if this mutation is going to impart a favourable character to the gene in which it is present, then this becomes a favourable variation. So mutation, or changes in DNA sequences which are heritable and which do provide favourable effect on to survival could be one of the reasons by which the variations are happening.

The other reason for the variations has been the process of sexual reproduction. We all know that we get a certain set of genes from our father, and a certain set of genes from our mother, and we are neither a carbon copy of our father nor our mother, we have a mix features because some genes have come from our father, some genes have come from our mother, and as a sum total and a combined effect. Now this has been one of the major reasons for variation and is one of the major reasons for success of survival in higher organisms.

Now how are these variations through sexual reproduction brought about? What is the process? We'll discuss this variation, and even in this variation, how are these variations brought about? We'll discuss them when we are talking about the process of 'Meiosis', and I'll come to that when I'm talking about meiosis. So, there are ways by which across evolution, over a period of billions of years, the DNA has evolved, and it keeps evolving, sensing demands of the environment, and these favourable changes have went on accumulating and over a long period of time, a point will come when the original species will end up giving rise to a newer species.

And what are the causes of extinction? Well, a lot of these are speculations because we cannot go back in history and try to redo what has been done, and we can't recreate a lot of environmental changes which must have happened a few billion years ago. But, we speculate that if a species fails to adapt itself to a changing environment, it's going to become extinct, or if it specializes to such a point that it cannot re adapt itself, then also it can undergo a cause of extinction.

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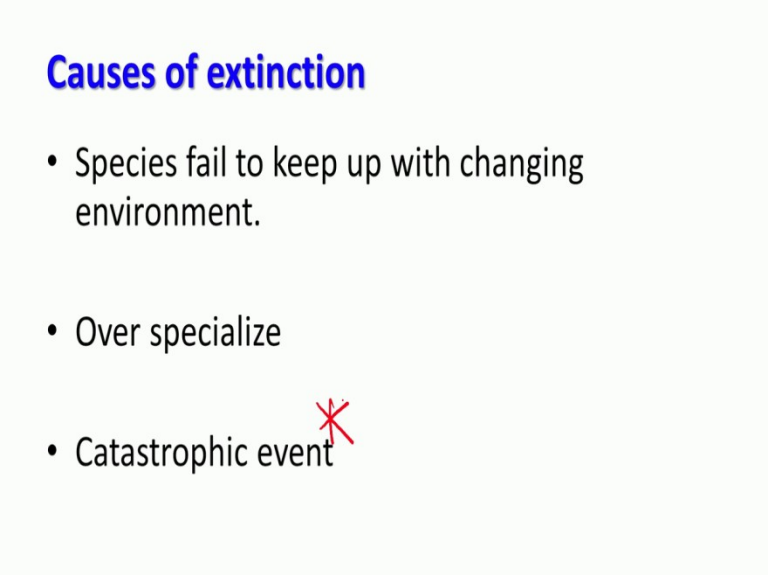


And then the classic example has been the disappearance of dinosaurs because of catastrophic events. So, there are ways by which life evolves, and then it not just evolves, it keeps on accumulating these changes and most of these changes which have managed to survive across evolution have been those changes, which have given a survival advantage to a given organism, and as a result the organism tries to retain it and the life tries to retain it for further passing it on to the progeny.

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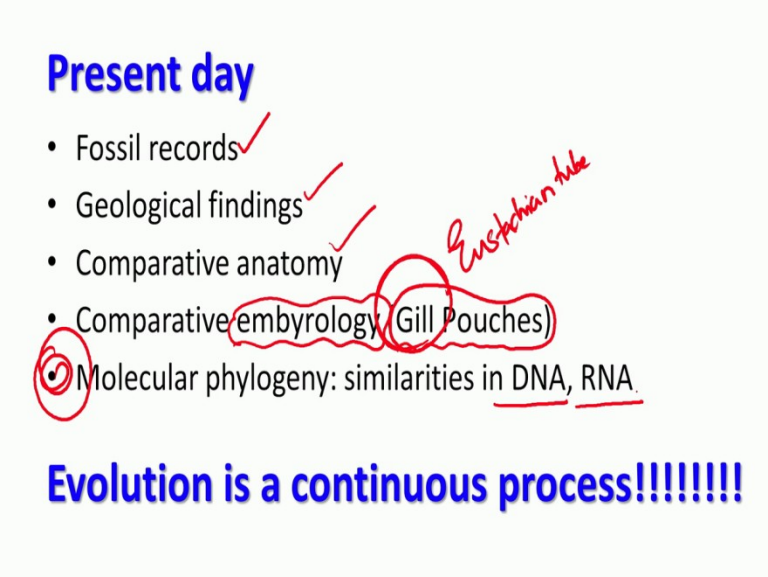


So in present day, we tried to understand evolution through fossil records, through geological excavations; we also tried to understand how the organisms would have evolved by comparing the anatomical structures, as I mentioned, in case of skeletal formation or comparative embryology. Now this is an interesting observation. For example, if you were to look at the embryonic development of fishes or reptiles, birds, all the way up to mammals, we find that the embryonic stages, all of them, right from fishes till humans express at a certain stage in their embryonic development, a structure called as the gill pouch, which is around this area.

But, in case of fishes, the gill pouch actually evolves and forms the functional gills through which the fish respires. Well in humans, though it is present in the embryonic stage, it later ends up becoming a 'Eustachian Tube', tube or a tube which actually connects your middle ear to the nose. To the throat, sorry. So, basically, what you find is that these gill pouches are present during the embryonic development all across from fish to the humans.

So you can do comparative embryology and try it group and see how evolution must have taken place, and the most recent is the advancements in molecular biology techniques wherein we are looking at the similarities in structure, the sequences of DNA and RNA, and are linking it to the process of evolution. For example, the DNA which will code for enzymes, for basic metabolic reactions are highly similar across evolution. Yet, in some organisms, it's a little more better

evolved than the lower organisms. So by comparing the actual DNA and RNA sequences, we can also estimate how closely the species are inter-related, or they have diversified.

So, there are ways to understand this evolutionary process. So I would like to again, end this, or rather summarize this talk by saying that evolution is a continuous process. For all you may know, as we are talking and as are our cells dividing, if mutations are taking place with time, and if these mutations accumulate and become favourable, it becomes a variation. So it is a continuous process. However, the end results of these evolutions are not going to be seen in our own lifetimes, but probably in millions of years down the line.

So, I would like to again say, that the crucial part in evolution has been the theory of Darwin, which is the ‘Origin of Species by means of Natural Selection’, where the natural selection is essentially provided by the environmental changes; and in order to adapt to the environmental changes, individuals in a population will develop variations. And these variations may either happen because of sexual reproduction, because of mutations and if the variations are favourable, it’ll keep on getting accumulated over successive generations, unlike Lamarck’s theory where he was only talking about doing the lifetime of a individual organism.

Darwin extended and he explains that these evolutionary changes accumulate over generations and then diversify into a newer species. So with that, we have talked about evolution, and if you are interested to read more and get a better perspective on how it actually happened? What was the voyage of Beagle which was taken by Darwin, I would recommend that you take your time out and go through some of these videos on You tube. Thank you and see you later.

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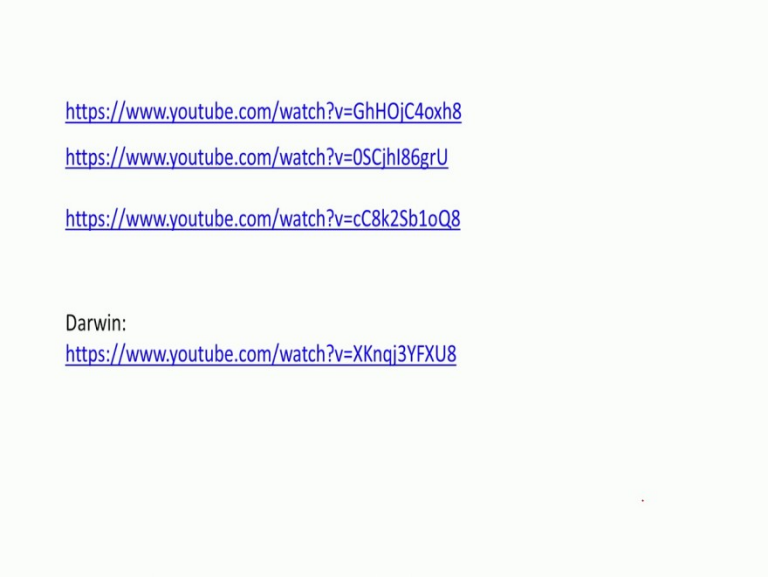

**Biology for Engineers and other Non-Biologists**
**Professor G.K.Suraishkumar**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Lecture Number 04**
**Cells**

Welcome to this set of lectures on "Biomolecules and their relationship to the Cell Structure and Function", which are important aspects. We'll go through a set of lectures which will cover appropriately sized, so that it'll be easy for you to assimilate aspects.

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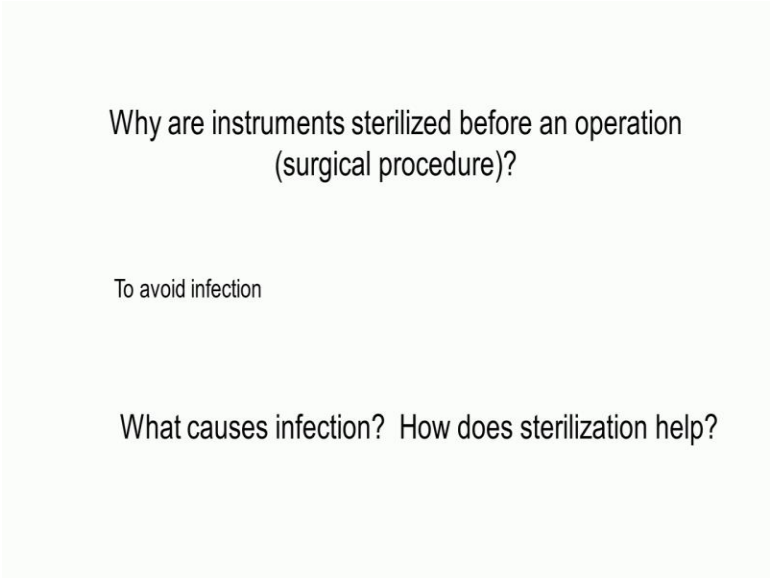


Let's ask the question. Why are instruments sterilized before an operation? What I mean by an operation is a surgical procedure. Why are instruments sterilized before the medical doctor cuts open the skin? All of us would know the answer to this question – to avoid infection. Okay? What causes infection? And how does sterilization help in preventing infection?

These would be the natural questions that come about, right? What causes infection? Let's answer that first.

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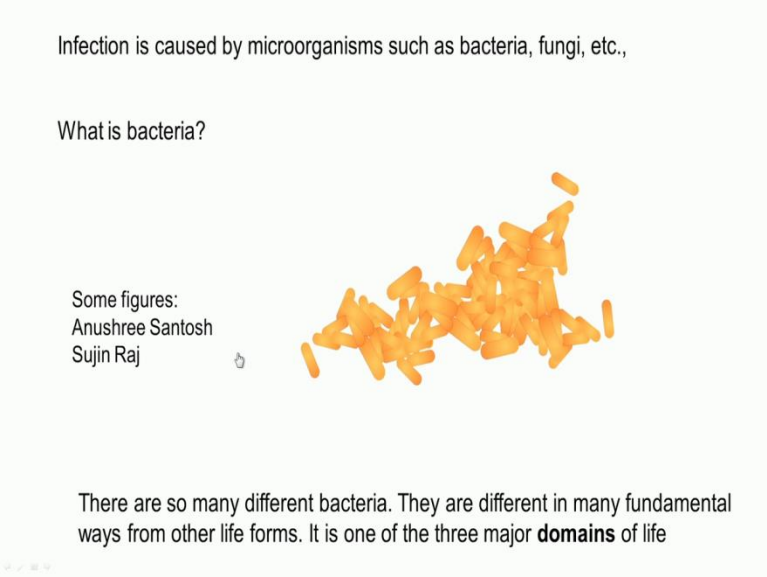


Infection is caused by micro-organisms, such as bacteria, fungi and so on, okay? I would should say some types of micro-organisms among bacteria, fungi; there are a lot of very useful types of bacteria, fungi, which actually reside inside our body and do a lot of useful things for us, okay? We should not forget that aspect. So there are harmful bacteria, harmful fungi, viruses and so on. But in terms of infection, let's stick to bacteria and fungi.

They can, cause a lot of infection. right? And, to, where is this bacteria and why should it cause infection when the surgeon is making an incision in the skin? That is because there is these micro-organisms, bacteria, and other such organisms are in the air around us. Studies have found that about ten power three to ten power four thousand to ten thousand bacterium per milliliter, okay, per cc, per cubic centimeter, are present in the air around us. They are present all the time, and they are interacting with us all the time. Our body has immune system which fights against this all the time, and as long as this balance is maintained, we don't get infection.

Whereas when the surgeon is operating, and when the surgeon is making an incision in the skin, if there are bacteria around, it will start settling in this wound and that will cause infection. Okay? So conditions need to be created such that there is not much bacteria around, not much bacteria on the tables, on the material that is in the operation theatre, certainly not in the instruments and so on. So, such things need to be taken into account, and it is also a nice exercise to carry out, to realize how much bacteria is present around, I did mention ten power three to ten

power four organisms per ml. You may want to do this calculation, find out the mass of air in the room that you are sitting in. Okay? You would be in for a surprise.

The density of air is 1.29 kg/$m^3$, if you would need that to make an estimate, you would be very surprised as to the a mass of air that is just present in the room that you are sitting. Why don't you do that? Now what is bacteria? Bacteria, it could be something like this if you look at it under the microscope. You know you'll see these rod shaped structures here. This could be one of the bacteria that is present in nature. There could be other kinds of bacteria that are present with different shapes, different, slightly different size, and so on so forth. And, there are so many different types/ kinds of bacteria. They are different from… in fundamental ways; we'd come to know what those fundamental ways are to certain extent when we go through the course.

They are different in many fundamental ways from the other life forms. And it is so different, and so big that it is bacteria, is one of the three major domains of life. Before I go forward, I should acknowledge that some of the figures in this course have been adapted by Anushree Santosh and Sujin Raj. Now let's get back to domains.

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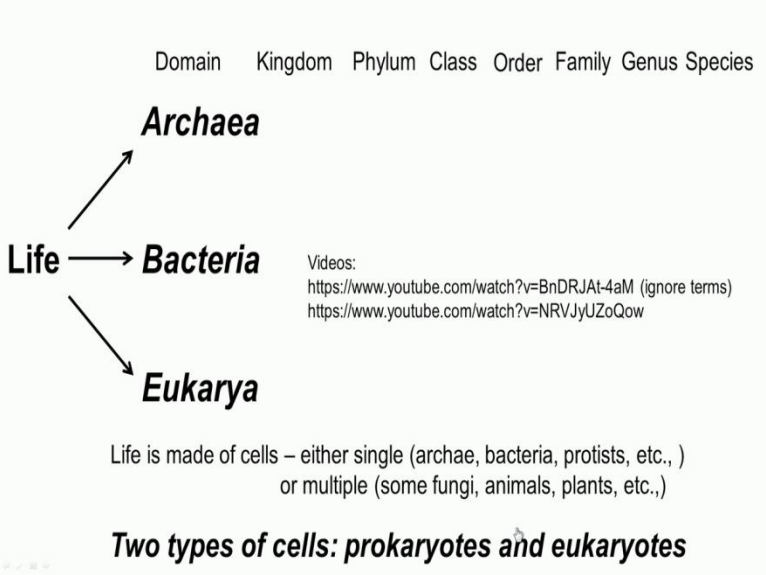


Life, bacteria is one of the domains. The other major domain is archaea, and eukarya, is the other, the third major domain. This is what I call as domain. There are three major domains in life, and each domain has many kingdoms, each kingdom has many phyla, phylum, plural,

phylum- singular, phyla plural. Each phylum has many classes. Each class has many orders, each order has many families, each family has many genuses, and each genus has many species.

Okay? Don't worry about this. I'll give you a mnemonic, which I picked up from one of the, one of the videos that I'm going to suggest to you. I think it is the mnemonic is 'Do Koalas Prefer Cheese Or Fruit Generally Speaking', yeah. 'Do Kualas Prefer Cheese Or Fruit Generally Speaking', let us have it that way. Okay? It is the way to remember this. Anyway, if you look at these videos, there are two videos that I'd like to recommend.

The first one is a small video you can watch. Please ignore the terms, they'll talk of DNA, RNA and so on and so forth. Please do not worry about the terms, you will know what those terms are only as a part of this course, if you do not already know them. If you know them then it's fine. And the second video is slightly longer, about, you could watch about three-fifths of that video, then it gets into too much detail and this would tell you all about the domains of life and some of these aspects also.

Then life itself is made up of cells, either single cells, as the case of archaea, bacteria and some protists, in the eukarya family, or it could be made up of multiple cells, such as some fungi, you know mushrooms and so on so forth, the fungi, animals of course, plants, humans, and so on, okay? So life is made up of either single cells, or an organization of single cells. Can anybody guess the number of cells in the human body?

It's of the order of about $10^{14}$. So you can imagine the number of cells and how they interact with each other to make life possible. There are two major types of cells; first type is called prokaryotes, the second type is called eukaryotes. What do these names mean? If you know the meaning of the names, probably it's a little easier to get more comfortable.

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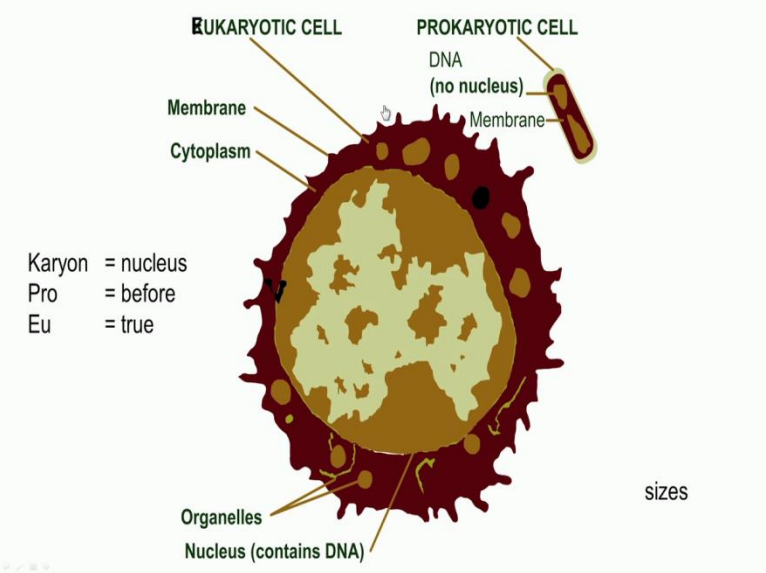


It so happens, karyon means nucleus. Karyon is the word for nucleus. Pro is before, this we all know. Eu means true, okay? So, prokaryote, before nucleus, eukaryote, something that has a true nucleus. That's what it means. This is a prokaryotic cell, a simple enough cell here, typically small, we'll come to sizes in a minute, it has some parts, let's not get too much into it.

It is of course separated from its environment through a cellular envelope that could have a cell wall and a cell membrane, it could have some DNA, you know kind of strewn around here in the cell which is not limited in any way physically in the cell; and this is prokaryote- before nucleus. There is no well-defined nucleus in a prokaryotic cell. Whereas in the eukaryotic cell, it's complex, it has a well-defined nucleus that contains many things including DNA, right? This is the basic difference.

Eukaryotic cell has a true nucleus, a prokaryotic cell does not have a well-defined nucleus. This is a well-defined nucleus. This has a membrane, and inside the membrane whatever is there is called the cytoplasm, and in the cytoplasm you have a lot of organelles, small things, that have, each one has their own function, and of course the nucleus is here, indicated here which contains DNA and other things.

So, this is good enough to know. The typical sizes, a prokaryotic cell; for example, a bacterial cell, is about five microns, typical size, okay? Five micron length, two micron diameter, or if it is

spherical it could probably be about five micron diameter, and so on. Many prokaryotic cells are of that size typically speaking. There are variations of course, but we are talking of some typical sizes here. Whereas a eukaryotic cell could be of a typical size, a mammalian cell for example could be of ten micron diameter, typical size, even a human cell goes anywhere from seven microns to about twelve to fourteen microns during its lifetime. Right?

So there is variation with time in the cell size also. But we're talking of typical sizes. If we talk of fungi which are eukaryotes that could be a few microns in size, fungal cell. If you talk of mold, you know, you cannot really talk of a single cell in the mold because it branches and so on so forth, let's not get into that, we just need to, kind of, have this in the background saying that the cells are not separated from each other.

If you talk of a neuron, which is a neural cell in humans, that could be two hundred microns, right? So it widely varies, the cell size widely varies. Typical sizes, about two to five microns this is about ten microns. These are good enough for our back of the envelope calculations. Why are we so interested in this cell? Because cell is the fundamental functional unit of life.

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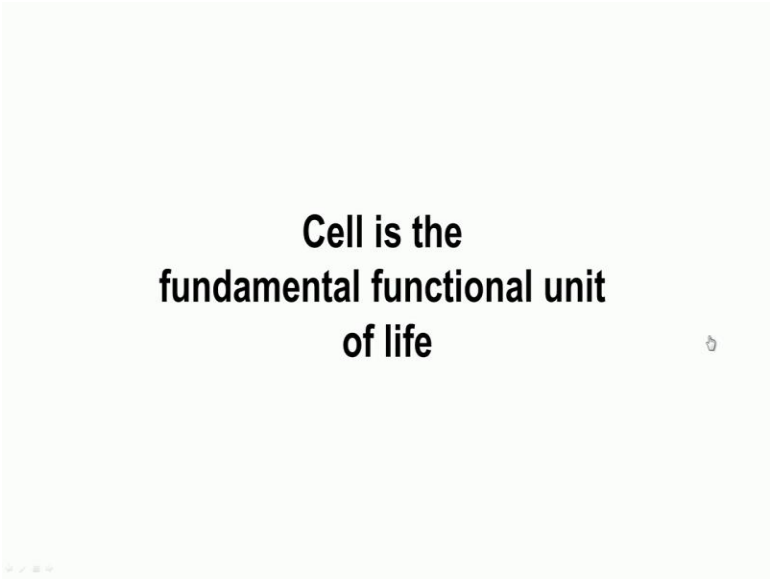


What does it mean, if we understand cell, and all its functions, all its properties, then we would be able to somewhat predict what happens to life. That is what it means. Same way that a unit cell in, in let's say crystal structure, the properties, with those properties you could predict

properties of the whole thing, and so on so forth. This is some fundamental unit of life itself. It is a biological cell. That's why we are so interested in the cell.

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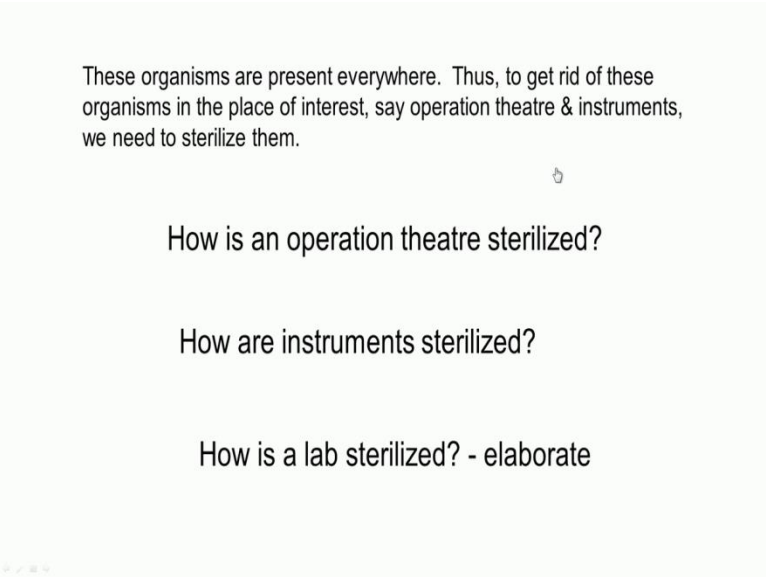


As I mentioned earlier, these organisms, remember we are talking, our story is about infection, infection in during surgeries, how to prevent them and so on so forth, let's stick to that story. The organisms are present everywhere. The harmful organisms are also present everywhere, and to get rid of these organisms in the place of interest, which happens to be the operation theatre, the instruments which are used to cut the skin and so on, we need to sterilize them. Right?

How are instruments sterilized? How is an operation theatre sterilized? Instrument sterilization, that, it's rather straightforward, so let me talk about that first, and then we'll get to the operation theatre sterilization, which is a whole field in itself. Instruments are sterilized by exposing the metallic instruments to high temperature and keeping it at high temperature over a certain period of time, the standard way of sterilization; autoclaving as it is called.

So the temperature is raised to about 121 degrees C, steam is used. As we all know, thermodynamic steam, you know 121 degrees C, you need a higher pressure to maintain the conditions there. So you have slightly higher than atmospheric pressure conditions in the autoclave. So you create such conditions and you keep it at that condition, one twenty one degrees C, at say one point two atmospheres, for about 15 minutes; it kills the cells, as well as kills the spores, and so on so forth, okay? So that's a very standard method of sterilizing

instruments, or sterilizing things that we use in the lab to grow organisms and so on. So instruments are sterilized that way.

But how do you sterilize operation theatres, okay, which are huge spaces? How do you sterilize labs which are huge spaces? You know in the labs you would want to grow only the cells that we are interested in. We do not want the others to grow, and compete for the food that is given to the cells of interest, okay? How is this done? It is, very interesting, the way it is done. It is done by using chemical vapors, okay? And, the procedure that I'm going to describe now, is used heavily in labs, because you can control the conditions there and kind of not let people enter the lab very strictly and so on so forth, you have a control over that, and therefore you could do that. Okay?

You will have to be careful if you are dealing with people and you want to use this procedure. It's not very safe. There could be related methods that are safer, but which are a lot more elaborate to use. The cells are killed by formaldehyde, okay? HCHO, formaldehyde. And the formaldehyde vapor is generated from, what are called formalin solutions. Formalin solution is nothing but forty percent formaldehyde in water, okay? This you get, you can buy it off the shelf. So what people normally do, let me describe the lab procedure here. First all the windows and doors are sealed except the passage through, or the door through which we need to get out.

And then, we use what are called aluminum boards, in which we have this formalin solution, forty percent formaldehyde solution. This is in the liquid, okay? This has to come out into the vapor to kill all the organisms, in the space, in the lab so that the space is made clean or the space is made clear of these micro-organisms that are present there.

And to make that possible, to generate formaldehyde vapors, what is used is the exothermic reaction between potassium permanganate, and the formalin solution. Okay? So these aluminum boards containing formalin solutions are placed in key positions in the lab, then everybody is sent out, and before you place the formalin solution, make sure that it is made sure that all the windows and doors are shut and taped so that no vapor escapes out, because this vapor is poisonous.

And then, the person uses a mask and so on, just for those few seconds, drops a few pellets of potassium permanganate, that are carefully weighed out for, required for the stoichiometric release of formaldehyde and so on, drops in the aluminum boards, farthest first, and then keeps

dropping in the boards that are closer to the door, and then quickly walks out the door, shuts the lab, and tapes the door shut. Okay? It is left there for a day or two, and may be a day more, just to make sure that none of the vapors are present, and then somebody gets in and neutralizes the present formaldehyde and so on so forth. Okay?

So it's an elaborate procedure that is used to sterilize the lab; we do it from time to time, may be once in a month or so, it is recommended that the lab is sterilized. A similar procedure can be used for operation theatres, it has been used for operation theatres. But in addition, you could swab with, let us say, seventy percent ethanol, you could use other cleaning solutions; mop the floors and so on so forth, that's normally done. That is how the space is gotten rid of these micro-organisms. The micro-organisms, some of which have the capability to cause infection in humans. That is how an operation theatre is sterilized, how the instruments are sterilized.

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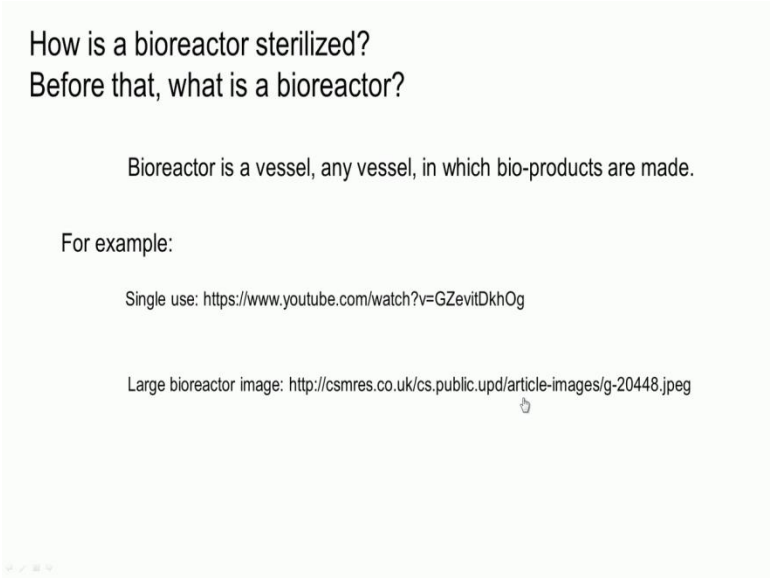


How is a bioreactor sterilized? And you would go, ‘where is this bioreactor coming from’? Okay? So this question, before that. “What is a bioreactor”? Okay? This is something that people normally used from an engineering perspective. So I thought it will be closer to you. Bioreactor, by the way, it is it is certainly closer to me, this is the area in which I used to work heavily. Bioreactor is a vessel, any vessel, in which bioproducts are made, it is as simple as that.

It is something that is used for production that uses organisms for production. These organisms produce many useful compounds, many useful substances, including a lot of medicines. For

example, if you see this figure, I think this is a video, YouTube it is a video, it will tell you how a single use bioreactor looks like, and what it is used for and so on.

Bioreactors could be very large, extremely large because of the use in production of high volume products, okay? And this link here gives you an image of one such large bioreactor. And many of these water treatment facilities have bioreactors that are large. Okay? Bioreactors are the basal ones that are used for water treatment. They are very large too.

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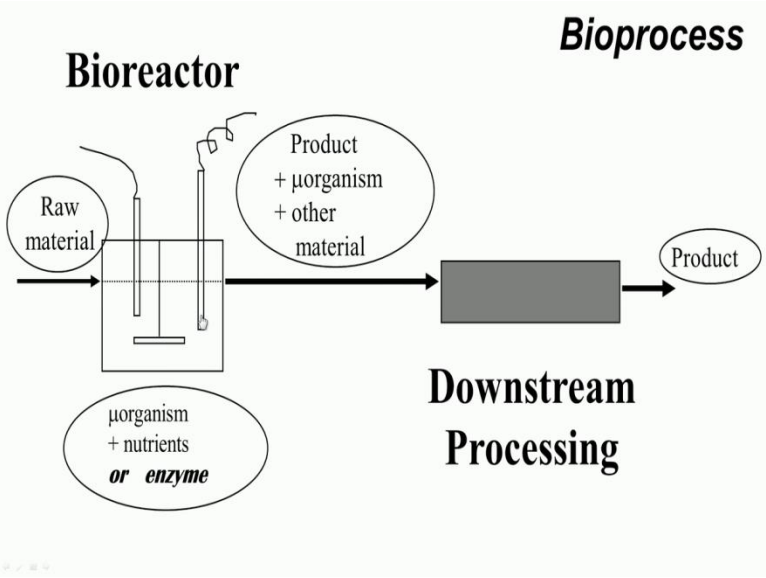


The bioreactor is a part, an important part of what is called a bioprocess, and the bioprocess is the one that is used to produce all these products of interest to us. There are very many products, we'll see some of those products in this course itself, okay? We have, for a bioprocess, the bioreactor aspect, and, what is called the downstream processing aspect. In fact, the downstream processing part could have about 90 percent of the steps of this bioprocess. Bioreactor is a small part of it, okay? But it's kind of a central part.

Raw material goes into the bioreactor, either in a continuous fashion or in a batch fashion, you know, you dump everything at one time. And, in the bioreactor, you have the micro-organism of interest, along with the nutrients for it to grow and make the product. Or, there could be an enzyme that is present, okay? Just take the name on its face value, and now you might have heard of enzymes, you will actually look at what enzymes are in detail in this course.

Or an enzyme here, in the micro-organism plus nutrients or an enzyme, and the raw material is converted into the product of interest. So what comes out of the bioreactor is a mixture of the product of interest, the micro-organism that is there which is producing the product and a lot of other material which is present in the nutrients, the medium as it is called, and so on.

And may be some other products are also produced, which may not be of much interest to us, we're interested only in the product of great importance to us, amongst the products that are present. So, this product needs to be separated out from all this to be able, for it's proper use, and that is done by what are called the downstream processing steps, a large number of steps, including distillation extraction and so on and so forth, till we get the final purified product, okay?

This is what the bioprocess is. The bioprocess produces a lot of useful substances, and, in the bioreactor, the cells that we talked about, the cells that are used typically the bacteria that we talked about, the fungal cells that we talked about, maybe mammalian cells that we talked about, right? They are used to produce products, and in the bioreactor, the cells are subjected to shear, okay? That is the next part of the story. We will stop here for, for this lecture, this part of the story, essentially our story went something like this, we wanted to know, what causes infection, and we said micro-organisms cause infection, and we saw how micro-organisms are organized for better understanding.

If you look at those two videos, it'll give you more reasons for, organizing it a certain way, and so on. Please look at those videos, on 'Taxonomy'. And then, we looked at the cell as the fundamental functional unit and there being two types of cells, prokaryotes, before nucleus, and eukaryotes, with a true nucleus. In other words, prokaryotes do not have a well-defined nucleus, eukaryotic cells have a well-defined nucleus. And, then we saw that the cell is the fundamental functional unit of life. Then we saw that these organisms are present everywhere, and started looking at how to get rid of them in an operation theatre. We looked at some means of getting rid of them. And then, we looked at what a bioreactor was. Let us stop this lecture here, there is good enough new information for you. In the next lecture, let us take things forward with shear of the bioreactor. See you then.

**Biology for Engineers and other Non-Biologists**
**Professor G.K.Suraishkumar**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Lecture Number 05**
**Biomolecules: Lipids**

Welcome! We are discussing 'Biomolecules and their relationship to the cell structure and function'.

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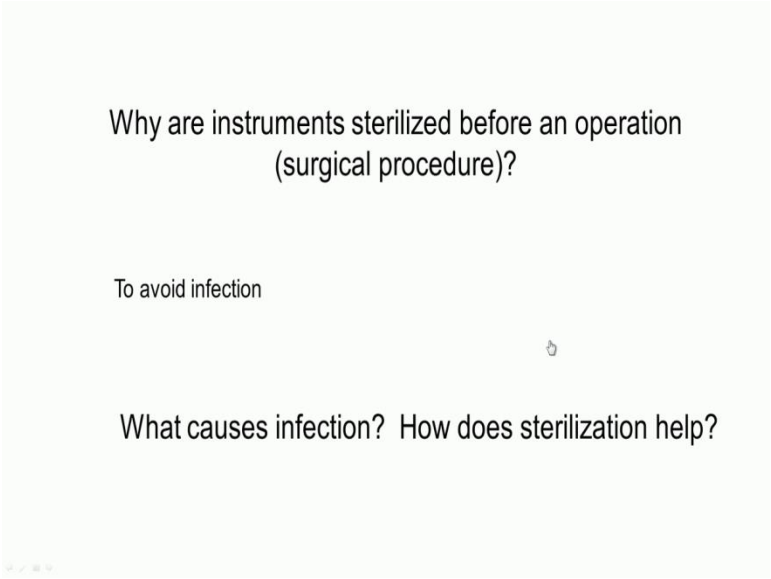


We saw in the last lecture, the micro-organisms, and to see that we had a storyline, we were looking at why instruments are sterilized before a surgical procedure and we asked a few questions and we came down to bacteria which can cause infection, which is one of the things that can cause infection.

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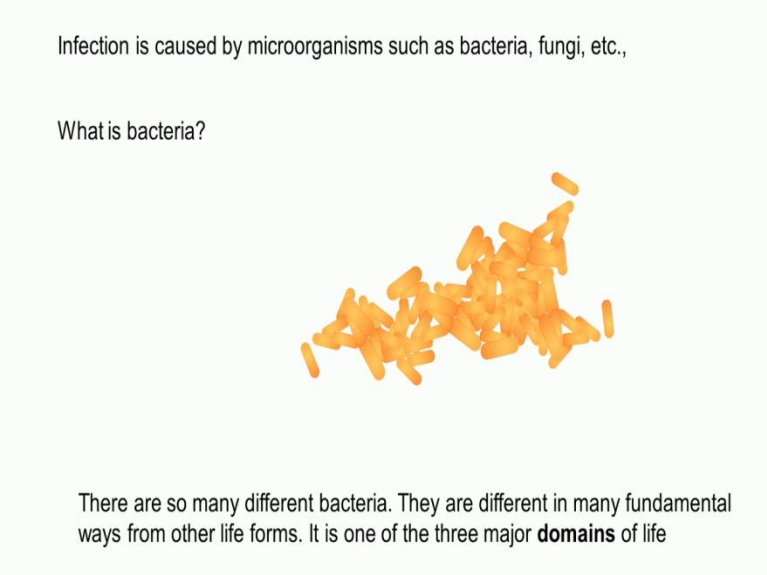


And there are so many different bacteria in the world, in life and so large that and so fundamentally different from other varieties that we call that a domain. Life, we said had three domains; bacteria, archaea and eukarya, which are different in some fundamental ways, and these are called domains.

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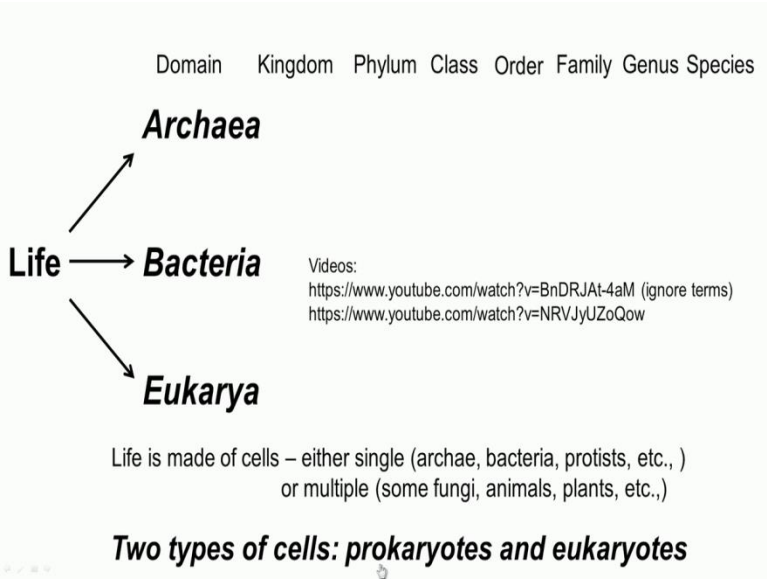

There are many kingdoms in a domain, there are many phyla in a kingdom, there are many classes in a phylum, there are many orders in a class, and there are many families in an order and there are many genuses in a family and many species in a genus. This is the taxonomy, as it is called, or the way organisms are classified so that there is no confusion. Hopefully you have gone through the videos here. We had mentioned that you could ignore some of these biological terms. They are just being, you can, kind of get used to it without really knowing what they are. We will know most of the important things as a part of this course, you don't have to worry about that.

So you could ignore the terms such as RNA, DNA and so on so forth at this stage and watch this, and watch about let's say two thirds of this video. I talked about mnemonic to remember this. Do Koalas Prefer, I said Cheese Or Fruit, I think the video says Chocolate or Fruit Generally Speaking. That's a mnemonic to remember this. And then we said that life is made up of cells, either single cell, such as in archaea, bacteria, and some part of eukarya called protists and so on, or they could be made up of multiple cells such as fungi, animals, plants and so on. I said humans are made up of about $10^{14}$ cells.

And basically, there are two types of cells, prokaryotes, before nucleus, and the ones with a true nucleus, we saw how they looked, and their sizes and so on and we also said that cell is the fundamental functional unit of life.

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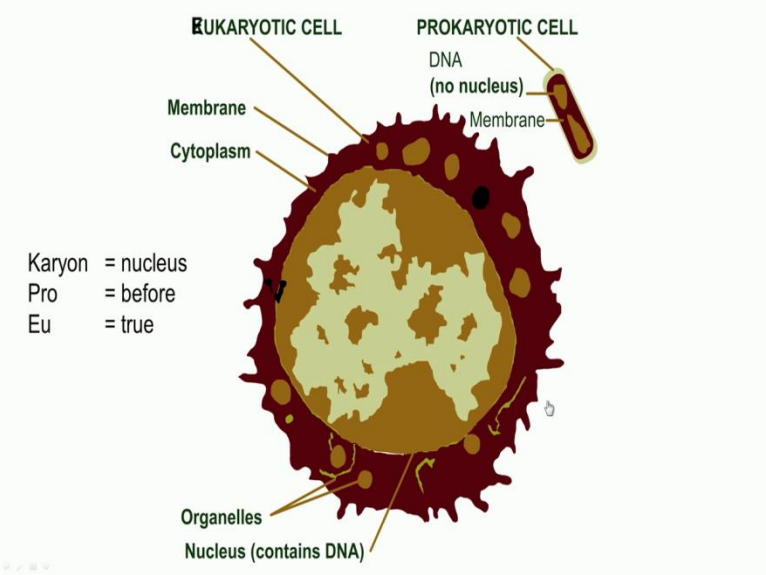

If we understand this, then we have probably understood life, and, we are nowhere close to this.

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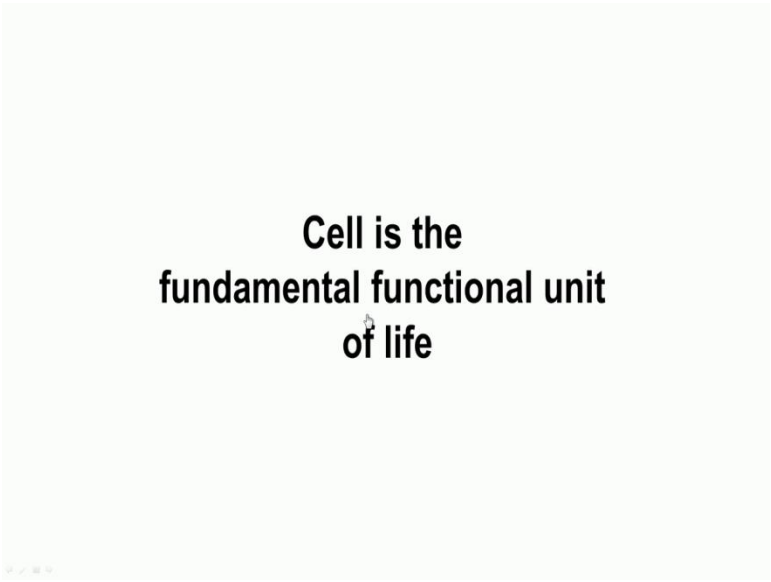


And then we talked about organisms and so on so forth and I told you how a lab is sterilized, how that can also be used to sterilize operation theatres and hospitals, and there are other means, other more safer means of sterilizing operation theatres and instruments.

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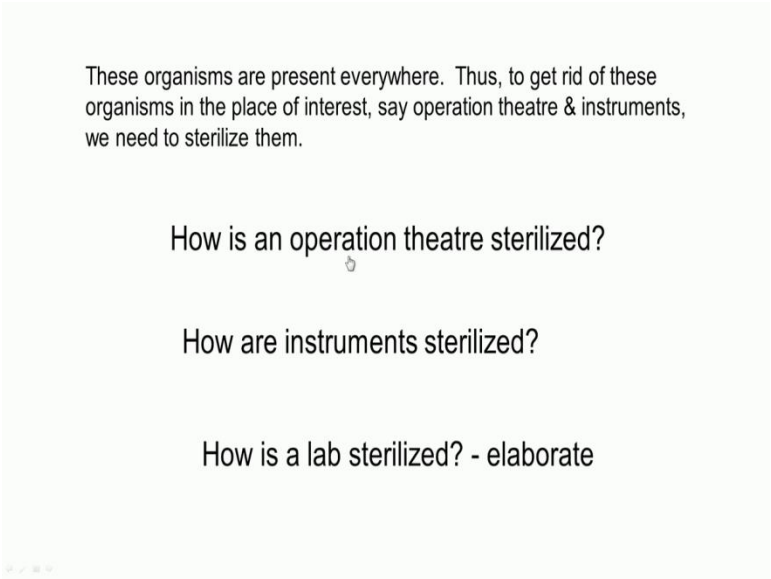

Then we talked of bioreactors; bioreactors are the production vessels. In fact, a bioreactor is a vessel, any vessel in which bioproducts are made, and there are so many bioproducts of use as we speak and there are many more potential bioproducts being developed.

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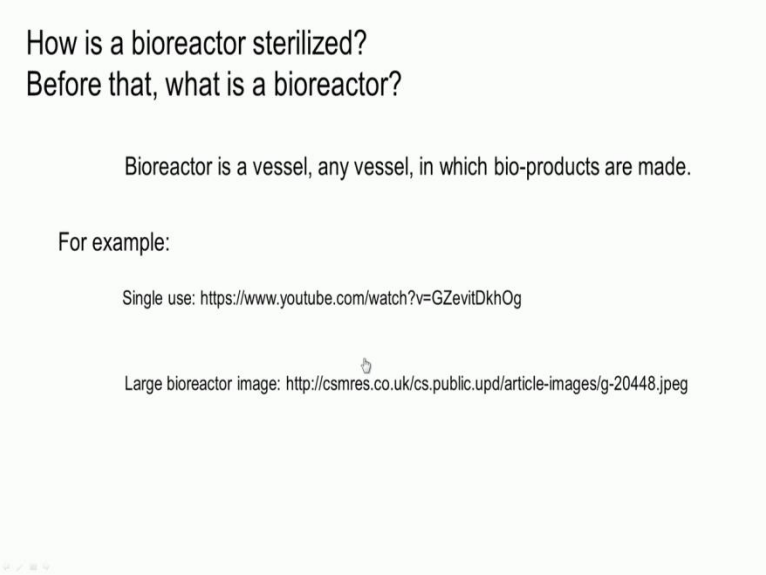


I had given you a video for a single use bioreactor and an image link for a large bioreactor, bioreactors can be very large depending on their application. And then I told you a bioprocess which is what is actually used to produce bioproducts, bioreactor is an important aspect of it.

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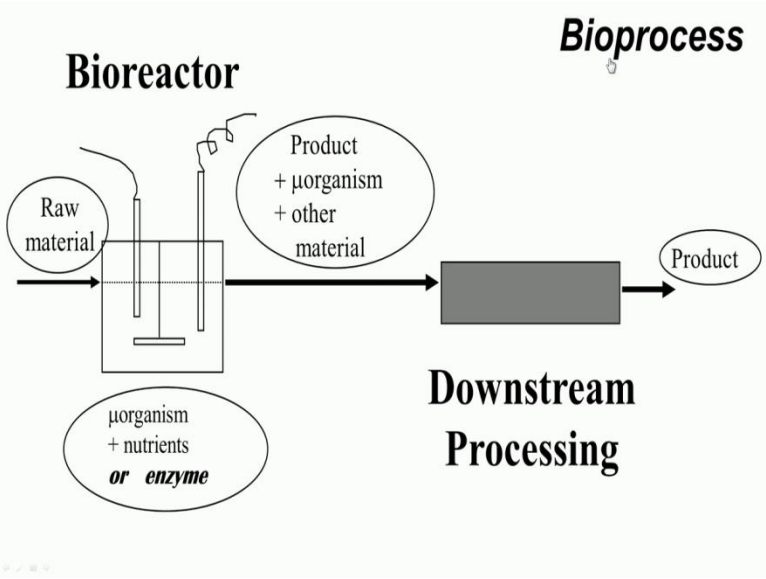

And then, we said that cells are subjected to shear in a bioreactor. This is going to be our second story. Our first story was about infection and so on so forth. We learnt that organisms cause infection and what organisms there are, how they are classified and so on. So our second story starts with shear in the bioreactor, okay?

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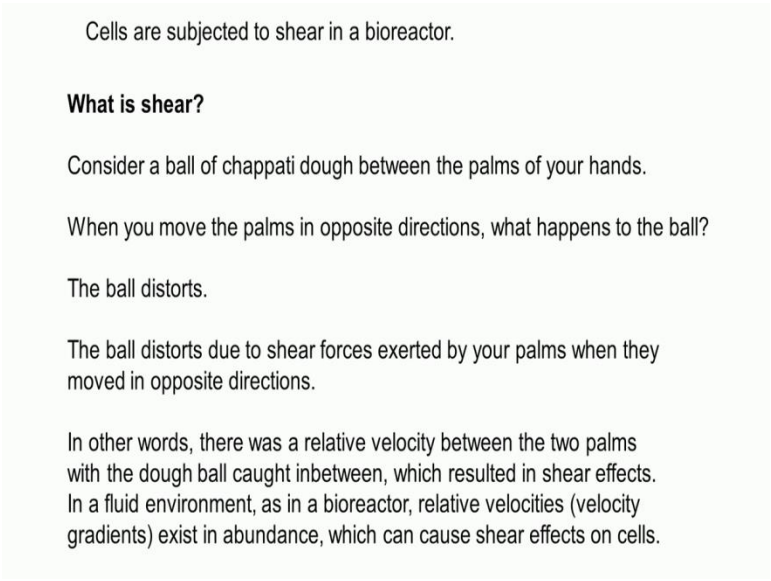


This term shear itself could be new, even to engineers who are students. If you have done, probably a degree in engineering, you would know what shear is or related fields, you would know what shear is. Let's start from the very basis, and then I'll tell you what shear is, that way we are all in the same plane. Let us consider a ball of 'chapati dough'. Okay? And place the chapati dough, puri chapati dough, in between the palms of your hands, place your palms in opposite direction. I think I've kind of step, given the various steps here. Slightly rest these palms and move the palms in opposite directions.

When you do that, what happens to the ball? The ball distorts, the chapati dough, okay, flexible, distorts. The ball distorts due to the shear forces exerted by our palms when they're moved in opposite directions. Okay? The ball distorts when, because of, what are called shear forces that are applied on the surface. These are exerted by the palms when they are moved in opposite directions.

Okay? In other words, if you have some background in physics, you would understand this a little more easily when there is a relative velocity between the two palms with the dough ball

caught in between, then it results in shear stress, or shear effects, as you can call. Right? So this is a requirement for shear to arise that there has to be a relative motion, which means one part of the dough ball in this case, the upper part needs to be experiencing a velocity that is different from the other end of the dough ball, let us say, and therefore there is a relative velocity between the two ends, and if that happens, there is a shear force that is experienced by the dough ball, okay?

In a fluid environment such as a bioreactor; bioreactor is a vessel in which bioproducts are made by cells. The vessel is typically stirred to keep the cells in suspension, it could be operated in many ways and so on, let us not get into that. So if it is stirred, there is going to be a lot of velocities, velocities in, speeds in different directions, different velocities of the fluid particles in the bioreactor. And such an environment is very rife for a lot of shear forces to act. So, in a fluid environment as in a bioreactor - relative velocities, or velocity gradients exist in abundance, which can cause, shear effects on cells.

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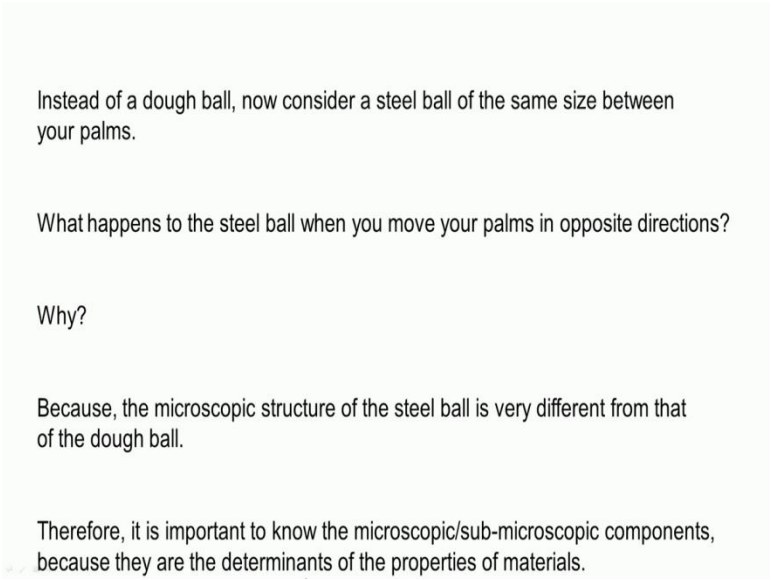


Now, instead of a dough ball, let us consider a steel ball of the same size between the palms. And what happens to the steel ball when we move the palms in the opposite directions? Pretty much nothing, right? It does not distort, in other words. Why? Because the microscopic structure of the steel ball is very different from that of the dough ball. You all know that. Dough is made up of something which we will, which you will probably be able to guess as a part of this course and

steel ball is made up of steel, it is made up of steel molecules or iron plus other combination and so on and so forth.

And the microscopic structure of steel is very different from that of the dough ball, and with the shear forces that one is able to exert, just by moving the palms in opposite directions, nothing happens to the steel ball. Therefore, it is important to know the microscopic or the sub-microscopic components because they are the determinants of the various properties of materials, right? This you would all be familiar with, different backgrounds, you would all be familiar with this concept that it is a microscopic structure, or a sub-microscopic structure and components and their properties that determine the properties of materials as a whole.

Now let us ask the question. What gets affected by shear in the cells? Let us say we know nothing about cells, we have been talking of various terms, I am sure, even in the other lectures by Dr. Madhulika Dixit, she would have mentioned a lot of terms, we will clarify all the required ones, in this, as a part of this course at different points in time. You do not have to worry about that.

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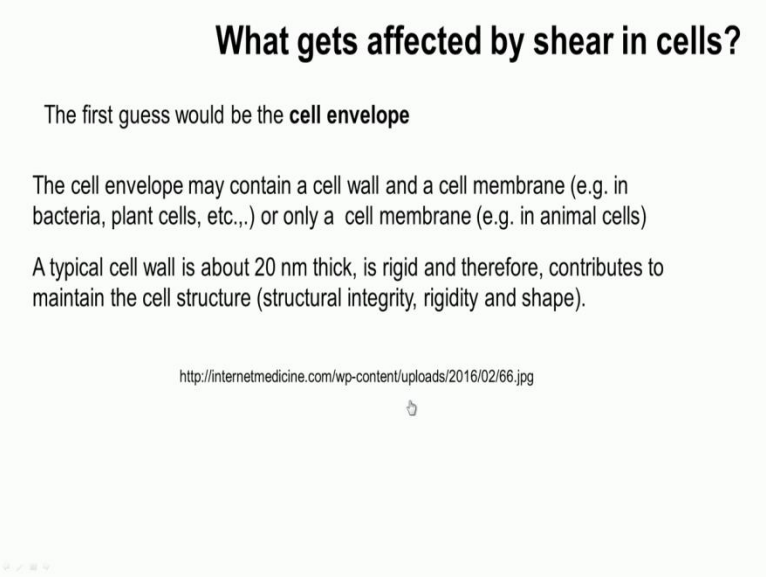


So what gets affected by shear in cells? The first guess would be the envelope that covers the cells, right? It is, it is a very rational guess. So what does the cell envelope contain?

The cell envelope may contain what is called a cell wall and a cell membrane. Remember these two are different things. One is called a cell wall, the other one is called a cell membrane, and both of them envelope the cell, right? The cell wall and the cell membrane both are found in some bacteria in plant cells and so on, or the cell wall could be completely absent, okay? Only the cell membrane distinguishes the cell from its surroundings, right? And that is the case with all our cells, all animal cells do not have a cell wall, they just have a cell membrane and that is what is exposed to the environment.

Typical cell wall is about twenty nanometers thick, okay? You can imagine the size, meter, then centimeter is $10^{-2}$ meter, millimeter is $10^{-3}$, micro is $10^{-6}$, nano is $10^{-9}$. This is twenty nanometers thick, is rigid and therefore contributes to maintain the cell structure such as integrity, rigidity, shape and so on and so forth, that is typically what a cell wall does. You could look at this particular link here; by the way I think this is the right time to show you the various links. I did mention a file, a pdf file that would have these links that can be clicked, I think control and a click would take you there. Let me show you that file. I think it is the right time to show you that. Right?

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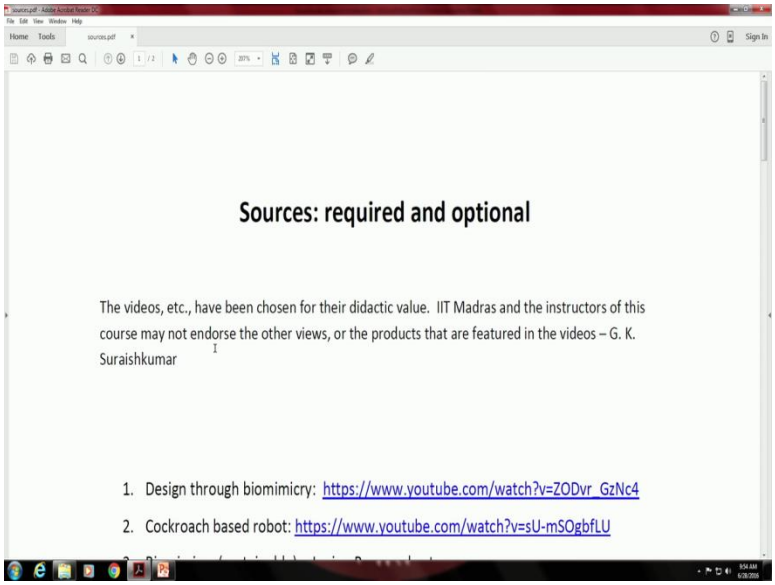


I, this would be available as a part of your resources, I think it is available right below or right above I think there, there is a link, a button on the right hand side of the video, when you play them. And if you click that, it will take you to this file. So these are sources, many of which are

required, and some of which are optional. I will point out which are optional. If it is, if I do not say it is optional- it is required, okay, required to appreciate the course better. As I mentioned, we cannot play these videos here, therefore we have given you the links so that you can go and take a look at them. The videos have been chosen for their didactic value, for their, for their value to teach.

IIT Madras and the instructors of this course, both Madhulika and I, may not endorse the other views that are expressed in the video or the products that are featured in the videos. Some of these very nice videos have been made by companies to sell their products. But we do not endorse the products.

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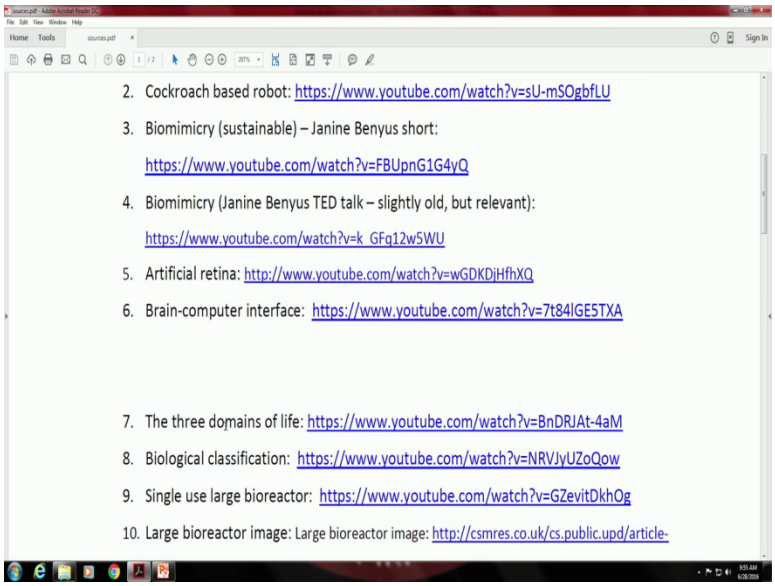


So we have seen all these videos designed through mimicry; I did not mention the second one, but please go through it, it's a very nice short video from the science magazine about a cockroach based robot, this is in the introductory lecture, and these were the various biomimicry lectures and then the artificial retina lecture, brain-computer interface lecture, and then the three domains of life, biological classification, single use bioreactor; these three were videos and the large bioreactor, this is an image, and the cell wall number eleven is what we are talking about right now. Okay?

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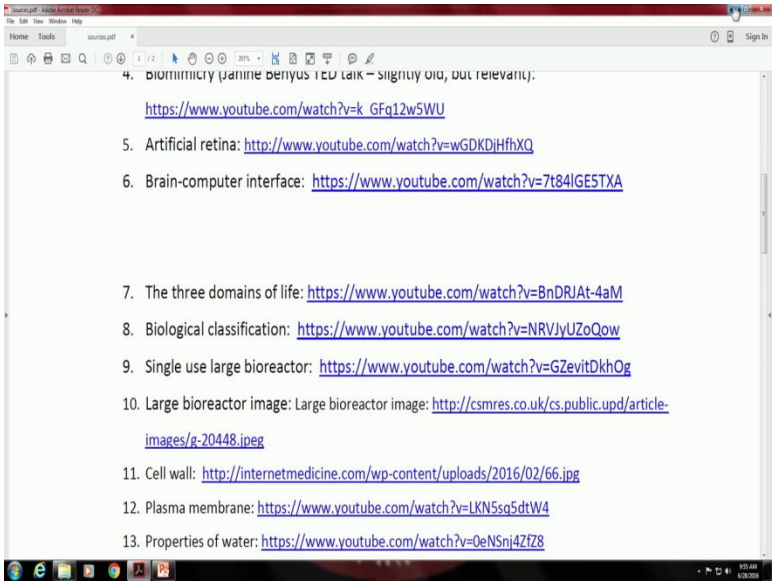


If you click on this, you will get an image that you could see. Let us get back. As a cell wall is predominantly made up of two kinds of molecules called ‘peptidoglycan’ and ‘teichoic acids’, okay?

This is the terms. I’m just going to float this around. Do not worry about the terms as of now. If it is important enough, we will go and see what it is; if it is not, just hear the terms so that if you hear the terms later, you will at least vaguely remember, and you know, I have kind of heard this somewhere. That is good enough. And if it is important enough, then you would be repeatedly exposed to it and you can pick it up.

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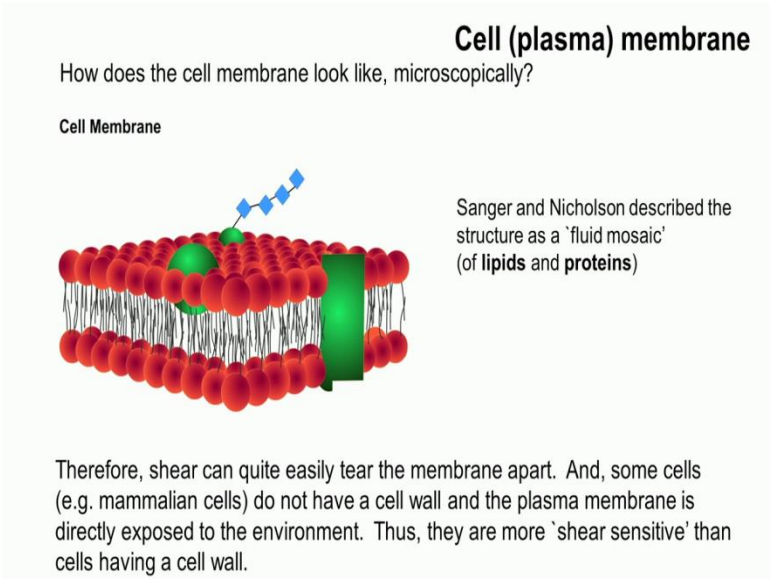


The plasma membrane; earlier we talked of the cell membrane, the, oh sorry, the cell wall, now let us talk of the cell membrane or the plasma membrane. This is where it is very interesting. How does the cell membrane look like, in a microscopic sense. It looks something like this, you know, this is a two-dimensional structure, this is shown as some sort of a, you know rectangular, a cuboidal kind of a structure. This is a cell, right, you take a small part of the cell, it look like this, and if you extend it in all directions and assume that it is covering some sort of let us say, a spherical shape or an oval shape, then you can understand how this fits in into the structure of the cell, right?

So you have the cell membrane consisting of two layers as you could see; the first layer is this circle here and some of these, the, lines here, the second layer is circle here and the other lines in the opposite direction, right? So in other words, it has a double layer here. It so happens that the nature of this is very different from the nature, the nature of the ball is very different from the nature of these lines here, we will come to that. In addition there are a few other things, there are these green things that are shown, and there are these blue things that are shown, we will get to that. The green things are actually proteins, we will see what proteins are, although we have heard some terms so many times, we will see what those proteins are. And these are carbohydrates that stick onto it, we will see what they are. Okay?

For now, let us focus on this double layer here, which has, if you take one molecule that comprises a double layer, it has this round head and a certain tail. Okay? So Sanger and Nicholson described the structure of a cell membrane as some kind of a ‘fluid mosaic’; mosaic is just these things put in together, so this is a mosaic of, what are called ‘lipids’. In fact, these molecules you know that we have been describing so much here, these are called lipids and there are proteins, and there could be other things also. So Sanger and Nicholson described this structure as a fluid mosaic of lipids which are these and proteins which are seen here. That is good enough for now, okay, that is what a cell membrane is.

Therefore shear, you know, can easily, can quite easily tear the membrane apart because they are all floating around, okay, you provide enough surface force, the shear is going to tear apart these lipids. And some cells such as, as I mentioned, the mammalian cells are cells, do not have a cell wall and the plasma membrane is directly exposed to the environment. And therefore they are much more shear sensitive than when compared to the cells that have a cell wall, okay, this is common sense. When you use these cells in the bioreactor, mammalian cells are used in the bioreactor for production of high value products such as monoclonal antibodies which are used for cancer therapy and so on.

And when you, when these cells are used in the bioreactor for production of these, they have, they are directly exposed to the various stresses because of the velocity gradients in the bioreactor and those shear stresses can break the cells apart. Okay? So what are these? Let us look at the nature of this molecule, it is an important class of molecules. Definition is very vague, okay? And that is the best that we can get, and let us start with the vaguest definition in biology pretty much.

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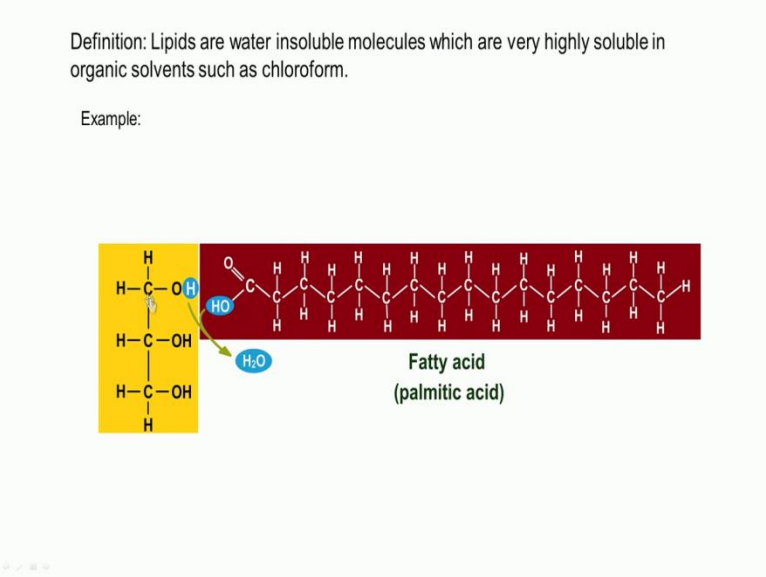


'Lipids' are water insoluble molecules which are very highly soluble in organic solvents such as chloroform, okay? I know it is vague, but let us stick to it. That is what a lipid is. And that is one of the four major classes of basic biomolecules that are present in life. Okay?

So for example, you all, okay let us take this molecule here, okay? This is a three carbon molecule, okay, if you know a bit of chemistry you would know this is glycerol, the one that is shown in yellow or some shade of yellow there, is glycerol. It has three OH groups here, three carbons here, and to one of these OH groups, a long chain carbon molecule with a COOH gets attached, okay? When this gets attached, the $H_2O$ gets out and the C joins with this O, so you have a CO, on the other side this O, on the other side, and this bond going onto this O to provide an example of a lipid, okay?

So you have a fatty acid, which is hydrophobic, and you have glycerol, which is hydrophilic and attached, you could in principle, get a lipid. C16 is the palmitic acid and all these bonds in between these carbon molecules are all single bonds. Therefore this is saturated fatty acid. Saturated fatty acid with glycerol is an example of a lipid. Now, instead of one fatty acid attached to one of this OH, what was initially OH of the glycerol.

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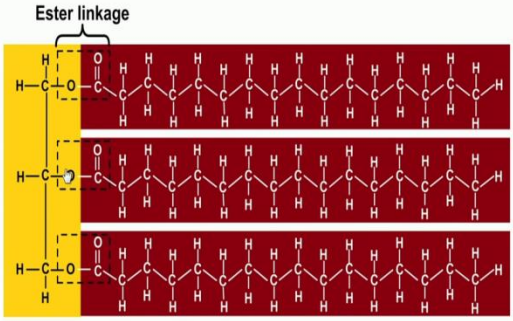


Triglyceride (fat)

If you have three fatty acids attached to the three oxygens of the glycerol through ester linkages, as they are called. Then you get fat. Right? All the fat that that causes a lot of social difficulty is nothing but this, okay, it is a triglyceride, you have three fatty acids attached to a glycerol molecule and that is your fat. Fat is a lipid. So fat causes whatever difficulties, and all that is a lipid. It is called a triglyceride, okay because you have you have glycerol, you have a glyceride bonds being formed here for three fatty acids and you have a triglyceride there.

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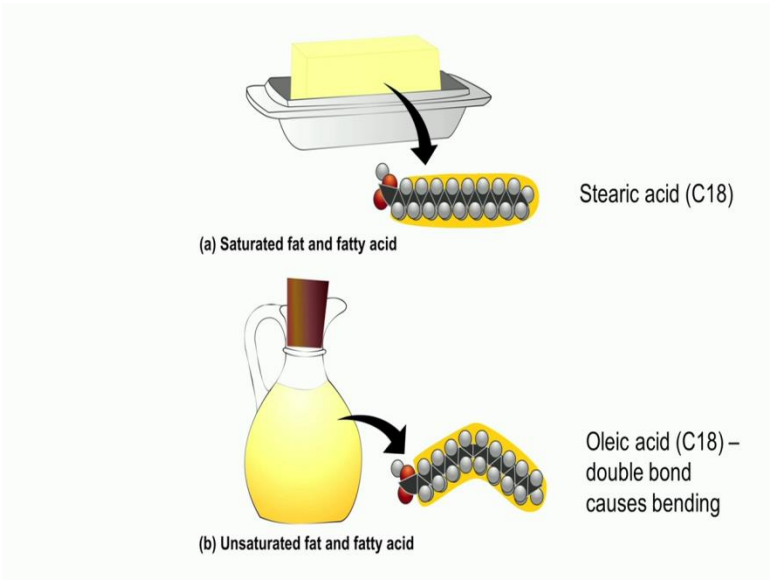


C18 stearic acid is nothing but butter, okay, it is saturated; if it is saturated you get butter. And C18, with a single double bond gives you oil.

Butter, saturated because all the bonds between carbon atoms are single bonds, and here one bond is a double bond, which actually introduces a kink in the structure and you have a fatty acid with single bond unsaturation, and then you get oil, okay? You know how useful these products are, and this is the molecular nature of these products. C18 is butter, C18 with a double bond is oil, unsaturated oil and so on, okay? All these saturated unsaturated terms that are used by doctors, actually refer to the nature of the bonds between the carbon atoms.

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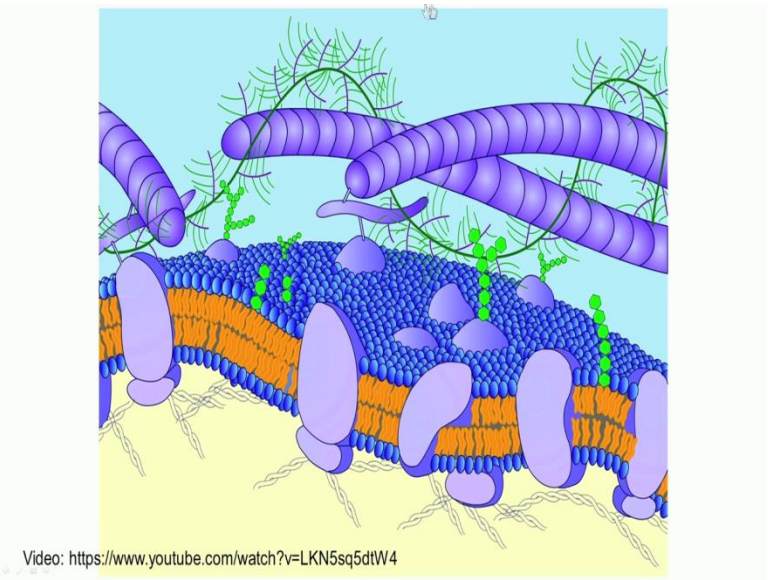


So this is the three dimensional view, let us just focus on some parts of it; there are many parts to it which let us not worry about. So this is the double layer plasma membrane as I said, it covers the cell, this is a part of the plasma membrane, assume that it is extending in various directions, and you see how it can cover the cell.

So this plasma membrane has a double layer of lipids with a lot of proteins sticking around a fluid mosaic of lipids and proteins, and then there are various other things which we will not worry about now. Okay? If you are interested, you could see this video, by clicking I think it is video number twelve, the item number twelve here, plasma membrane, you could see this, and that will improve your appreciation of the plasma membrane.

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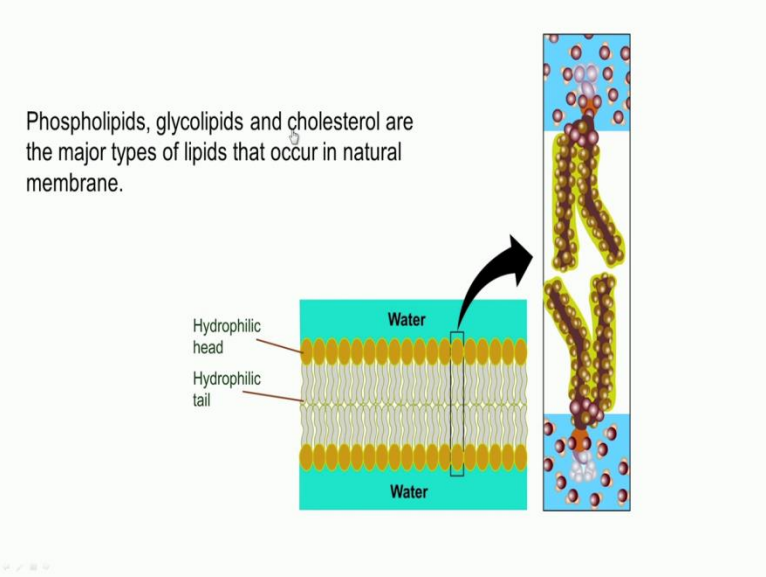


So let us go a little deeper, let us try to understand this a little better. So I am taking a part of the plasma membrane, a two dimensional view. So this is the bi-layer of lipids, you have the hydrophilic heads here, hydrophilic just means water loving, and therefore when it is exposed to water, it will tend to orient itself towards water, and you have these hydrophobic tails; in other words, this is a part, this could be a part of the glycerol molecule and this could be the various fatty acids, okay? Typically there are two fatty acids here and this is what has been blown up here, right, these are the fatty acids, this is probably saturated, this is probably unsaturated, and you have some other molecules here which are hydrophilic, they like water, and these are hydrophobic molecules, and this is a structure of one of this pair blown up, okay? Nothing else.

Phospholipids, glycolipids and cholesterol are the major types of lipids that occur in a natural membrane, okay? In other words, we, we saw the triglyceride, right, the glycerol molecule being converted into triglycerides. That was an example. In the phospholipids, this is different as we will see later. There is another set of molecules called glycolipids where this part is different and cholesterol which is completely different molecule, we will see that in a little while, okay? Cholesterol, is a very common, commonly known molecule, it was supposed to cause a lot of difficulty. People have, The American Heart Association has changed its view on cholesterol now, but it is popular anyway.

So you, it it will be nice to see what it is all about, okay?

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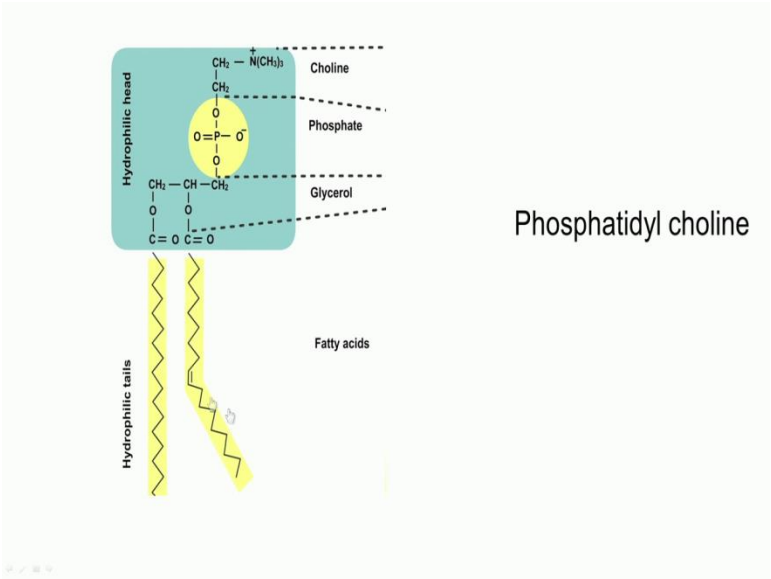


So this is a phospholipid, particularly phosphatidyl choline. These are the fatty acid chains, the hydrophobic tails that are here and this is the glycerol molecule as you can see here, the three carbon chain here and two of those O molecules are attached to the fatty acid.

The third molecule of O is attached to a phosphate group, '$PO_4^{-}$'group, and then there is a choline group that is on top of that, okay? This is called phosphatidyl choline. As you can see this is a hydrophilic end and this is a hydrophobic tail, so this fits into the various needs of a lipid. And this is an important constituent of natural membranes.

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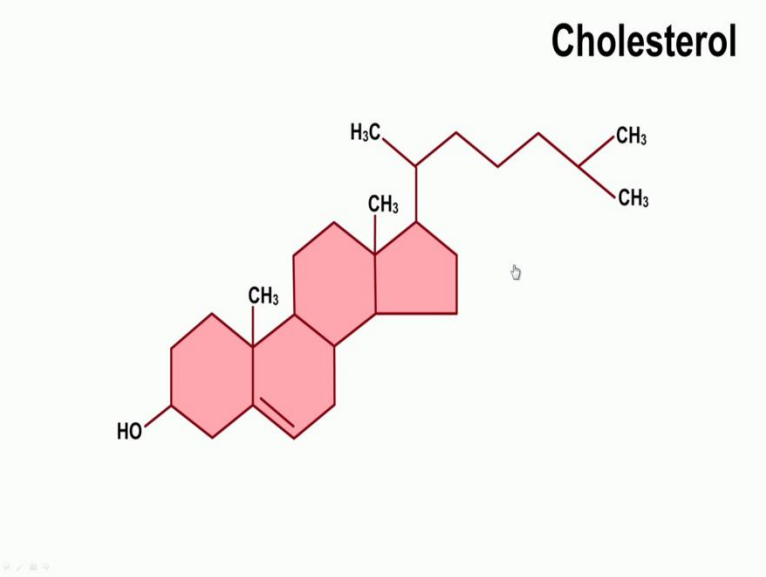


This is cholesterol, right? You see all this here, all this is the hydrophobic part, and this OH is the hydrophilic part, okay, and the cholesterol is also a lipid that doctors thought caused so many problems earlier.

How do lipids in the membrane determine shear sensitivity? How sensitive the shear is, There are various types of lipids. So depending on the types of lipids that are present, the shear sensitivity should vary, okay, because the properties of the constituents determine the overall properties of the whole. Right? So let us see how that is and let us see an application of that towards our needs.

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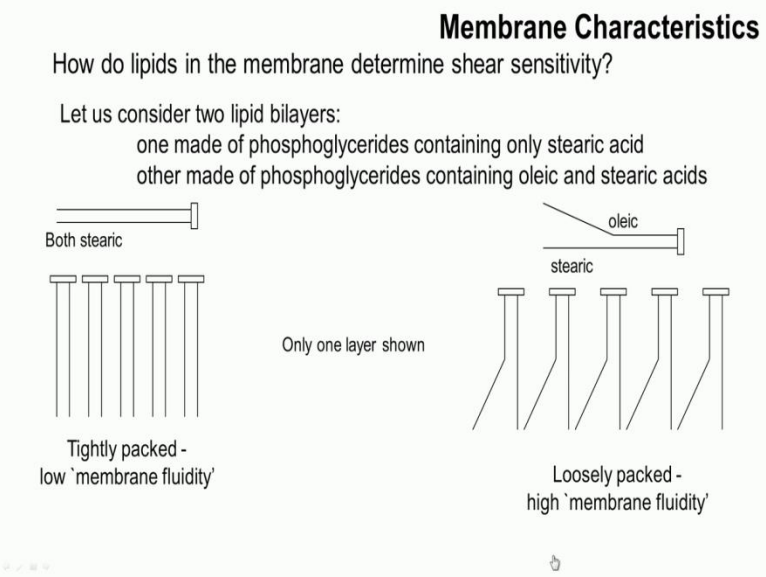


For that, let us consider two lipid bi-layers; the first bi-layer is made up of phosphoglycerides containing only stearic acid, okay, C18. C18 saturated; and the other is made up of phosphoglycerides containing oleic and stearic acids. Oleic, as you know has one unsaturation, stearic acid, has no unsaturation. Because of that, if both are stearic, okay, this, this is the hydrophobic tail, hydrophilic head; if both are saturated, such as stearic acid, the structure is going to be something like this. Whereas, if it is one oleic and one stearic, the stearic is going to be straight, the oleic is going to have a kink, and therefore it is going to occupy more space compared to stearic here, okay?

And therefore, if we tightly pack lipids containing both stearic acids, it is going to pack something like this, okay? Reasonably tightly packed, I am just showing one layer for clarity, you can imagine the other layer on the other side; whereas if we have both stearic and oleic present on the phosphoglycerides, then it is going to pack like this. The area that is the space that is occupied by each of this is going to be higher, therefore the packing itself is going to be much less dense than the one with both stearic acids, and therefore the 'membrane fluidity' is going to be high due to the loose packing, whereas here the membrane fluidity is going to be low due to the tight packing.

So it is quite easy to see that the tightly packed thing is going to resist shear much more than the loosely packed thing. A small amount of, smaller amount of shear force can tear this apart

compared to this, okay? So that is very simply how the structure of the cell membrane determines its shear sensitivity, and the structure of the lipids or the constituents the nature of the constituents determine the structure of the membrane and the structure determines its property. For example, 'membrane fluidity'; loosely packed structure will be more shear sensitive. Suppose we think of making the cells more robust to shear, okay, shear is our story here. Suppose we think of making the cells more robust to shear, what do we do?

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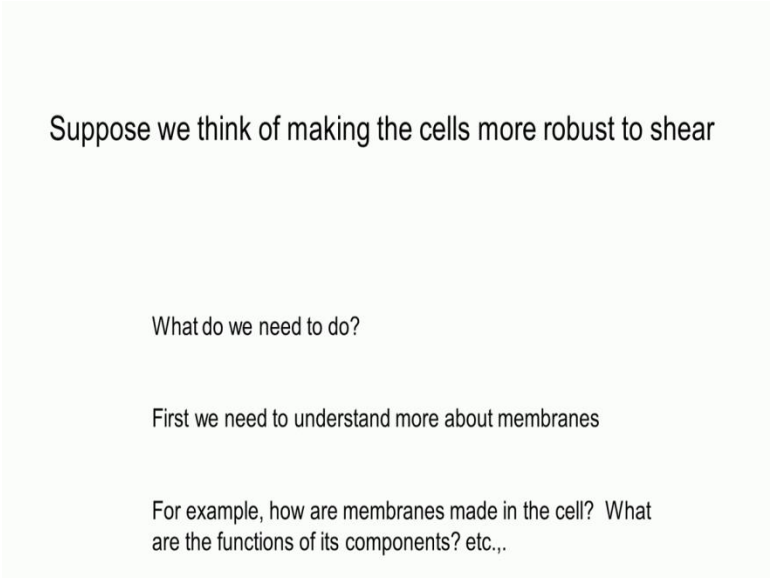


We need to understand, of course more about membranes and how are membranes made in the cell and so on and so forth, what are the functions of the various components, and then somehow make the cell to make or to generate the kind of lipids that we need to have a more densely packed membrane, and therefore more shear sensitive cell lines, or mammalian cells, maybe, okay, if you are going to use them in the bioreactor, right? So this is one way of thinking about it. Fine, I think we will stop here for this lecture, we will continue further when we meet next. Very briefly, in this lecture, our story was about shear. We saw what shear was, let me quickly go back to what we saw.

We saw what shear was; I told you in some detail what shear was and that it can cause damage to cells, especially in an environment such as a bioreactor environment which is full of shear, and the microscopic or the sub-micoscopic components need to be known because they determine the properties of materials, and then we ask the question what gets affected by shear in cells and we

came up with our first guess, a cell envelope which consists of a cell wall and a cell membrane. We very briefly looked at the cell wall and then we looked at the cell membrane in some detail. We saw that it was a fluid mosaic of lipids and proteins and we saw what are lipids, which I said was one of the four major classes of biomolecules and they are defined as, lipids are defined as water insoluble molecules which are very highly soluble in organic solvents such as chloroform.

And then we saw a few examples, our fats are actually lipids, and then butter is a lipid, oil is a lipid, and then we looked at some of the details of phospholipids, and cholesterol and there is another type, glycolipids and all these three are commonly found in natural cell membranes. Then we saw how the membrane characteristics determine membrane shear sensitivity, and we also looked at a case where we could possibly modify the lipids to modify the shear sensitivity. Let us meet again later and when we meet again, we will continue with another story. See you then.

**Biology for Engineers and Other Non-Biologists**
**Prof. G. K. Suraishkumar**
**Department of Biotechnology**
**Indian Institute of Technology Madras**
**Week - 02**
**Lecture - 06**
**Biomolecules: Carbohydrates, Water**

Welcome to the next lecture on "Biomolecules and the relationship to the structure and function of a cell." As we said, cell is the fundamental functional unit of life. We have seen two stories so far, the first one was about infection, what causes them and so on. And we picked up a few things that are fundamental from that story, such as microorganisms are there everywhere, the various types of microorganisms, the various types of cells, the two major types of cells and so on. And the second story was about shear, shear, in a bioreactor and the cells being exposed to shear, and what could we do to make the cells more robust to shear.

We said that we could do that by understanding what causes the shear sensitivity and when we looked at that we came across a fundamental biomolecule- one of the four major classes of biomolecules called lipids, defined in a vague fashion, its defined, lipids are biomolecules that are insoluble in water but soluble in organic solvents, let's leave it at that that doesn't matter and we saw what lipids are fat is a lipid butter is a lipid oil is a lipid and lipids make up the cell membranes. This is what we have seen so far, this is where we left off last time. This time, let us start with another story.

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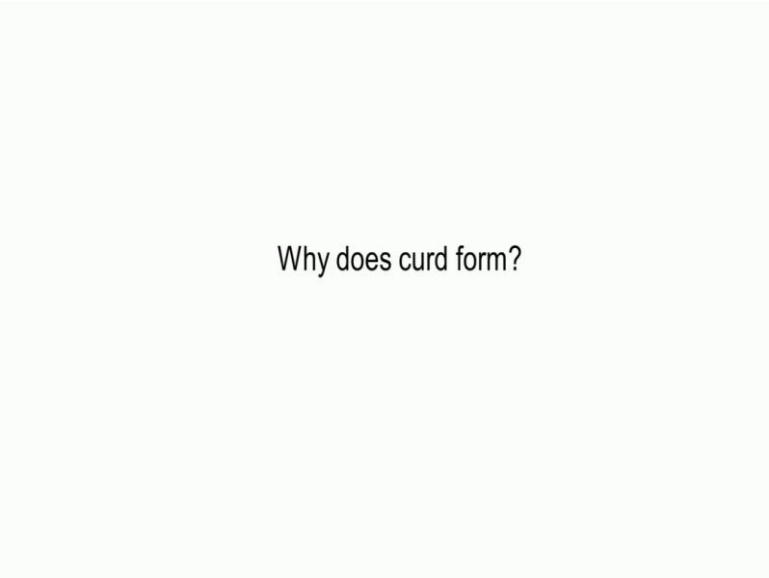


The story is rather simple in its inception but it goes on and it takes various branches, various forms, and we will probably learn some fundamental aspects from the story, various

fundamental aspects. We will keep going off-track from the story into some side stories and then come back to the main story, pretty much at the end of this module. Okay. Why does curd form, is the question that we are going to ask to begin the story. So before that how is curd made, curd or yoghurt as it is called in some countries, how is it made, it can be made at home, you take a vessel and you put some milk into it, right.

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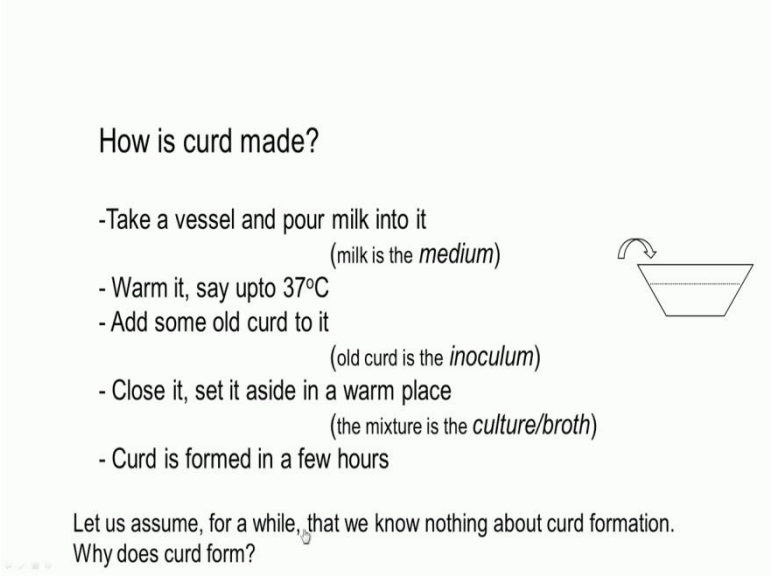


Take a vessel here, pour some milk into it, milk is called the medium; this is a term that is used in biology, biological engineering and so on. It's called the medium, warm it up to, let's say, 37 degree C in this particular case, add some old curd to it, okay. Old curd is the inoculum, as it is called in other term; don't worry about it if this term seems alien, you will get used to it. Close it, set it aside in a warm place, and the mixture that has this inoculum medium initially, and then something happens with the inoculum, something happens with the medium and all that is called the culture or the broth, in fact the, the broth changes as time goes on when it is set aside in a warm place and curd is formed in a few hours, okay.

The curd makers that maintain this curd at 37 degree C by plugging it into an electrical outlet you can even get curd in about an hour and a half hours, two hours; very nice curd gets formed in maybe a couple of hours, if a curd maker is used. At home it takes probably overnight or maybe a few hours if you do it in the morning and so on, okay. Let us use this as the basis for our story. Let us assume for a while that we know nothing about curd formation and ask the question, why does curd form. Let us investigate this just by using the tools that some of us might know, and some of us might be familiar with its basic, the tool is basically logic with some information, the scientific method, and so let us investigate curd formation

using this method. Since curd is forming from milk, something in the milk must be turning into curd, right, that's very logical.

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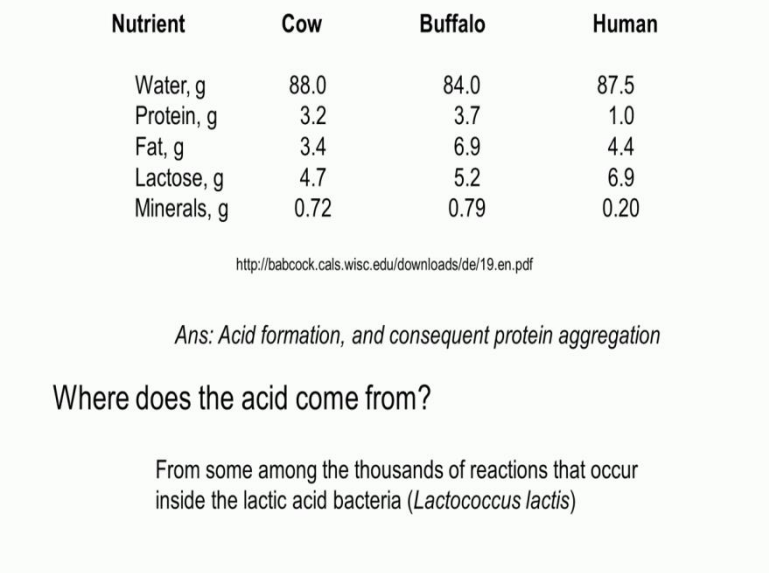


So what does milk consist of, if you look at the data in this particular case, this is the source of the data, you don't have to worry about this paper is this just additional information, if you're interested you can go and read this paper, it is not included in the list of references, but you could take it from here and read this paper.

Milk typically contains predominantly, water, protein, fat, lactose, minerals, predominantly here the cow's milk contains for every hundred grams, 88 g of water, 3.2 g of protein, 3.4 g of fat, 4.7 g of lactose and 0.72 g of minerals, okay that's cow's milk. Buffalo milk is slightly different and human milk is slightly different, okay. So this is the composition of milk. Since we are getting curd from milk, something here must be undergoing some changes to provide curd.

So what could that be? The answer is acid formation and consequent protein aggregation, is what causes curd formation, okay, this is the answer. Let me give you the answer right away, and then dig deeper. Acid formation and as a result, protein aggregation is what causes milk to turn into curd. Where does the acid come from? Okay, that's the question that we are going to ask. Where is the acid coming from? The answer is, from some among the thousands of reactions that occur inside what is called the lactic acid bacteria, in the terms of genus and species, it's called *Lactobacillus, Lactococcus lactis*.

This lactic acid bacteria *Lactococcus lactis* is what was present in the inoculum, the old curd that we added to the milk and each cell has thousands of reactions that go on at any given time, and from some of those reactions, acid comes. And that acid gets out into milk and causes protein aggregation and that when done in a controlled fashion gives you milk. (Again) same thing happens when you squeeze a lemon into milk, okay you form paneer, right, you form cheese, cottage cheese. That happens quickly when you do that slowly in a controlled fashion with a lot of other flavours that get released as a part of this process then it becomes curd, that's it.

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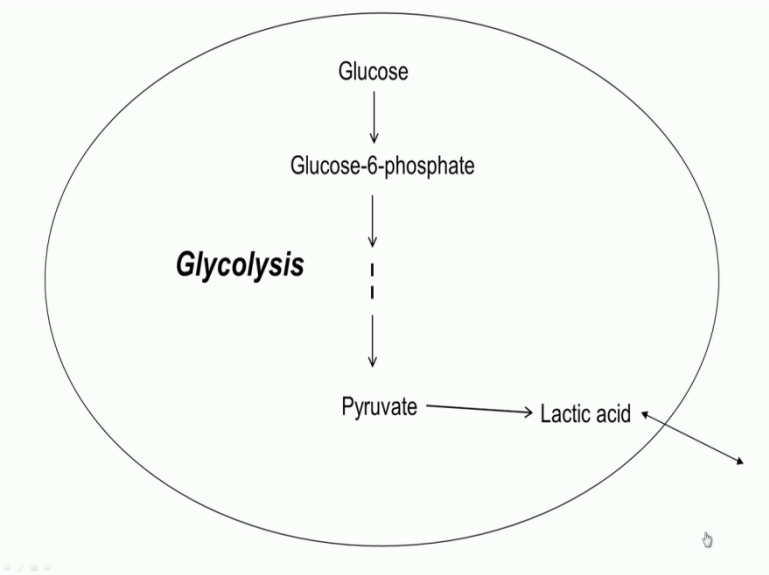


Let's dig a little deeper. So this is one set of one of the thousands of sets of reactions that occur in the cell, its typically starts with glucose, and this particular set of reaction ends with pyruvate, glucose gets converted to glucose six phosphate, gets converted to fructose six phosphate and so on and so forth until it gets with pyruvate. Pyruvate gets converted to lactic acid which gets out of the cell, this is the cell that is here, you could consider this as each *Lactobacillus* cell. This set of reactions, glucose to pyruvate is called glycolysis and each one is catalysed by an enzyme, we will come to that a little later. So glucose to pyruvate, has, these set of reactions has a name it's called glycolysis and as a result of this lactic acid gets formed as a result of two other reactions from pyruvate, lactic acid gets formed which causes acidification and formation and curdling of milk.

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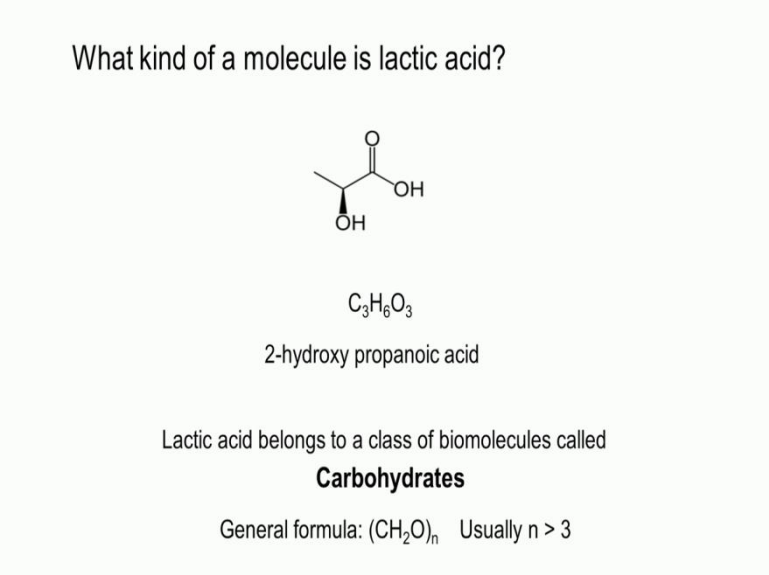


Now, what kind of a molecule is lactic acid which is what is causing the curdling? That's our set question. This is how lactic acid looks like, you see here these are the carbon atoms, here there are two OHs here, this is a COOH group here and this is an OH group here, okay, there are, this is a COOH and a OH group here. Therefore if you, if you write down the molecular formula, it is going to be $C_3H_6O_3$. You need to give importance for COOH, there are all the naming conventions that you could follow and so at the two position you have hydroxy group, therefore two hydroxyl, this is a C3, and therefore propanoic acid, okay, basic chemistry, organic chemistry. And therefore, this is a hydroxypropanoic acid, lactic acid belongs to a class of biomolecules called carbohydrates.

And what is a carbohydrate? It has the general formula $(CH_2O)_n$, you could represent it as $(CH_2O)_3$, then it becomes $C_3H_6O_3$ and so on. And usually n is taken to be greater than three for a carbohydrate. These are all usual things that happen, the normal things that happen. So, anything with a formula $(CH_2O)_n$ is a carbohydrate and carbohydrates are a large class or an important class of biomolecules. Earlier we saw lipids as one major class of biomolecules, now we are seeing carbohydrates as the second major class of biomolecules. And these are all present in the various, as a part of the reaction intermediates that happen in the cell.

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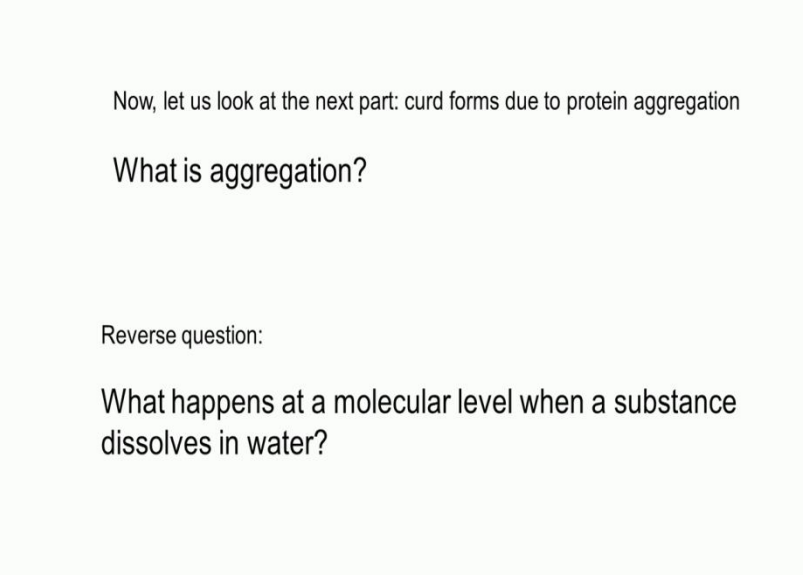


Now, let us look at the next part. We said acid formation and then protein aggregation, okay. Now what is aggregation? Okay, what do you mean by aggregation from a microscopic sense. To understand that let us ask the reverse question. What happens at a molecular level when a substance dissolves in water, okay? We said curd forms when these molecules aggregate, right, the protein molecules aggregate and get out of water. To understand that a little better let us, let us look at what happens at the molecular level when a substance dissolves in water.

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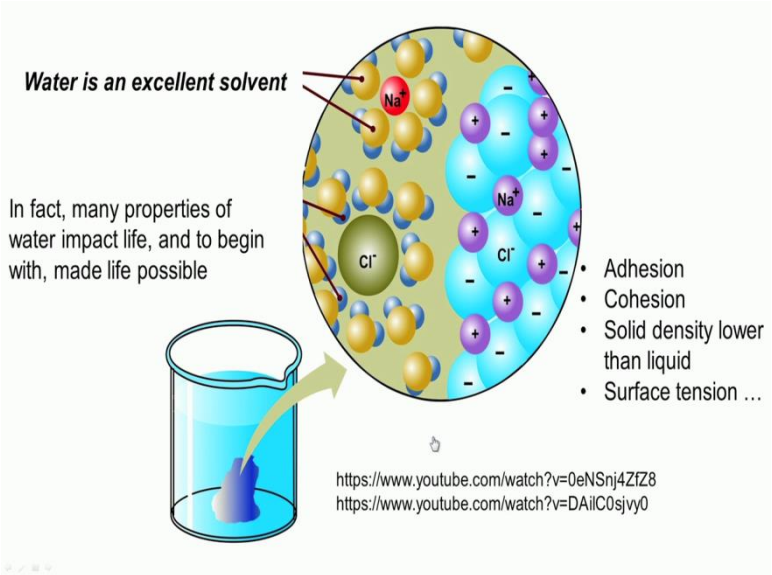


To understand that, let us look at an example of what happens when common salt dissolves in water. As we all know common salt is NaCl, okay. And when it is dissolved in water it splits

up into its ions Na and Cl and as you can see here, Na gets surrounded by a set of water molecules, Cl gets surrounded by another set of water molecules I mean another set of water molecules oriented differently. Na is positively charged and therefore a certain orientation happens to the molecules of water that surround it which is different from the orientation that happens to the molecules of Cl that surrounds it, okay.

So any substance that dissolves in water needs to have a set of water molecules that surround it, that's essentially what dissolution is at a molecule scale. Water happens to be an excellent solvent, you know we are (to) off to one of our side tracks in the story, water is an excellent solvent. In fact many properties of water impact life and make life possible to begin with okay, without water probably there won't have been a life as we know it. Why is that?

Water has very many important properties that make life possible, okay. Water, at a molecular level, has very many important properties. The first property is cohesion which is water molecules sticking together. Because of the hydrogen bonds between the various molecules of water they tend to stick together a lot more than probably many other substances, many other compounds. So cohesion is a very important concept. So cohesion, is sticking together of the same kind. Adhesion is water sticking onto other surfaces.

And adhesion to and cohesion together, determine a lot of things that happen with life okay. I will give you a video which very nicely explains how adhesion and cohesion are entirely responsible for the way the water gets distributed to various parts of tree, and what happens in various other things that, of life that are relevant for water and relevant in the context of water and so on. So let me not get into this for the time being but adhesion is the interaction of water molecules, the stickiness of water molecules to other surfaces, cohesion is sticking together of water molecules themselves, okay.

This results in a high surface tension, for example, cohesion and makes even some insects to be able to walk on water, the water strider and so on. This third one is a very important aspect, the solid density; the density of ice form of water is lower than the liquid density, okay. The water has highest density at around 4 degree C that we know. And water becomes a solid ice at 0 degree C. When it becomes a solid the structure of water actually opens up because it needs to have a crystal structure with the water molecules being kept at a certain distance because of thecrystal needs, and the density actually goes down a little bit, and therefore the solid ice density is lower than the liquid density, is very rare. Not many other

substances in nature have this property where the solid is, solid form is less dense than the liquid form, at least at certain temperatures.

What happens because of this in cold countries, ice forms right, ice forms on lakes, rivers and so on and so forth. And when ice forms because of the lower density of ice, ice floats. It doesn't sink. Because it floats, the water below can support life that depends on water. If it solidifies probably it won't be able to support life. And so this very property that the density of the solid is less than the density of liquid is what makes water, what makes life possible in all those water bodies, lakes, rivers and so on and so forth, in winter.

We talked of surface tension and so on as an outcome of cohesion, and I would like you to watch these two videos, you can take these as compulsory, which explain nicely how these properties determine life, how these properties of water determine life and why water is such an important molecule which makes life possible, okay. I think these are given in, under item 13, these two videos both are under item 13. Please go and take a look at these videos, they are very nice videos.

Okay let's continue. Now we said that if a substance dissolves in water, it means that it is surrounded by a layer of water molecules, okay. So if a protein is dissolved in water as in the case of a protein in milk, then there is a layer of water molecules that surround the protein and keep the protein in solution, right.

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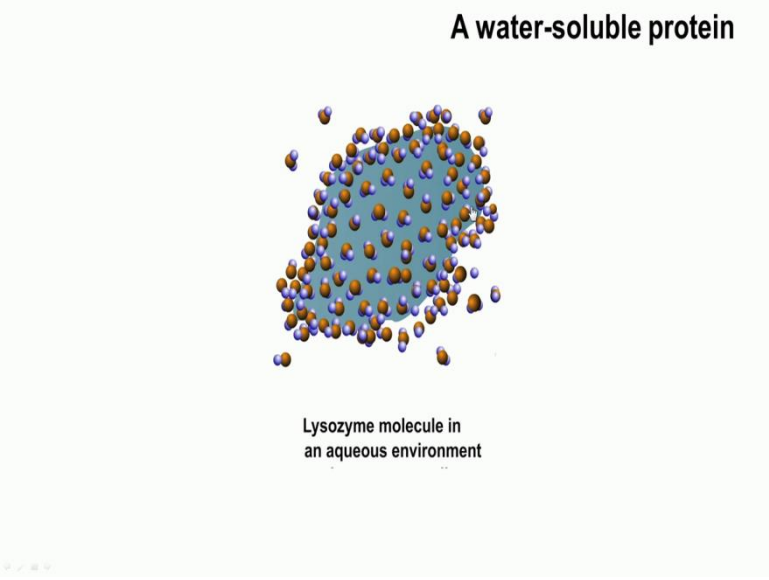


This is an example of a protein called lysozyme which is an important protein in the human body. This lysozyme is in an aqueous environment, water environment, aqueous is water,

aqueous environment and it therefore it is a let us say, dissolved in water and which means that is surrounded by water molecules. So we looked at what happens when a substance dissolved in, is gets dissolved in water, but our original story was why curd forms and we said acid formation, we saw acid formation earlier, and which causes protein aggregation, so let us look at protein aggregation. Now that, now that we understand why something is in solution, aggregation happens when molecules fall out of solution, okay.

They are, they get attracted to each other because of the lack of water molecules that surround them and then they come together and fall out of solution. That's typically what happens when a substance falls out of solution. The reverse happens when the substance goes into solution, and when there is not enough water surrounding it, and they attract each other, then it falls out of solution.

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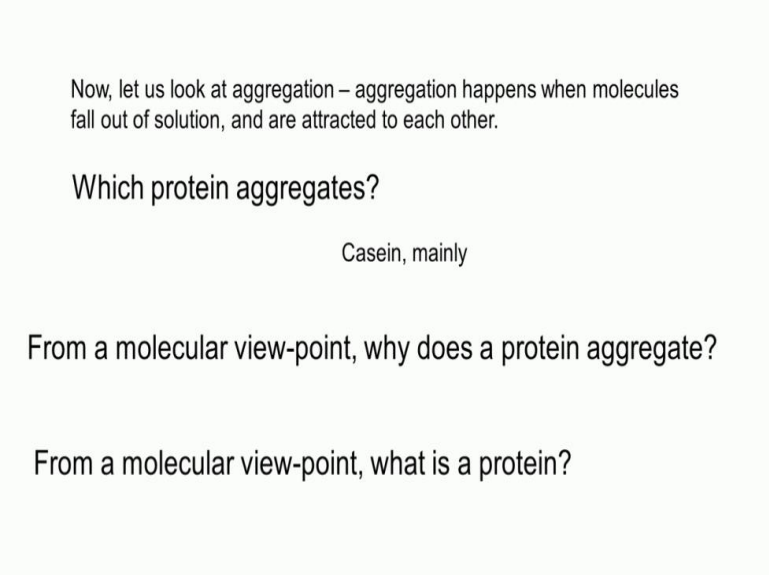


Which protein aggregates? In the case of curd formation, the answer, many of you may know it, it's called casein, this is the protein that mainly aggregates when curd gets formed. From a molecular point of view, why does a protein aggregate, that's the next question that you're going to ask.

And before that, we realise we don't really know what a protein is, and therefore we are going to ask the question, from a molecular point of view, what is a protein, okay. And I think we will stop here, this lecture we looked at carbohydrates and then we looked at what happens when something dissolves in water and some properties of water. I think that is good

enough information for now, fundamental information for now. You munch on it, and then (we) when we meet next, we will take things forward, see you then.

**Biology for Engineers and Other Non-Biologists**
**Prof. G. K. Suraishkumar**
**Department of Biotechnology**
**Indian Institute of Technology Madras**
**Week - 02**
**Lecture - 07**
**Biomolecules: Amino Acids, Proteins**

Welcome to the next lecture in this module which is on biomolecules and their relationship to cell structure and function. We are progressing in terms of stories here in this module. We are into our third story and some (side) side stories and as a result of these stories we are picking up some basic information.

Our first story was about infection and so on, the second story was about sheer in bioreactors which led us to lipids and what lipids are and things like that, one major class of biomolecules. The third story is why curd gets formed which led us to carbohydrates because curd gets formed by acid formation that leads to protein aggregation, what carbohydrates were - the second major class of biomolecules and we stopped at the point where we reached the third major class of biomolecules which happens to be proteins, proteins or amino acids as you call it. You will know the difference very soon.

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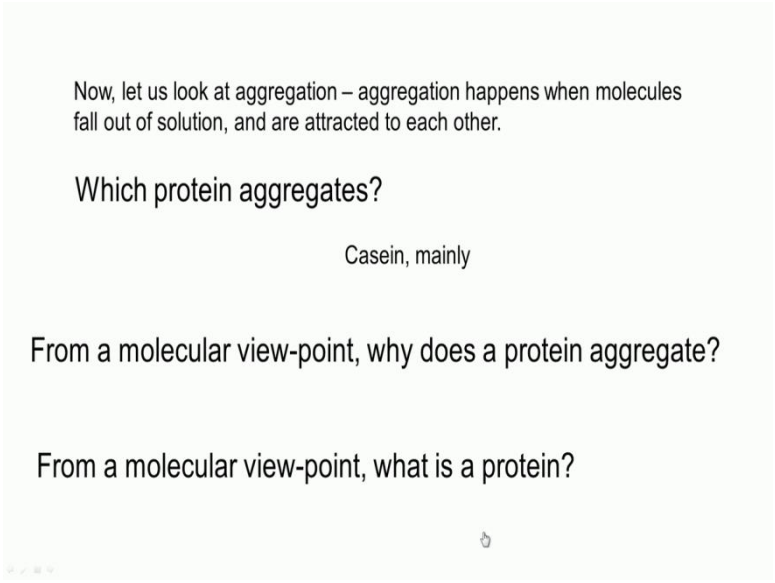


This is going to be a third major class of biomolecules, totally there are four. So in the last class, last lecture we left at a point, from a molecular viewpoint, what is a protein.

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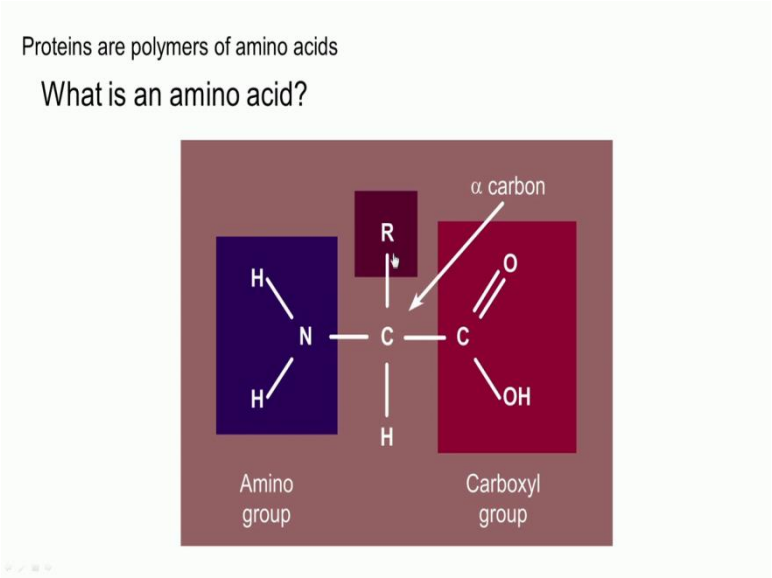


Proteins are polymers of something called amino acids, okay? That's what proteins are. They are polymers of amino acids. And what is an amino acid, okay? It's very simple; this amino acid is very simple. You have the central carbon atom here an alpha carbon atom. This is called an amino acid, right?

So you have an amino group here and a carboxylic acid group here. So an amino acid, the amino group is an $NH_2$ shown in blue here, the carboxyl group is a COOH which is shown in red here, okay? So, these two are attached with the opposite ends of the carbon molecule, the alpha carbon molecule, here. In addition you have a hydrogen atom here at the third and something called an R group as the fourth bond of the carbon, you know that carbon valency is four.

So you have amino group, carboxy group, carboxylic acid group, so amino acid comes from that and you have H and various R groups. So differences in the R groups are what gives rise to the differences in the various types of amino acids, right? And you have polymer of these amino acids, then you get a protein.

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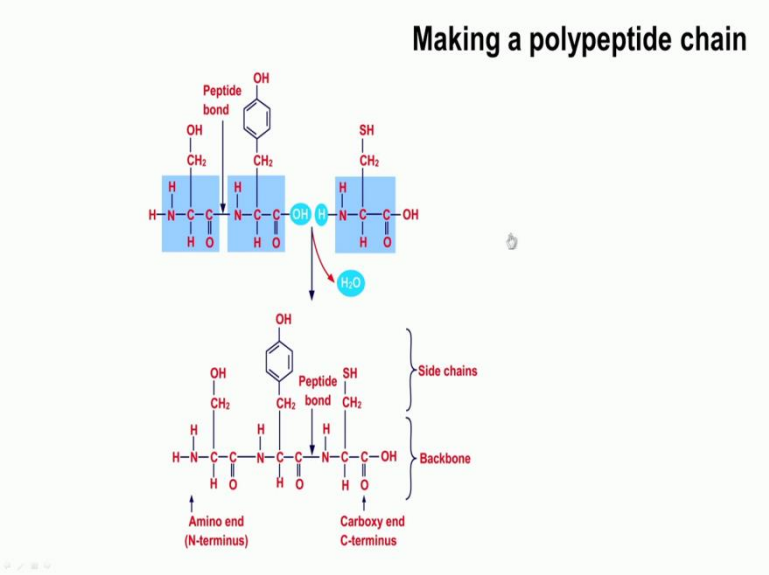


That is what is described here. A polypeptide is a small, small protein you can say, okay let us say that for approximately now. A polypeptide is nothing but a shorter form of protein. A polypeptide formation is given here to illustrate how a protein gets made. As you can recognise here, this is one amino acid, and the R group happens to be $CH_2SH$, right? When this meets, let us say, let us just focus on this amino acid here, which is already combined to another amino acid through what is called a peptide bond; let us look at the interaction between this amino acid and this amino acid.

This has a COOH group here this carbon atom; the $NH_2$ group here which is actually bonded on through the peptide bond to the other amino acid. The H group here and the R group in this case happens to be the CH2 OH across a (benzene), across an aromatic ring here. So when these two interact this OH of the COOH here, and this H of the NH2 here, condense out, the water comes out and it results in the formation of the CN bond here, the bond between this and this, this is called the peptide bond, okay?

This carbon carboxylic acid $NH_2$ this forms the backbone, and these various R groups form what are called the side chains of this protein molecule here as a part of these various amino acids. And also it's quite easy to see that there has to be one free end here of amino group, okay? If you assume that the growth happens this way of various amino acids attaching to form larger and larger polypeptide chains and the proteins and so on, so you have any protein will have one free amino end, okay, which is called the N terminus, and one free carboxyl end, carboxylic acid end which is called the C terminus. So a protein has a C terminus and an N terminus, it has a backbone consisting of the various peptide bonds and carbon atoms,

carboxylic acid molecules and so on. And the side chains that are part of each amino acid R group that constitute the protein. This is how a protein gets made from the various amino acids.

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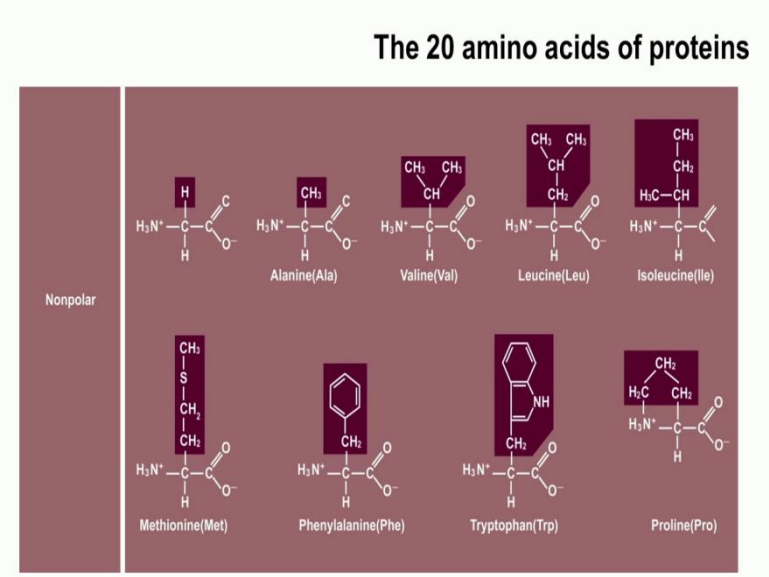


It so happens that there are twenty R groups, that exist in nature and therefore there are 20 different types of amino acids that exist in nature, right? There could be other types of amino acids, but they are not naturally occurring amino acids. And therefore there are 20 different types of amino acids in the proteins which are essentially polymers of amino acids. This has been grouped under these various heads depending on the nature of this R group here, okay? If the R group happens to be a nonpolar group, then it is grouped under this head. For example, this H group, CH3 group, CH3 and so on. I think there has been a slight shift here. That doesn't matter. Don't worry about the names now.

In other words, all these are nonpolar groups, the R groups and therefore they are grouped under this. Let's count the number of amino acids – nine of the 20 are amino acids with nonpolar R groups.

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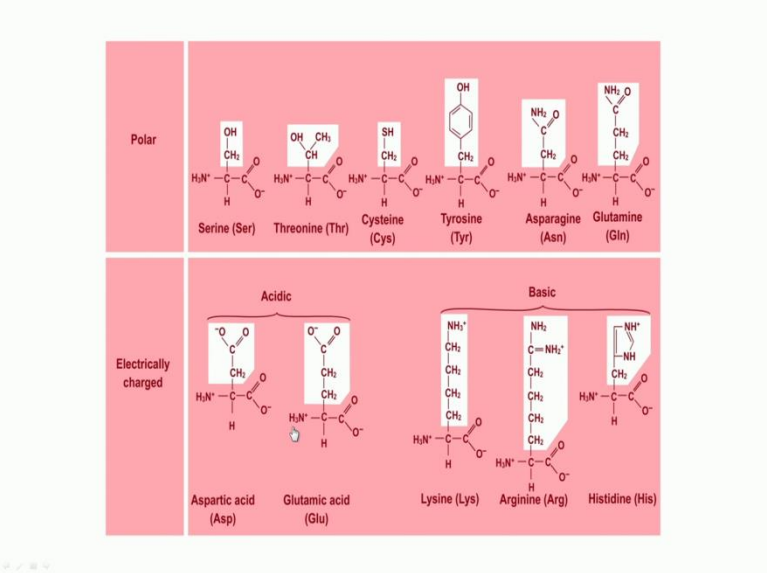


There could be polar R groups, such as $CH_2OH$ for serine, CH $CH_3OH$ which is threonine and so on. These are all amino acids with the polar side group and this has also been categorised as the side groups that are acidic and basic, if they are acidic and basic they need to be electrically rather the electrically charged groups are the ones that are on aspartic acid and glutamic acid and the basically basic groups are the ones that are on lysine, arginine and histidine.

You don't have to worry about the details here, I just mentioned the various things, as and when necessary, we can look at individual amino acids depending on our need, and a look at their properties. But these are the properties of the side groups that determine the properties of the proteins themselves. So we saw amino acids with nonpolar groups, we saw amino acids with polar groups, and electrically charged groups which could either be acidic or basic, that's good enough. Don't worry about anything else for now, you don't have to remember any names, don't worry about it.

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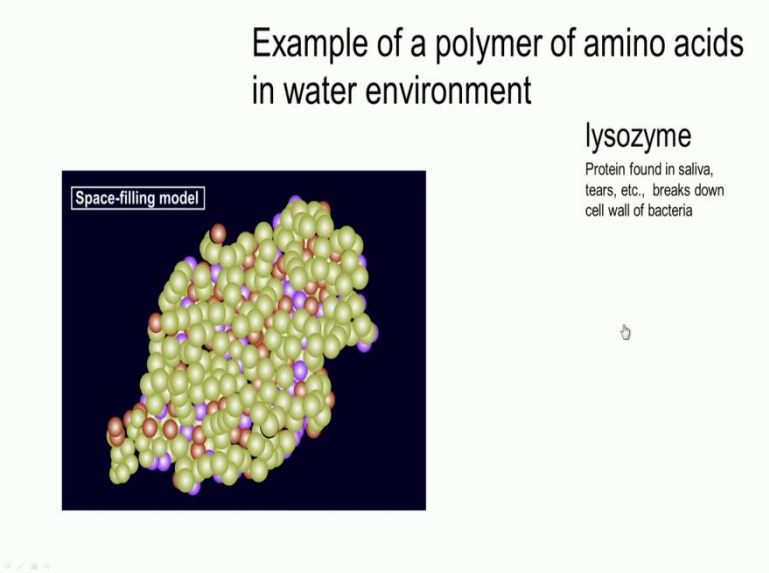


So this is the example, an example of a polymer of amino acids in a water environment, space filling model of lysozyme, water environment, this is surrounded with water, it has to be in water. This lysozyme, it's actually a protein that is found in saliva, tears and so on; and it also breaks down the cell wall of bacteria.

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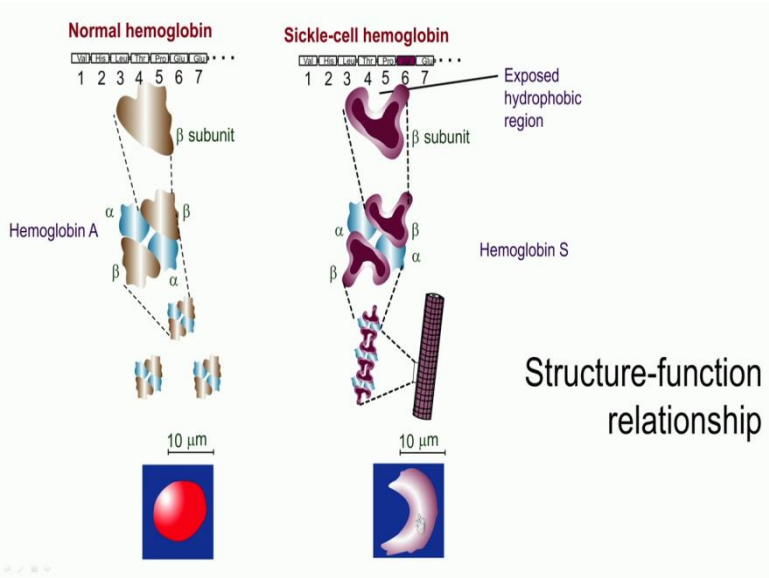


The nature of the amino acids, actually determines the nature of the proteins. Let us take an example here in the case of haemoglobin. The haemoglobin consists of various different amino acids here a long chain of amino acids all connected together by peptide bonds, okay? Some of these are given here, valine, histidine, leucine, threonine, proline, glutamine, glutamic acid glutamic acid. And because of this it forms various units and four units need to

come together to form haemoglobin molecule and if its folds correctly, we'll come to all these things later, the folding and so on. If it folds correctly, then it will do its job properly, right? It's folding correctly is what is going to result in a functional red blood cell which can carry haemoglobin, okay? If there is a slight change in the amino acid sequence here, for example this glutamic acid, (glu) at the sixth position, has been replaced with something else.

Then we get a disease, we get what is called sickle cell anaemia, right? That's the only reason why we get sickle cell anaemia; this glutamic acid at the sixth position has been replaced by another amino acid. What happens to that? What happens as a result of that? The, we get a disease that's the result. Why does that happen? That is because; there is a lot of interaction that happens between the various parts of the protein, the various amino acids in the protein, okay?

We all saw that the amino acid, the side groups of the amino acids could be so different. They could interact with each other depending on their chemical nature and you have a long chain of amino acids, so one amino acid here on one side chain could find another side chain attractive here, and therefore it will start interacting here as a result it will bend the entire protein here, right? This part of the protein would be bent because this side group wants to interact with this side group, okay?

And that is what results in protein folding by itself, okay? Its (it) happens spontaneously, because of the need for the chemical interaction between the side groups. And this folding, the way it folds because of the various side chains and so on so forth, is what gives protein its functionality. If that doesn't fold properly, then the protein will not be functional. And that is what happens here, in the case of normal haemoglobin it folds properly to carry haemoglobin and you have a functional red blood cell.

Just one change here, glutamic acid going to some other amino acid, changes the structure, changes the folding of the haemoglobin protein and thereby changes the structure of the haemoglobin protein, as a result - a sickle cell structure of the red blood cell results which is unable to carry oxygen through haemoglobin. And that results in sickle cell anaemia.

So the structure function relationship is so pronounced in the case of proteins, a proper structure is what results in proper function and this is a very nice example of that. I think we will stop here for this particular lecture, we saw, we started out by asking what proteins were, then we said that proteins are polymers of amino acids, then we saw what amino acids were,

it has a central carbon atom, an amino group on one side, a carboxylic acid group on the other side, so amino acid, and then you have a H group here, and various different types of R groups, one for each amino acid on the other side.

Then when you put all these together you have the protein and when you put all these together, there is a need for interaction of one R group with the other and probably other kinds of interactions with the water around it and so on so forth. Therefore the protein molecule folds and the folding of the protein molecule is what results in its function. And a very striking example is the case of haemoglobin in the normal case it results in the normal ability to carry oxygen to various parts of the body, a normal red blood cell.

In the case of one change at the sixth position, the amino acid being different, we get sickle cell anaemia, that is because of sickle cell haemoglobin being formed, which has different folding and therefore different structure, it does not have the functionality that is associated with the normal haemoglobin. And it also results in a sickle cell shape of the red blood cells and the disease. We will stop here; we will continue our stories further when we meet next. See you then.

**Biology for Engineers and Other Non-Biologists**
**Prof. G. K. Suraishkumar**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Week- 02**
**Lecture - 08**
**Biomolecules: Enzymes**

Welcome. We are discussing biomolecules and their relationship to cell structure and function. We have our own stories through which we pick up these things; we have had a few stories so far. The main messages from the story so far has been that there are microorganisms they can be categorised into various different domains.

The major biomolecules, I keep mentioning this although I haven't told you everything so far, the major biomolecules are four, we first saw lipids which are water insoluble substances that are soluble in organic solvents, reasonably vague definition but that's what it is, we saw some examples of it. And then we also said that lipids form an important component of the membrane, of the cell membrane, cell membrane is an important cell envelope; some cells have only a cell membrane to distinguish themselves from the environment, some cells have a cell membrane and on top of that a cell wall too, and maybe there are other layers, that we're not talking about now, let's not get into that.

The nature of the lipid itself makes it possible for lipids to act as lipid bilayers and therefore to act as effective envelopes between the cell and their surroundings. I probably briefly mentioned that in the passing earlier, let me talk a little bit about that and then go further. The, if you look at the way the lipids have organised themselves in the membrane there are, let's go here it might be a better picture; there are lipid bilayers.

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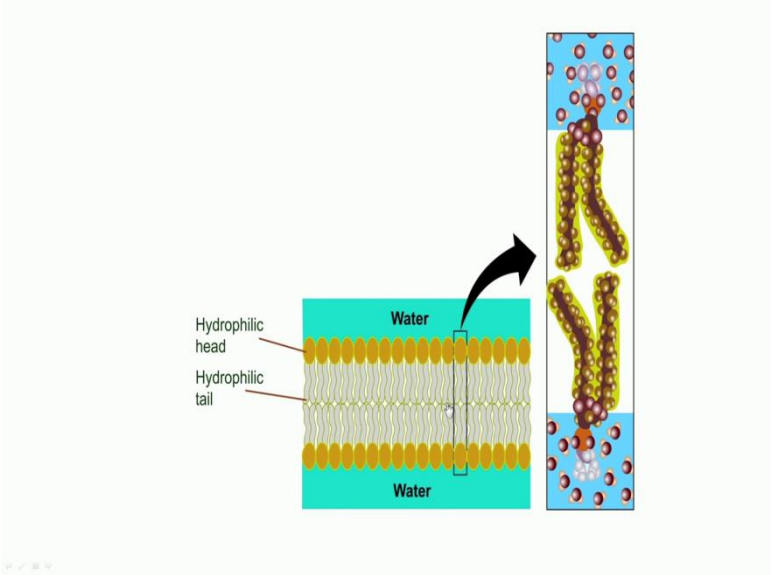


This is water extracellular. This is water intracellular, right? And this membrane in three-dimensions, separates the intracellular the extracellular. If you look at the orientation of the lipids here, the hydrophilic heads are oriented towards water naturally because the hydrophilic means water loving and they would like to be oriented towards water. And hydrophobic, like likes like, therefore they orient towards each other and by their very nature there is a hydrophilic layer here, there is a hydrophobic core here, and a hydrophilic layer here, separating the intracellular from the extracellular parts.

And this bilayer can self-assemble, nobody needs to put in any energy to assemble these layers by the very nature of their molecules, here is water, here is water and therefore the water loving parts will orient towards water and the water hating parts, the hydrophobic parts, this is hydrophobic here, hydrophobic part will come together here. If we think about it, this forms an effective barrier to the cell from its surroundings. Substances cannot very easily pass through the membrane; molecules cannot very easily pass through the membrane because of the nature of the membrane.

Here you have water loving-water loving so that's okay. But when water loving molecule comes here it needs to pass through a water hating core and the rates of pass through the membrane would be slowed down significantly. In a typical cell, there are actually proteins, you know the, cell membrane is fluid mosaic of these lipids and proteins, some of those proteins actually function to transfer substances from the outside to the inside, glucose goes from outside the cell to the inside of the cell, through some such proteins and many other

things have transporters that make it possible for such substances to move from the outside of the cell to the inside of the cell at reasonable rates.

So by the very nature of these molecules they act as effective barriers to outside components and they protect the cell. So this is one of the important structure function relationships compared to lipid. We have already seen that proteins, amino acids, polymers of amino acids are proteins and they are a large class of important biomolecules in the cell and there is a structure function relationship, one of which we have seen.

By the very nature of amino acids and the polymerisation of amino acids into proteins, different parts of the proteinaceous chain would attract depending on the side groups that are there and their interactions with water, all these combined, there could be interactions between molecules that are on different parts of the chain. Because of that the protein molecule folds, and because of the proper folding the protein molecule is able to act as an enzyme or even carry out its other functions, non-enzymatic functions and so on. So by the very nature of the polymers of amino acids they get their function.

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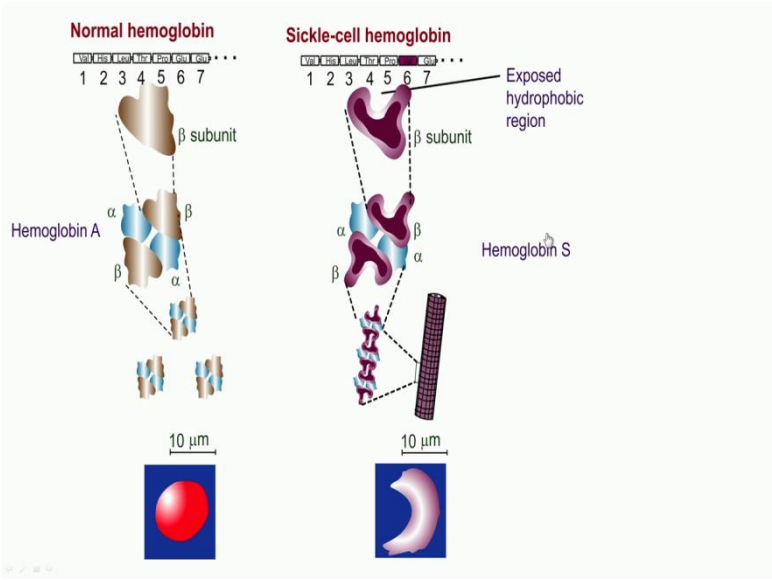


And if the appropriate folding of the haemoglobin chain leads to the haemoglobin protein when it folds properly, then it is able to carry oxygen and the red blood cells looks nice and clean like this disc shaped structure, whereas if in the sixth position the glutamic acid gets changed to valine for some reason, then the structure entirely goes out of proper orientation

and it is no longer able to carry oxygen properly, not just that it makes the shape of the red blood cells sickle shaped, and we get (sickle cell) sickle cell anaemia because of this.

So there is a good structure function relationship between these molecules. We have so far seen lipids, carbohydrates, carbohydrates as you know are intermediates in metabolism, they are a large energy stores and so on so forth. We will look at that in in some detail later in the lectures. And then of course proteins which (are) which form very many different functions in the cell because of the nature of the structure.

Having said these I think in the last class we stopped here the structure function relationship. Let's take things further from here in this lecture. Let us, okay, we will take a side story now, okay? We are still in the major story of curd making, we are asking different questions and trying to answer them. As a part of answering them, we are uncovering very fundamental aspects of biology, biological molecules and so on.

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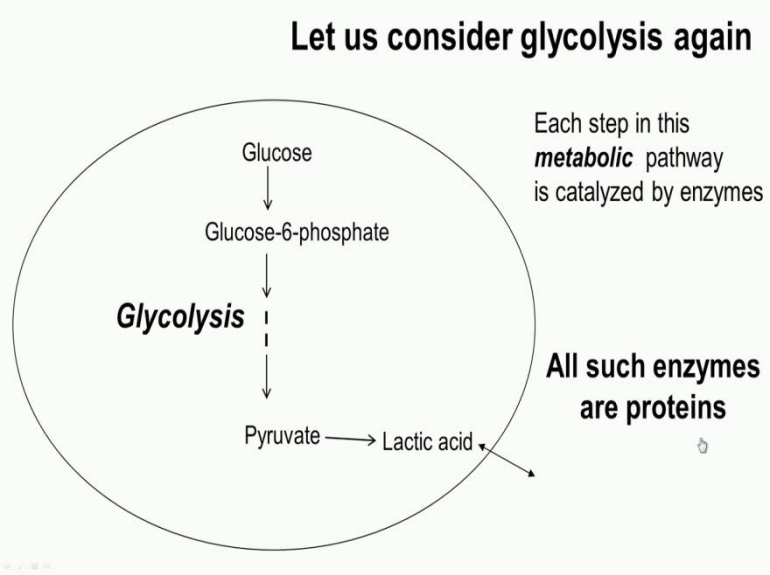


Now our side story here is glycolysis again, you know, we know that glucose going to pyruvate through glucose 6-phosphate, fructose 6-phosphate and so on so forth, is called glycolysis, these set of reactions and the curd formation happened because of the acid, the lactic acid that is produced from pyruvate through a couple of steps, gets released into the medium and the pH (we) we will get to that (and) and this causes curd formation, right?

If we look at this glycolysis, focus on glycolysis, all these are carbohydrates, fine. However, the conversion from one carbohydrate to another, glucose to glucose 6-phosphate, glucose 6-phosphate to fructose 6-phosphate and so on so forth. Each step is, of this so called metabolic

pathway, glycolysis, is catalysed by enzymes, okay? Each of these steps is made possible at the temperature of the cell because of a catalyst called enzymes, and all such enzymes that we're concerned with, are proteins.

There are other molecules in the cell that have enzymatic activities such as RNA, we will keep that aside for the time being, we'll just focus on the majority of enzymes in the cell in terms of glycolysis, in terms of metabolic pathways and they are all proteins.

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Proteins play huge roles in every cell and they make the various functions of the human body possible, okay? I would like you to watch this particular video if you look at the number here, it is number 14, role of enzymes in the human body.

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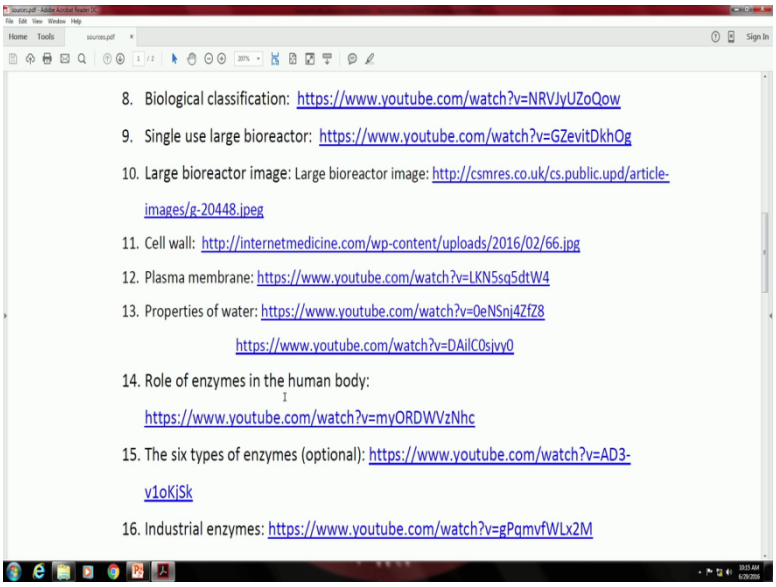


It's a nice short, let's say 3 to 4 minute video, I think. If you watch that you will get an idea as to the variety of functions that enzymes perform in the human cell and different human cells put together as the human body, and the, why humans do what they do, the biochemical basis is all given, is all made possible because of the enzymes, biochemical basis is the enzymes. So please take a look at this video.

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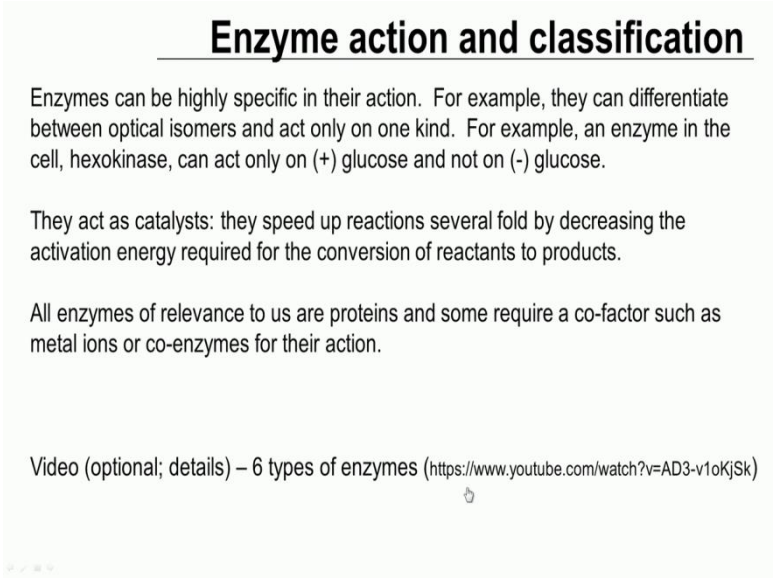


Let us talk a little bit about these enzymes which are specialised proteins or a class of proteins. Enzymes can be highly specific in their action, and that's what gives it its power. For example, they can differentiate between optical isomers and act on only one kind of

optical isomer, okay? The enzyme hexokinase, that's the name of the enzyme, it can act only on (plus) glucose and not on (minus) glucose, you know, D-glucose, L-glucose, dextro (rotary) rotary, leavo rotary, dextro rotary, leavo rotary and so on .

The hexokinase can distinguish between these two, it will act only on (plus) glucose it will not act on (minus) glucose. It can be that specific. And by the way, in nature in the cell, there are D-carbohydrates and L-amino acids naturally occurring. So L-carbohydrates and D-amino acids are not natural, usually, okay? There are always exceptions, usually you can consider this. So they are very specific in their action.

They act as catalysts, you all know what a catalyst does, it speeds up the rate of the reaction several fold at that temperature pressure condition and thereby makes the reaction possible. Here, say, they speed of reactions several fold by decreasing the activation energy required for the conversion of reactants to products, you all might remember the reaction coordinate here, the energy coordinate here, the reactants are here, the products are here at a lower energy level, there is an activation energy that needs to be crossed for this to happen.

The enzymes just bring down the activation energy as any catalyst does. All enzymes of relevance to us are proteins and some enzymes require something called a cofactor, such as metal ions or what are called coenzymes for their action. You have an apo-enzyme which is the proteinaceous part and to which you add a cofactor, metal ions or coenzymes as they are called to give the whole enzyme. Just have this in mind as information, so the enzymes may not be active in the absence of these cofactors which could just be a metal ion - Manganese ion.

This video, this is optional clearly, this gives you the six types of enzymes. The six types that the enzymes are classified into in a standardised fashion. If you want you can take a look at it, it's a lot of detail, if you're really interested you can go and take a look at this. When you have an enzyme- a catalyst, it speeds up the reactions, makes reactions possible, make cell reactions possible at the temperature pressure of the cell, which is very moderate, right? How do we quantify the activity of the enzyme, okay?

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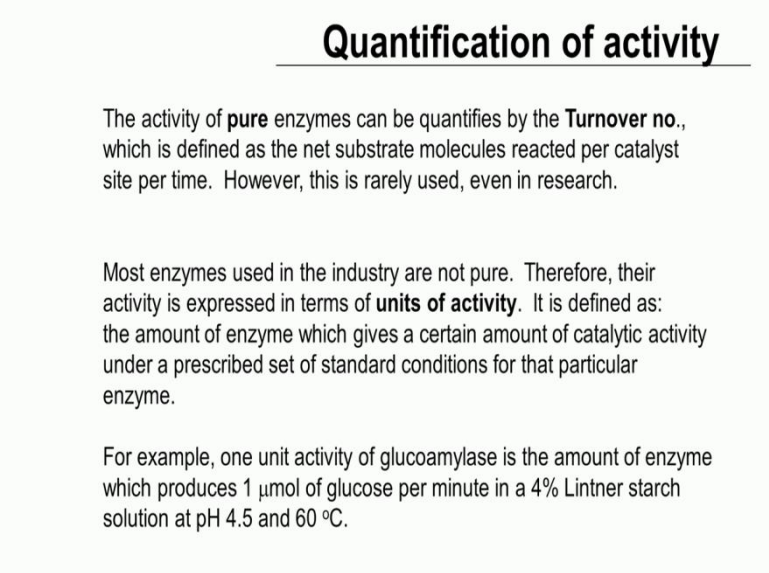


Its quantification is a very important aspect if you want to do something further with it, okay? Knowing information is fine, then we will have to find means of quantifying the information which leads to better analysis and better manipulation application and so on so forth. That's why quantification becomes very important. As engineers, some of you would know this already I just thought I will verbalise this clearly.

The activity of pure enzymes can be quantified by something called a turnover number. Turnover number is defined as the net substrate molecules reacted per catalyst site per time, okay? The way the enzyme action comes in we said was because of the three-dimensional folding. The three-dimensional folding creates pockets that are very specific to the substrate molecule or the reacting molecule, reactant.

The reactant comes and sits there and then it gets converted to a product by various different means and it is the appropriate fit of the substrate molecule to the fold in the enzyme, a part of the enzyme that makes this possible, okay? So it is defined as the net substrate, the number of net substrate molecules reacted per catalyst site per time, okay? But it's rather difficult to find out the number of molecules reacted per catalyst site per time through regular investigations and therefore it is rarely used even in most research, okay? None of us use turnover number when we talk of activity of enzymes especially when we are trying to use enzymes.

Most enzymes used in the industry are not pure, that doesn't matter. Their activity is expressed in terms of units of activity, right? And this is one of the reasons why we don't

look at turnover number because you need highly pure enzymes to even measure turnover number which itself is not very straightforward. And on top of that you have the regular enzymes not being very pure, okay?

So we settle for something called units of activity. Units, a Unit of activity is defined as the amount of enzyme which gives a certain amount of catalytic activity under a prescribed set of conditions for that particular enzyme, okay? It is highly variable and applicable only for that particular enzyme, okay? And people have, kind of, agreed on this. This is how it is right now. For example, one unit activity of glucoamylase is the amount of enzyme which produces 1 micro mol of enzyme, 1 micro mol of glucose per minute in a 4% Lintner starch solution at pH 4.5 and 60 degree C, okay? (It) it can be as specific as that; amount of enzyme which produces 1 µmol of glucose per minute in a 4% Lintner starch solution at pH 4.5 and 60 degree C. So, this is what we use to quantify the activity of enzymes.

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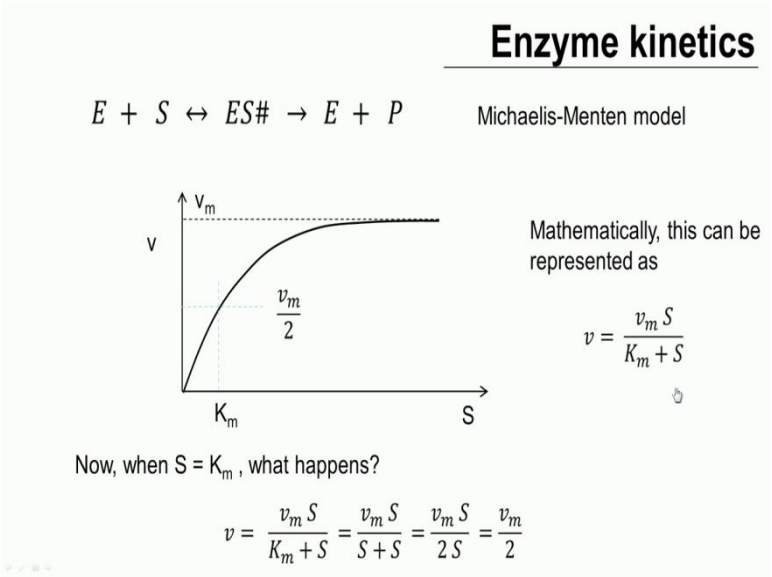


The speeds at which enzymes act are important to know, the kinetics is important to know of especially for design. Suppose we are designing an enzyme reactor to carry out an enzymatic reaction, we need to know how long to wait for the reaction to take place if it is a batch kind of system, or even if it's a continuous kind of a system for design of flow rates and so on and so forth, we need to know the kinetics. So let us look at a simple kinetics, simple enzyme kinetics, there are very many different kinds of kinetics, which are, which govern enzyme action; we will just look at one, for example, okay?

This model is as follows; the enzyme reacts with the substrate to reversibly form what is called the enzyme substrate complex, okay? There is a reversible reaction here, forward, there is a forward reaction here, there is a backward reaction here, there is an equilibrium at certain time and enzyme plus substrate gives you the enzyme substrate complex. And this irreversibly goes to give the enzyme molecule back and the product from the substrate, okay? This model is called the Michaelis-Menten model and it is the simplest model in terms of enzyme kinetics.

If we work out the rates by using this model and material balance considerations, okay, it's a reasonable derivation, about two pages long and if we (plot) if we look at how the variation is with the substrate concentration, variation of the rate of the reaction with substrate concentration, it will be something like this. It's a rectangular hyperbola, okay? It is reasonably linear here, then it goes like this and asymptotically reaches what is called a Vm, Vmax.

Mathematically, this kind of behaviour can be represented as V = VmS/ (Km + S). This kind of a formulation would yield this behaviour. So VmS/ (Km + S) is a good rate expression that one can choose as a first approximation for enzyme kinetics, okay? This is of course a first approximation, this is called the Michaelis-Menton model.

Now, when the substrate concentration becomes equal to Km, let us see what happens to this expression, right? If it becomes equal to Km, V = VmS/ (Km + S), so Km is S, so let's substitute S for Km, so you get V = VmS/ (S + S), you get V = Vm/2. What does this mean graphically? At S equals Km, the V that you find, would be Vm/2.

So Km is called the substrate concentration which gives half maximal rate of the reaction, right, and Km is the Michaelis-Menton constant. So this is a nice expression to know, the first approximation to know for enzyme kinetics, and it is useful in many different ways, I just mentioned one.

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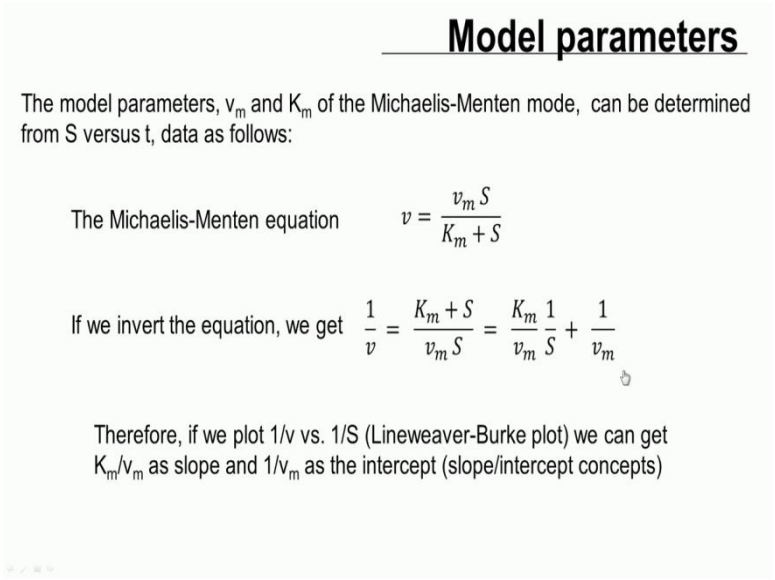


To find out, Vm and Km, as you can realise Vm and Km will be dependent on the enzyme of the substrate and for each combination there will be a Vm and a Km, so how do you find that out, how do you find out these model parameters here, Vm and Km. To find that out, what we do is something like this, one of the things that we can do is something like this because we'll have to find that out from data.

These model parameters can be determined from the substrate versus time, the variation of substrate concentration versus time data. We do an experiment where we carry out an enzymatic reaction and then we monitor the substrate concentration with time and that's the data that we have, we have the substrate concentrations at various times. From that data, how do we get the model parameters, is what we are going to look at now, at least a basis of that.

The basis is as follows; the Michaelis-Menton is V = VmS/ (Km + S). If we invert this, you know, invert the left hand side and therefore we invert the right hand side, we get 1/V = (Km + S)/VmS, just inversion, so if we separate out the terms, this term would become Km/ VmS and this term, 1/Vm.

So this is of the form y = mx + c or if we plot 1/ V versus 1/ S we expect to get a straight line with Km/Vm as a slope and 1/ Vm as the intercept. Such a plot is called the Lineweaver-Burke plot, plot of 1/ V versus 1/ S, you calculate velocity from the substrate concentrations and then plot 1/ V versus 1/S, you get Km/ Vm as a slope, and 1/Vm as the intercept. And I think this audience would know the slope and intercept, right? There is the equation of a

straight line y = mx + c, and m is the slope, c is the intercept. That's why we tried to plot it this way, so that from a straight line, we can get these parameters.

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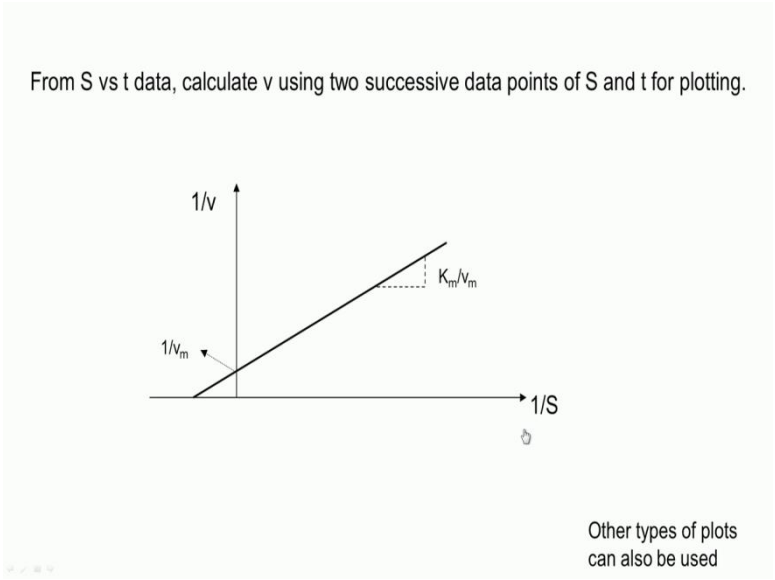


So from S versus t data calculate V using two successive datapoints S, of S and assign it to the midpoint of the time interval there and then 1/ V versus 1/ S, straight line, Km/ Vm as a slope and 1/ Vm as the intercept. Other types of plots can be used, there are some difficulties with this in the sense that the data here is not very reliable and it will determine the slope and so on. Let's not get into that, this is an introductory course. So this is one way of quantifying enzyme kinetics.

We talked of enzymes in the cell and how to quantify the enzyme activity and how to get how to represent the kinetics mathematically and how to get those parameters, the model parameters. Now let us look at the industrial uses of enzymes, they are very widely used, if you look at this video, it'll tell you the various ways or some of the ways in which enzymes are used and their usage is billions of dollars per year kind of level. For example, if you, okay let me just point this out to you. The industrial enzyme is video number 16, you might want to click on that and take a look at that.

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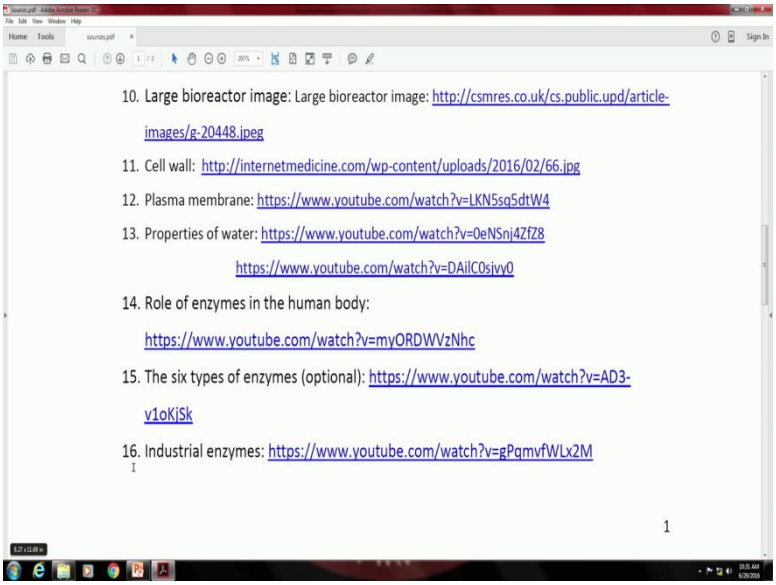


That's this video, okay? Please do that.

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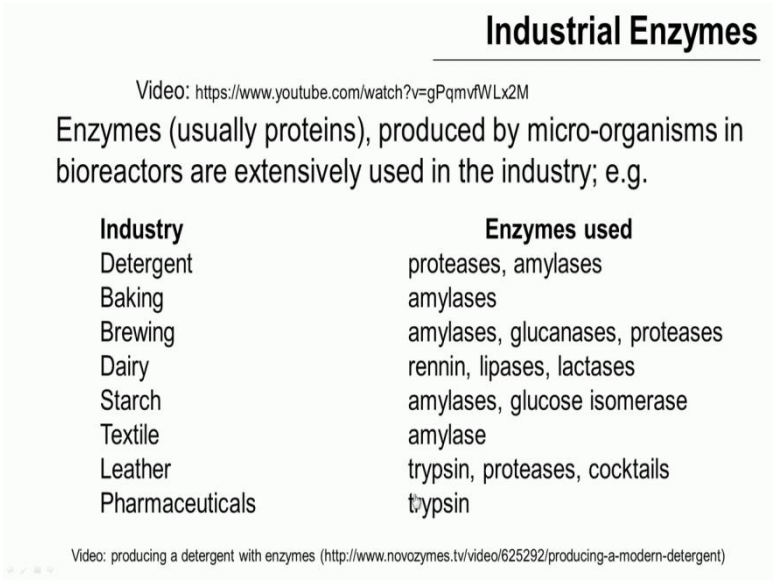


Enzymes, as we said usually proteins, are actually produced by microorganisms in bioreactors and that is pretty much a source of enzymes nowadays, okay? The other sources such as you know earlier there used to be other sources such as animals and so on. They are not very extensively used nowadays, most enzymes are produced by microorganisms in bioreactors.

For example, the detergent industry uses proteases and amylases for better cleaning power. Baking industry uses amylases; brewing industry uses amylases, glucanases and proteases;

dairy industry, rennin, lipases, lactases; starch industry uses amylases, glucose isomerase; textile industry uses amylase; leather industry, trypsin, protease and cocktails of enzymes and so on; pharmaceuticals, trypsin and so on, okay? So, heavily used as I said billions of dollars' worth per year is what is involved, the amount of money involved in enzyme use; enzyme manufacture, enzyme use.

So this video gives you some idea as to how enzymes are used in detergents, please take a look at this video, it's by novozymes but look at the the details in that video. Also have in mind our disclaimer for all these recommendations.

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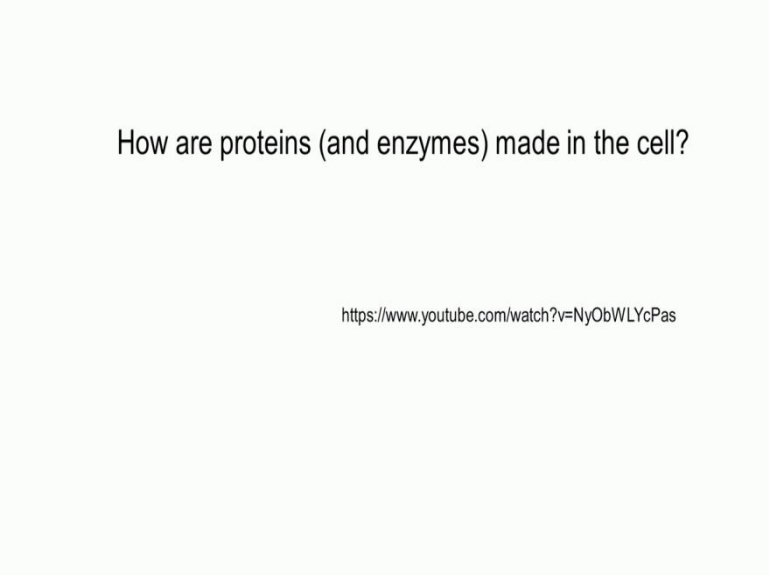


When we meet up next, this is the story that we are going to ask in the larger story of curd making, how are proteins and enzymes made in the cell, okay? We saw that enzymes are being used, heavily used in the cell, for catalysing various different reactions and so on, enzymes produced by microorganisms are used in everyday products and so I mean, that's a very large industry, the next story we're going to see how the proteins and the enzymes are actually made in the cell, okay? Let's meet up later. See you then.

**Biology for Engineers and Other Non-Biologists**
**Prof. G. K. Suraishkumar**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Week- 02**
**Lecture - 09**
**Biomolecules: Nucleotides**

Welcome to the next story in the larger story of biomolecules. I think this would be the last lecture, last story in the biomolecules where we tie up all the loose ends and so on so forth. We will begin the story by asking this question, how are proteins and of course enzymes, we are looking at enzymes that are proteins, made in the cell, okay?

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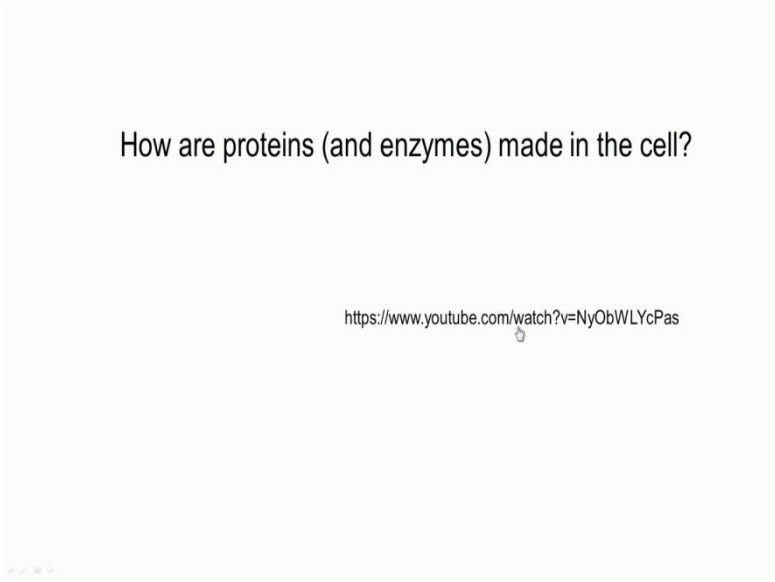

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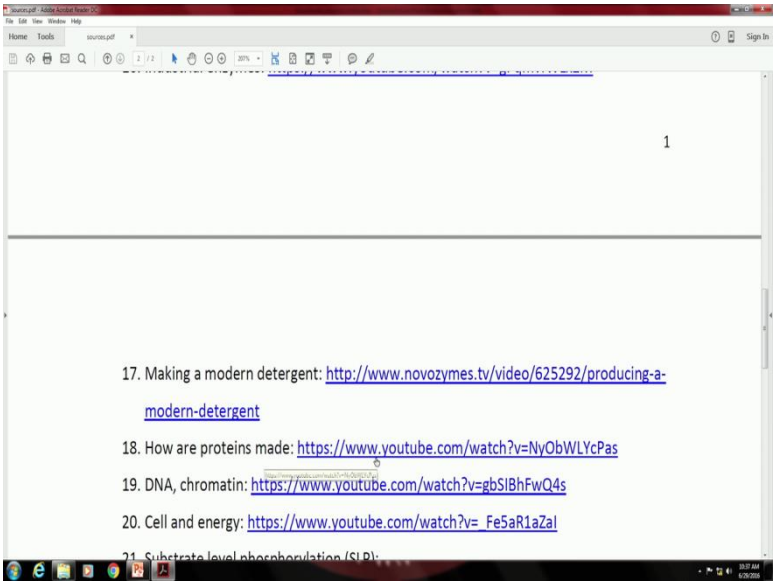


To answer this, I would point you out to required video here, I think the video in the list is number 18, it's a very interesting video and important video in terms of the principles for the course, so I would require you to see that video. Please see that video, it's only about 3and a half minutes long, okay? And it's made nicely. It was made as a part of the human genome project, but it very nicely explains how proteins are made, okay? Very briefly speaking, this video will say that the information in something called the DNA, deoxyribonucleic acid, gets converted or transcribed to the information in the messenger RNA and that gets translated to the information in the proteins.

That is the base (information) base message in this particular video. Please take a look at it, it will provide you with a nice context, it will probably clear up quite a few of those nagging questions that you may have had at several points in time and so on, okay, it's very interesting aspect of life in general.

So we said that the information in what is called the DNA, deoxyribonucleic acid, gets converted to information in the mRNA, okay? What exactly is DNA, okay? All of you, I'm sure, would have heard of DNA, it's a very popular term nowadays and that's how information is stored in the cell, the information that needs to passed on from one generation to another generation, okay? In fact DNA is the code through which this information is passed down generations. So what is DNA and how does the structure of the DNA make it possible for information to be passed on, okay?

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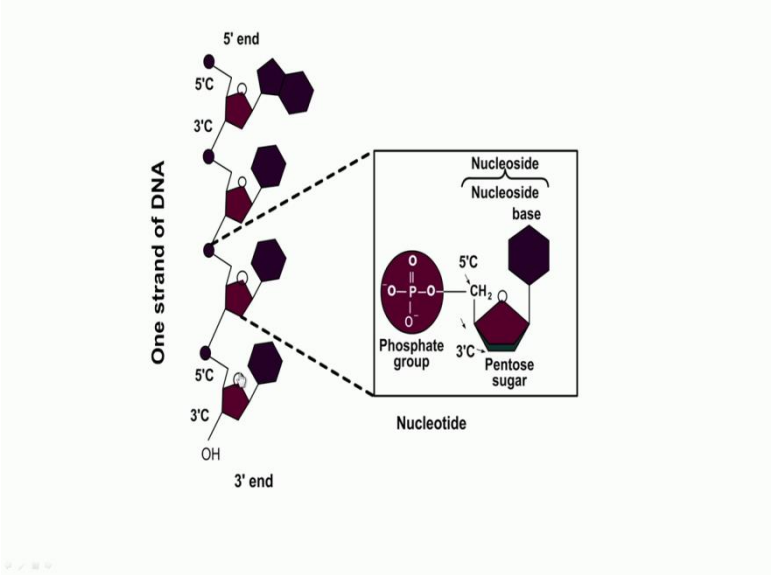


DNA is a polymer of the monomer called a nucleotide; deoxyribonucleic acid; but let's call it nucleotide here. If you look at a nucleotide, it consists of three major parts. You have what is called a pentose sugar here, a pentose carbohydrate here. This is an oxygen, one carbon atom, two carbon atoms, three, four and the fifth carbon atoms here, okay? So this is called the three prime position, this is called the five prime position of the carbon atom and to this five carbon sugar, to the first position, you have what is called as a base, a nitrogenous base that is attached to it. The combination of the base and the pentose sugar is actually called a nucleoside.

To a nucleoside if you add a phosphate group, it becomes a nucleotide, okay? And this is the base unit for DNA. Repeating; you have a pentose sugar, you have a nitrogenous base that is attached to this, and you have a phosphate group attached to this. You get the monomer of DNA which is a nucleotide. So you can see here this is the pentose sugar, the nitrogenous base and the phosphate group, okay? So this, let us start here.

The pentose sugar, right, and the nitrogenous base and the phosphate group to, this is a three prime end which is free here, the five prime end of the sugar is attached with the phosphate group. The three prime and of another sugar, ribose sugar of a nucleotide, gets attached with this phosphate group and that's how the chain gets built up, the polymer gets built up, okay? So you have one three prime end that is free, the five prime end attaches to the three prime end of the next nucleotide, similarly the five prime end attaches to the next, to the three prime end of the next nucleotide and so on and so forth to form the polymer of DNA.

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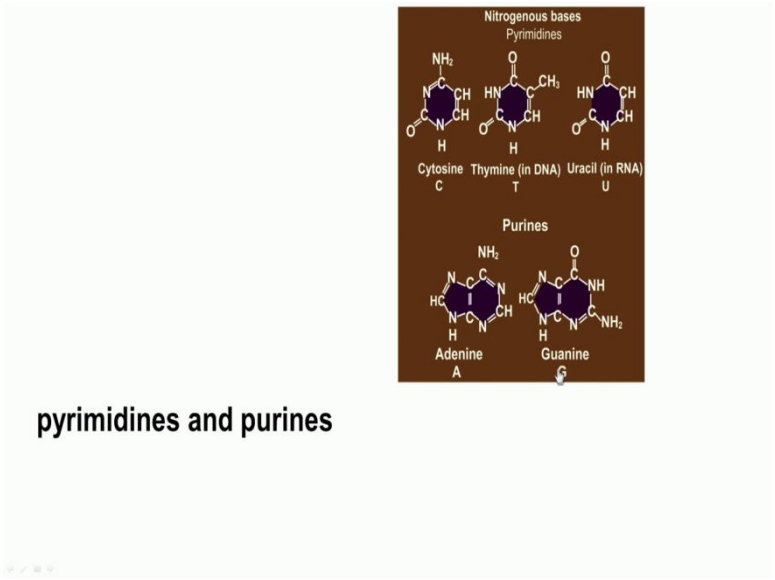


The bases, we said that it contains a sugar, the base and the phosphate group, the bases are of five kinds. They are, they could be cytosine, thymine and uracil or adenine and guanine. Some of these terms would be familiar to you. These are what they are actually. You could have pyrimidines which are these one-ring nitrogenous substances and purines which are slightly bigger nitrogenous substances.

So this base here could be one of these kinds, either a cytosine or a thymine or uracil or an adenine or a guanine, okay? It's so happens that in DNA you have a only CTAG, Gattaca remember the movie? CTAG and in, in what is called RNA, you have the uracil instead of thymine. The pentose sugars are of two kinds, DNA has what is called a deoxyribose and RNA has what is called a ribose, okay? The only difference between these two sugars is the two prime position.

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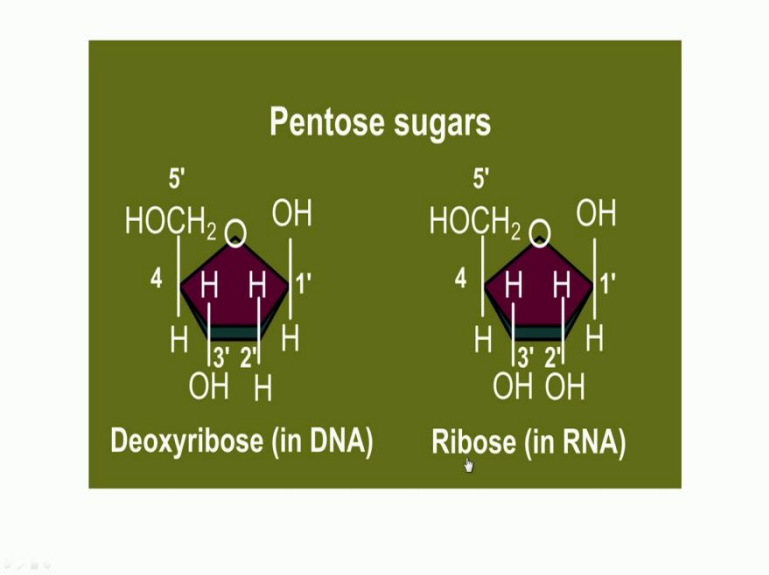


In the case of deoxyribose you have an H in the two prime position, in the case of ribose you have an OH in the two prime position. That's the only difference between or that's one of the major differences between DNA and RNA in terms of the sugar here, right? Okay let's go back a little bit. So you have a nucleotide which could be a DNA or a RNA, you have a pentose sugar. If you have a deoxyribose sugar you have DNA, if you have a ribose sugar you have RNA.

In the case of a base, if you have adenine, thymine, guanine, cytosine, those are the bases found in DNA, you have adenine, uracil, guanine, cytosine, then you have the bases found in the RNA, the phosphate group of course is the same. So that is what a nucleotide is all about and that is what passes on the information from one generation to another.

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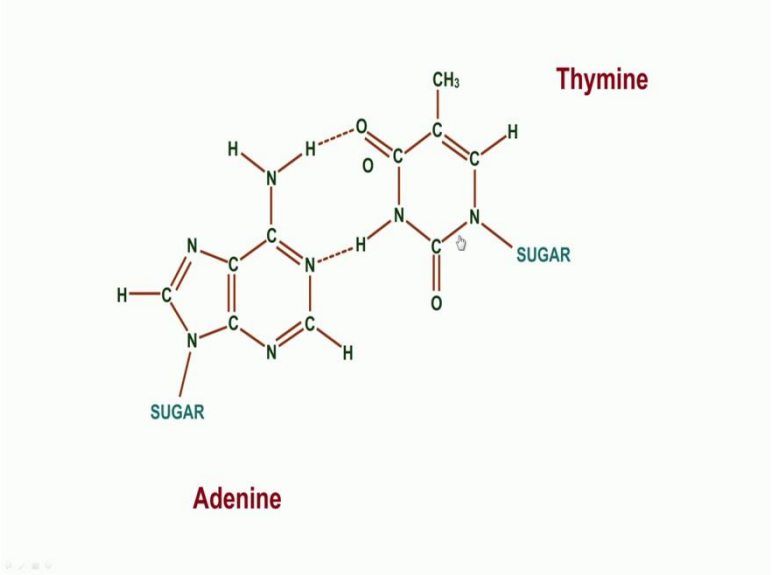


One of the ways by which information transfer from one generation to the other is made possible, is through this. This is adenine, this is thymine, right, you have the adenine and thymine represented here in part, you have the sugar attached, then you have the nitrogenous base alone shown and then of course you have the phosphate group which is not shown.

Adenine, thymine; if you go back, adenine is here which is a purine, thymine is here which is a pyrimidine, okay? Adenine and thymine, by their very chemical nature can form hydrogen bonds between these two, okay? There are two hydrogen bonds that are formed between adenine and thymine and this is what is called base pairing, okay, nitrogenous base, base pairing, this is a base pairing, a hydrogen bond formation between adenine and thymine. There are two hydrogen bonds that are formed between adenine and thymine.

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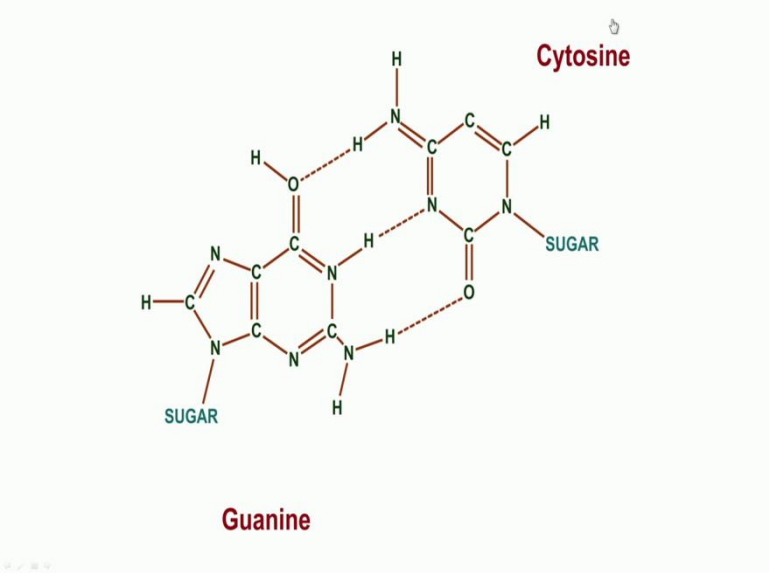


And guanine, cytosine, okay, guanine is a purine, cytosine is a pyrimidine; you have the hydrogen bonds that are formed between cytosine and guanine, okay? So this hydrogen bond formation or base pairing as it is called, is what gives DNA its structure and it makes it possible to do what it does. How is that happening? Let us look at one strand of the DNA here like this, let us look at the other strand of DNA here that is like this.

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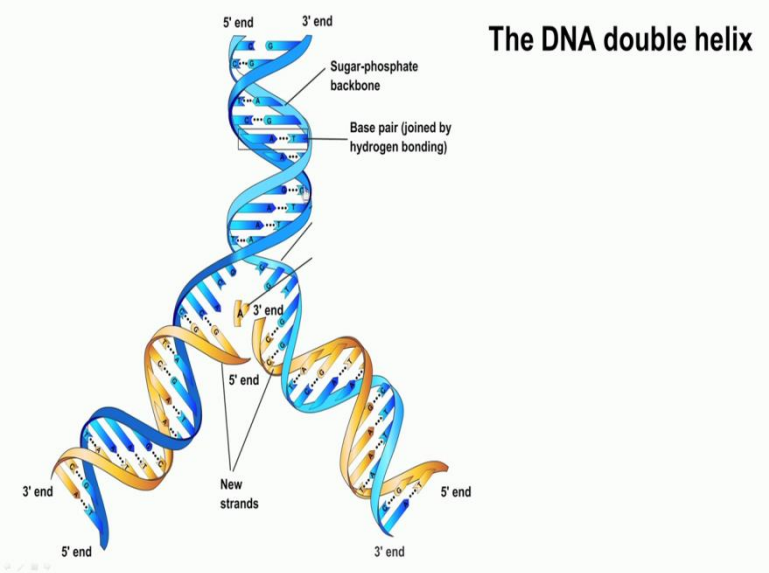


We know that adenine pairs up with thymine and guanine with cytosine. So the adenine on one will pair up with the thymine of the other and pull it together and so on and so forth, the guanine of one pair with a cytosine, adenine - thymine, guanine - cytosine pairing and this pairing gives the dual strands of the DNA a helical structure, okay? So this is what

automatically results because of the various constraints in size and so on and so forth, the base pairing or hydrogen bonding between the bases, gives it its double helical structure and the double helical structure makes other things such as replication of DNA as well as transcription, translation and things like that possible, that we will see in later lectures, okay? So this is how the double strand is getting formed here and this double strand becomes essential for its various functions.

The DNA in a single cell, if you stretch out the DNA, can be about 2 metres long, right? The 2 metres long DNA is somehow packed into the nucleus which is sub (microscopic) sub micron sized, okay, the cell itself we saw was the (bacteria) the in a eukaryotic cell that is a typical size is 10 micron, right? The nucleus is much smaller than that and in the nucleus this DNA gets packaged, right? Whatever is 2 metres long when stretched out, gets packaged there. How does that happen?

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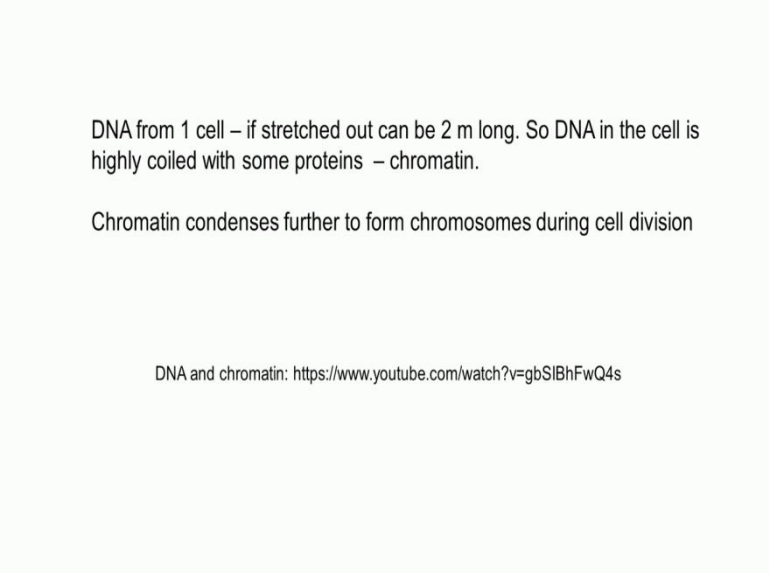


That is because the DNA is in a coiled form, okay? You can imagine this, you can take a piece string, okay, cotton string and try twisting it, then twists and twists and twists and twists and it becomes smaller and smaller and smaller, okay? That's pretty much what happens with DNA too and the coiling and super coiling and super super coiling around proteins which are called histone proteins, essentially results in it being packaged into a size that can fit into the nucleus.

And, so DNA exists in what is called a chromatin form which is nothing but this coiled form, okay? Chromatin condenses even further to form visible chromosomes, the coloured

substances, during cell division, in other words they take up dyes during this stage and that's why they are called chromosomes, coloured bodies. During cell division chromatin condenses further to yield visible chromosomes under of course the microscope, not, not to the naked eye, okay?

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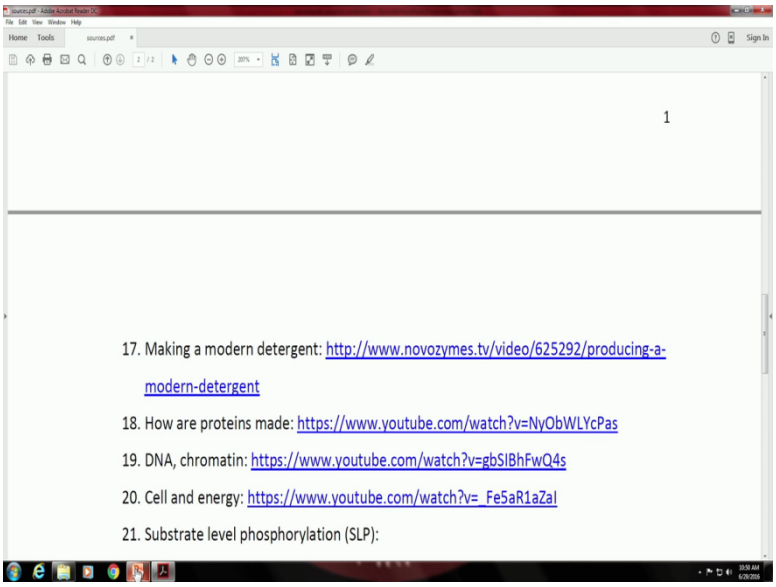


Please take a look at this video, DNA and chromatin, which gives you the details of this packaging, it is an animation which is a nice animation which shows you how the 2 meter long DNA gets packaged into a nucleus by coiling and super coiling around histones, another proteins, okay? This is, the video is number 19 here, please take a look at that video.

Now, let us look at a slightly different aspect. We said, cell is the fundamental functional unit of life and it's a very busy place, a lot of reactions happening all the time, thousands of reactions happening all the time.

There is transport of substances into the cell, out of the cell and so on and so forth. We already saw lactic acid going out of the cell and so many things happen there and any process requires energy. So how does the cell get the needed energy to do all these things and maintain life? This happens because a large amount of energy in glucose, okay, if we split glucose, or if we oxidise glucose, a large amount of energy gets released at one time.

The cell may not be able to use all that energy at that time. Therefore it requires energy in units that it can use directly, right? So that is what happens here, the large amount of energy in glucose gets packaged into appropriate energy in what is called an Adenosine Triphosphate or ATP. ATP you would have heard, right? This, this stands for adenosine triphosphate, this

happens to be the energy currency of the cell. And this process happens through what is called a substrate level phosphorylation of ADP or an electron transport phosphorylation of ADP, we will look at a little bit in detail about these things. And this is how the cell gets its energy to do its various functions.

Please take a look at this video to get some idea, it's a slightly longish and an old video, but I think it's a nice video which gets to all the which touches upon all the necessary aspects for an introductory kind of an exposure, please take a look at that, it's about 9 minutes long.

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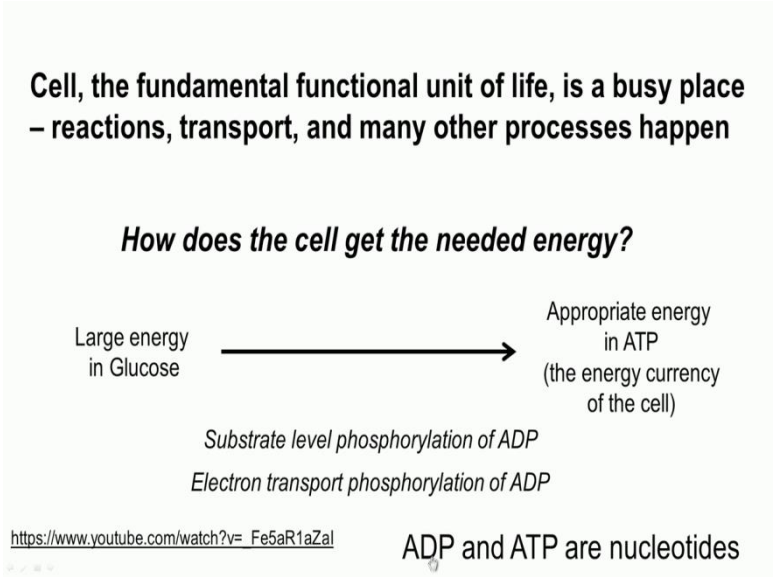


And we said ADP getting converted to ATP and so on so forth, adenosine diphosphate getting converted to ATP. It so happens that ADP and ATP are nucleotides. They have the structure, sugar, a pentose sugar, a base and a phosphate group or phosphate groups attached to it.

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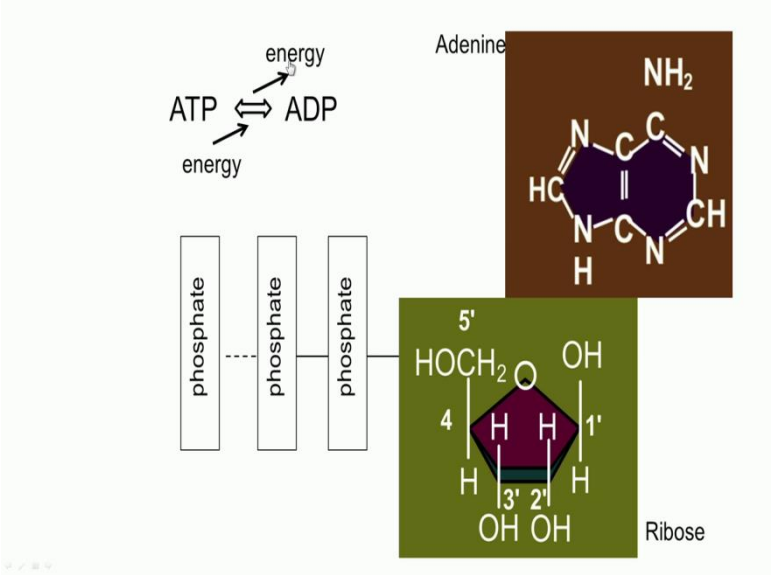


So, a ribose sugar, in this case ATP, okay? So this is somewhat closer to RNA, the ribose OH in the 2 prime position. The adenine, okay, ATP, (aden) adenosine triphosphate, so the adenine, nitrogenous base it is attached to the first carbon atom here and the attachment is not shown, the bond is not shown here. This one needs to be removed and this one needs to go and attach to nitrogen here.

And to the 5 prime carbon, oxygen and so on, you have phosphate groups that gets attached. If you have 2 phosphate groups then this is called adenosine diphosphate, adenosine diphosphate, okay, ADP and if you have 3 phosphate groups attached you have adenosine triphosphate. So ATP and ADP - the energetically important molecules in the cell, are also nucleotides.

ATP gets converted to ADP, by the release of energy and this energy is rightly sized, right, to carry out, to fulfil the needs of various different cellular processes, reactions maybe and so on so forth. So it's about 7.3 kilocalories per mole and that's about the right size for various things. ADP gets converted to ATP and that's what we are going to, that's how ATP gets formed and that would require input of energy, okay? So ADP getting converted to ATP to kind of store up the energy currency and which is ATP - 3 phosphate molecules. A breakage of this would yield energy that can the fuel or that can provide the energy for the various cellular operations.

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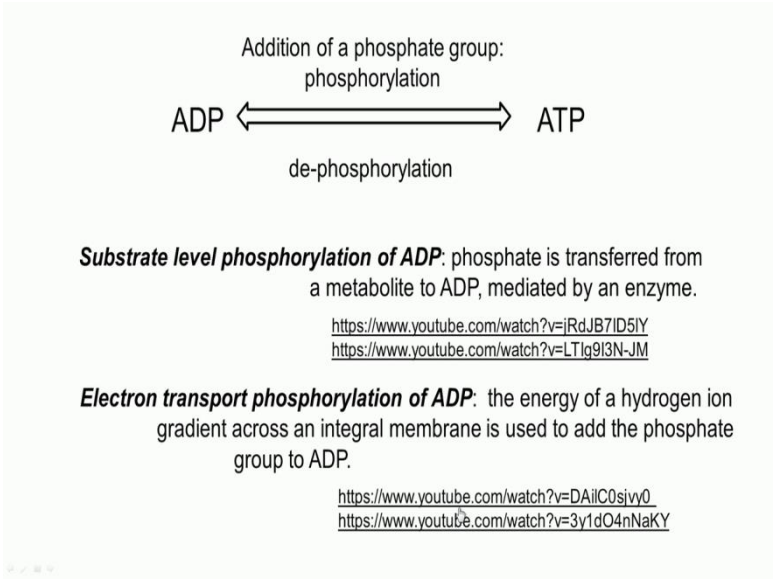


So let us look at this process in some detail, ADP getting converted to ATP. ADP getting converted to ATP we know is by addition of a phosphate group, it is called phosphorylation. Phosphorylation of ADP yields ATP, and dephosphorylation of ATP yields ADP and it is this dance that provides the energy for various things and also makes the energy currency in the cell.

This phosphorylation of ADP to ATP is carried out by 2 major means; one is called substrate level phosphorylation of ADP. The phosphate group is transferred from a metabolite to ADP and is, the process is mediated by an enzyme, that's what is called substrate level phosphorylation. For example, glucose 6 phosphate can transfer its phosphate group to ADP to form ATP. Let me not give you that example. Let's say a phosphate molecule, can get, can get dephosphorylated and in that process, provide the phosphate group to ADP enzymatically to create ATP, right? And that is called substrate level phosphorylation of ADP. We are not getting into details of this, is a lot that can, that one can know about this process, we are not getting into this.

However, it might be helpful to see these videos, if you're interested in knowing some details. So I would say that this is kind of optional videos. The electron transport phosphorylation of ADP, right, the energy of the hydrogen ion gradient across an integral membrane is used to add phosphate to the phosphate group to ADP to form ATP. This by itself is a very very interesting process, this was what Mitchell proposed, I think in 1966 which won him the Nobel Prize, which provided the coupling between the hydrogen ion gradient which provided a way by which ATP can be made, which was kind of missing at

that time; he proposed this as a hypothesis and it explained a lot of things, okay, and won the Nobel Prize also. So it's that important.

And you could look at these 2 videos to get some more insights about electron transport phosphorylation, okay? These are details, I don't want to get into details in this introductory course, however if you're interested you can get into these by yourself.

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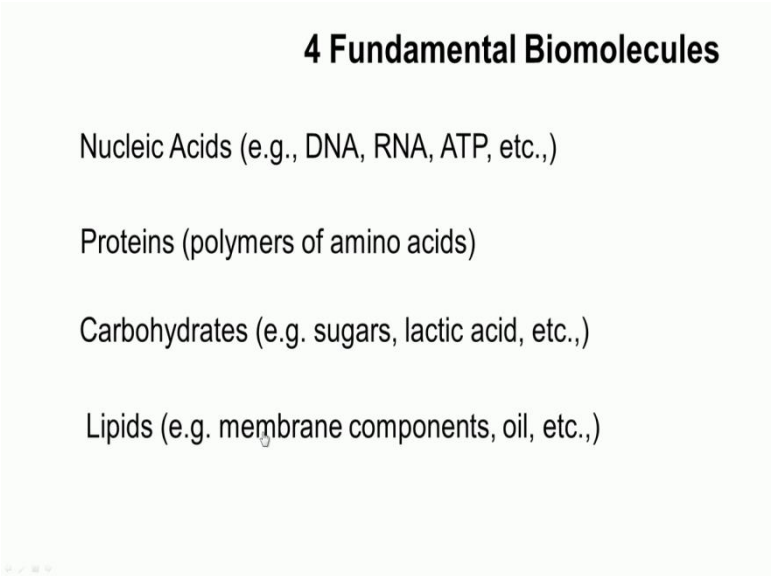


So to sum up, there are 4 fundamental biomolecules last, from last to the earliest, nucleic acids, DNA, RNA, ATP and so on, proteins, polymers of amino acids, carbohydrates, sugars, lactic acid and so on that we have seen and lipids which are membrane components, oil, butter and so on, right?

So these are the 4 fundamental biomolecules, all life is made out of these biomolecules and the structure function relationship is important in these biomolecules, many of these biomolecules, and that is what determines life itself. That's why we were so interested in knowing about these 4 fundamental biomolecules.

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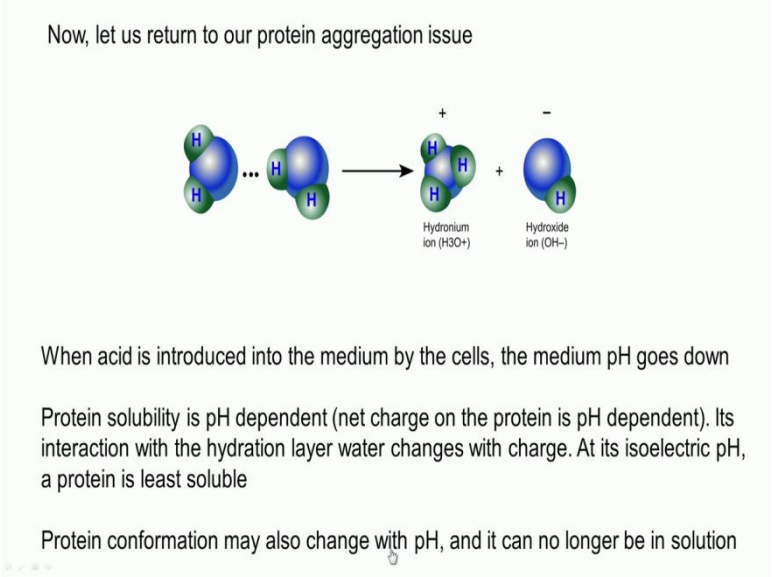


Now we are going to wrap up everything together and by, by coming back to our original story which is curd formation, why does curd form. We said acid formation, followed by protein aggregation, protein that we are looking at was casein. Then, let us look at the protein aggregation to a certain extent, we took off on side routes and those stand out to be very enriching in terms of knowing about fundamental biology, biological molecules and so on. So let's come back to our initial story and finish up there. We have water here, you know $H_2O$ and there is hydrogen bonding between water molecules that we know now. A small part of it is ionised at any time into hydronium ions, $H_3O^+$, and hydroxide $OH^-$, okay?

And a very small part, $10^{-7}$, is what it is and that's why pH is 7, negative log of the hydrogen ion concentration, right? So, that gives you an idea as to the concentration of the species that is dissociated. And this happens naturally, and this is what gives water its pH. So this is what happens. When acid is introduced into the medium by the cell, the medium pH goes down further, the hydronium ion, hydroxide ion concentrations vary; H plus which forms hydronium ions and therefore this starts going up, the pH starts going down. The protein solubility is pH dependent because the net charge on the protein is pH dependent, right? You know that the charges on the protein, this is a zwitter ionic molecule, therefore it is pH dependent and so on.

And at various different pHs, the net charge in the protein could be very different because of the nature of the amino acids that make up the protein. Therefore the way it interacts with the hydration layer; we said that if a protein is dissolved in water, there is a hydration layer around it; the interaction with that hydration layer changes with charge, changes with pH, okay? At the isoelectric pH, isoelectric pH is a pH at which there is no net charge, the interactions would be very different and at that time, the interaction between the proteins could be higher, so they fall out of solutions; there is no longer much of the hydration layer around it.

Therefore they fall out of solution, they aggregate because of the interactions between them. And that is essentially what happens, protein conformation may also change with pH, and it can no longer be in solution. Therefore the casein molecules, the casein protein molecules there have different interactions with their hydration layer. At one point in in pH, they came out of solution, they interacted with each other and formed, they curdled and that's how curd gets formed, right?

So this is this is one of our base stories with which we started out, so let's finish up there. The take-home message is something that we saw earlier; the 4 major kinds of biomolecules, their the very nature of the biomolecules gives it a certain amount of function, the way they're put together, the structure, gives it its major function and so on, okay? And of course we started out with the fact that there are a large number of microorganisms and there are ways to organise them to make better sense of them, right? So that is the story on biomolecules, that is the module on biomolecules, and when we meet up next we will take up another aspect. See you then.

**Biology for Engineers and Other Non-Biologists**
**Prof. Madhulika Dixit**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Week- 02**
**Lecture - 10**
**Cell structure and function: Prokaryotes**

So welcome back to these series of lectures on biology for engineers and non-biologists. In the last 2 videos we did talk about the origin of life and then the evolution different forms of life. Now since its inception, it is very clear that life has been an enigma and it continues to be an enigma for a lot of biologists as well as other researchers. Now what we do not understand is how is it that different cells behave differently or different forms of life behave differently.

And the reason is very simple because if you look around yourself in this world of life biology, you find huge amount of complexity. You have a simple, single celled organism like amoeba or you have a much more complex organism like human beings. So there is a huge complexity and how do you try to understand this complexity, and most of the researchers use a very reductionist approach, wherein you try to study life in bits and pieces and try to identify the unifying features which can connect to this thread of life.

So with that background in the next 2 videos what we are going to talk about, are the very fundamental unit of life which is the cell. Now how do you define cell; as I just mentioned it is the most fundamental unit of life and it is this cell if you understand how this fundamental unit of life works you can kind of extrapolate a lot to the various organisms and their mechanisms of working.

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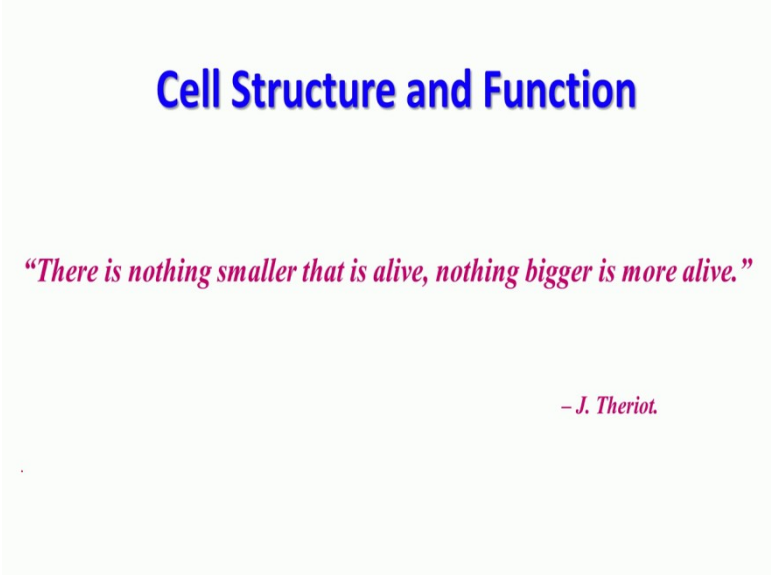


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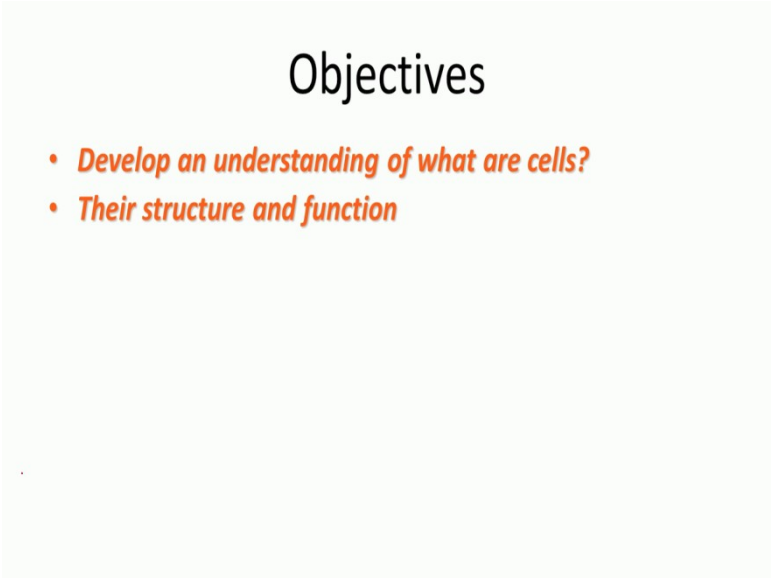


So in this video we are going to talk about cell structure and function and I would like to start this particular video in this series by quoting J. Theriot that "Nothing which is smaller is more alive and nothing bigger is more alive." So in other words it is the most fundamental unit and it is the unit of life. So what are the objectives of this video? The objectives of this video are to develop an understanding of what are cells and what is their structure and how this structure interconnects with the function of the cell.

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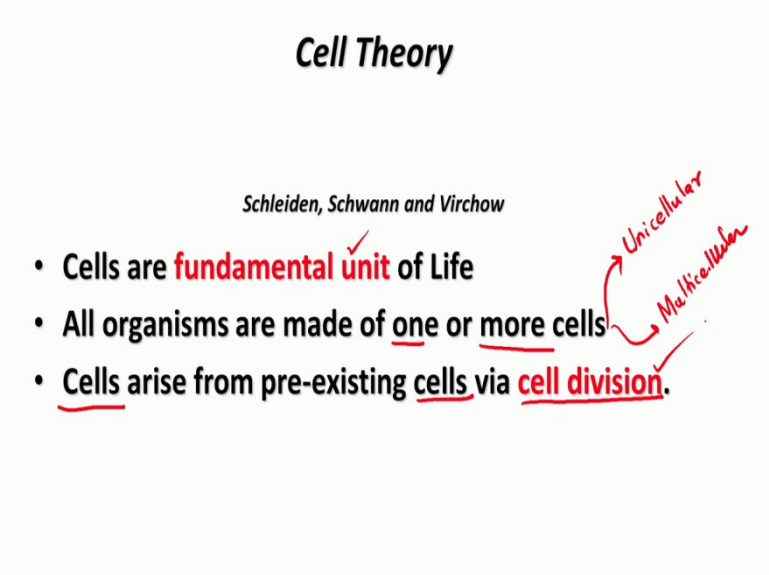


Now, way back in mid-1800s, 3 German scientists came up with a theory which is called as the cell theory and this holds true even today and as I said it starts by stating that cells are the fundamental unit of life, if you understand cells and how cells function you can kind of understand how life can function. Also the second point of this theory is each organism, each living organism is made up of at least one cell or more than one cell. So this is what leads to what you understand as unicellular organism or a multicellular organism.

And then the third and the most important feature which we have been speaking about since the first-class is that each living cell will give rise to new cells via the process of cell division that is one set of cells will always arise from pre-existing cells through the process of cell division or what you call as reproduction. So what are the properties of cells and how are they similar to the things that you see around yourself and life, as I told you, life is complex but so are cells.

But what I want you to bear in mind is that just like life a cell is a machinery or a dynamic system where each and every component of that system is in its equilibrium with the other also it is the cell where the various components of the cell talk to each other, communicate with each other and then the effect that you see is a sum total of all these reactions, all these interactions which are happening within a cell.

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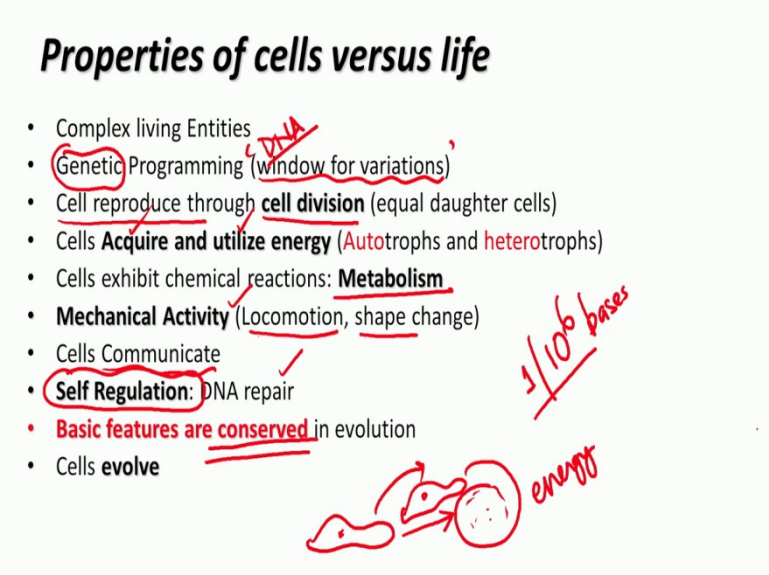


So yes, cells are complex entities and the beauty is each cell which is the most fundamental unit whether you take a single celled organism or a multicellular organism has its entire information packaged which is what you call as the genetic material packaged in the form of DNA in some organisms it can be RNA too, and it is this genetic material which has been discussed in evolution, is the molecule which provides the window for possible variations and adaptations.

So each cell has its genetic code, its information stored in the DNA. Now, how that DNA is stored is what determines different types of cells and I will come to that a little later. The other thing about cells like any other organism whether human beings or dogs or plants is that they reproduce through the process of cell division and they give rise to new daughter cells. Cells like any living organism would like to acquire energy for its sustenance and not just acquire energy, if the need be, utilise that energy for life processes.

For example if there is an amoeba sitting somewhere on the soil and it finds a food particle, somewhere in the neighbourhood, it will try to approach that food particle. It will try to move towards that food particle but the process of this amoeba trying to move towards food particle involves a whole myriad of processes; in other words not only does the amoeba to move forward, it has to change its shape, it has to go and then engulf this food; now all this is a energy intensive process.

So the amoeba will have to have machinery in place where as and when the organism needs it, the energy is supplied. So it not only should acquire energy from outside sources such as

the food which it is trying to eat, it should also conserve energy and then utilise that energy for movement and other body functions.

Now, all this is only possible in a living cell because, as I said, cells are made up of a whole series of reactions which are taking place, which is what we call as metabolism. So the metabolism happens, there is a process in place to acquire energy either from outside or synthesise the food on their own and then utilise that particular food for generation of energy.

Mechanical activity is another part of life as we mentioned which may either be involved in the process of locomotion or in this case of amoeba such as shape change. Now if an organism is living, it's never living in isolation, it has to communicate with its environment. It needs to sometimes relay the information from outside to inside or vice versa. In such a situation a cell or an organism needs to have a system in a place which is capable of doing communication so this fundamental unit of life is also capable of communicating with the outside world and responding to it.

And the best part among all these is that all these processes, whether it is of locomotion, whether it is of generating energy, it is a process of communicating, you find that the cells are highly regulated. These are one of the best features wherein a cell keeps in control its entire processes which has very few margins of error. A classic example is the process of DNA replication; we will be astonished to know that every time a cell's DNA duplicates, the process will see to it that it duplicates its copy exactly. So much so that the error rate at the time of DNA replication will be probably 1 in say 10 million bases.

So for every 10 million reads, probably a 1 error may happen which obviously contributes to the variation in the daughter cell, but this is the fidelity of the process and this is simply possible because the cell has complete machinery to take care of even the repair of any parts of the body or the parts of the cell structure as well as the processes. But whether we take a single celled organism or we go across all the way to higher end organism, what is interesting is to note and this is what we all cling on to is that certain processes of life are fundamentally conserved.

When I meant fundamentally conserved, for example how the DNA will store information, in what language all this information is coded, has remained conserved across these billion years of evolution, whether you talk about bacteria or you talk about human beings, you find that that genetic code by which the information is stored is highly conserved.

Similarly, a lot of other metabolic reactions which govern life are also conserved across evolution. So what are these cells and why it has been such an enigma to understand life, and let me bring your attention to one major limitation that we have as humans is a resolution of a human eye. It's not possible for us to see things which are below a few millimetres in size.

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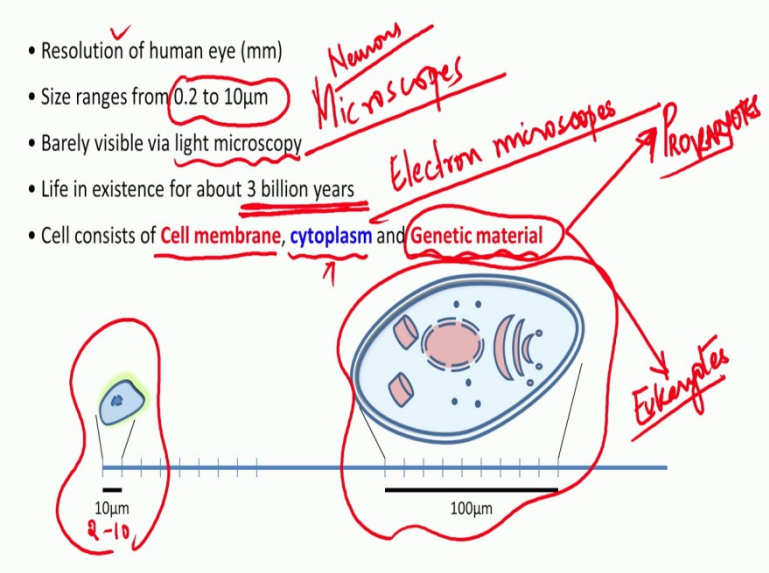


Now this has been one of the major limitations in our understanding of how life works because a lot of these organisms are much smaller; they are about the size range of anywhere between 2 to 10 microns or they can be as big as 100 microns but yet this size is way below the resolution of human eye and this surely required some sort of invention and that came in mainly through the invention of microscopes. Had it not been for the optics and development of different kinds of microscopes, it would not have been possible for researchers to understand how life really works and how the cells really work.

So, coming back to the size of the cells what we find as of today is that most of these cells with few exceptions, have a size in the range of 0.2 to 10 microns. You have exceptions, for example neurons that we would see are much bigger in size than 10 microns. But they are exceptions. Most of the cells across the living world are in this size range and as you can obviously see this size range is way below the resolution of human eye and we need the aid of different kinds of microscopes, some of them are visible through light microscopes some of them aren't.

The ones which are not visible through light microscopes, we then resolve to electron microscopes,where, instead of using light as the source of visualisation we use a beam of

electrons to visualize. But whatever the approach you take, one has to appreciate one thing and that is life has been in existence for 3 billion years. And I want to keep stressing on this point because what as biologist or as researchers who may be non-biologist as well, what we are trying to understand are systems and not just one system, multiple systems or probably millions of these systems because there are at least 2 million living species which have been already identified each species being a system by itself that this life has been in existence for last 3 billion years so we are trying to understand the history of life which has accumulated in last 3 billion years.

And it's not easy to understand unless we have appropriate tools at our disposal. So let's come back to the cells. Any cell of any organism which is existing on earth today has 3 common features is that is each cell is surrounded by an outer boundary which is called as the cell membrane. The cell membrane helps in segregating the internal content of the cell from the outer environment. The second most common feature is the cytoplasm. Now this is a fluid, a jelly like fluid which holds the entire cell together and it is in this jellylike fluid, the genetic material which is our DNA or RNA in some cases is present. So each cell consists of an outer boundary, the outer membrane, the jellylike fluid called the cytoplasm and the genetic material within the cell.

Now how this genetic material is actually organised within a cell determines 2 major classes of cells or 2 major groups of cells, prokaryotes and eukaryotes. So we divide different types of cells into 2 major categories, prokaryotes and eukaryotes. Today we will talk about the features of prokaryotes and then slowly move on in the next class to the differences between prokaryotes and eukaryotes.

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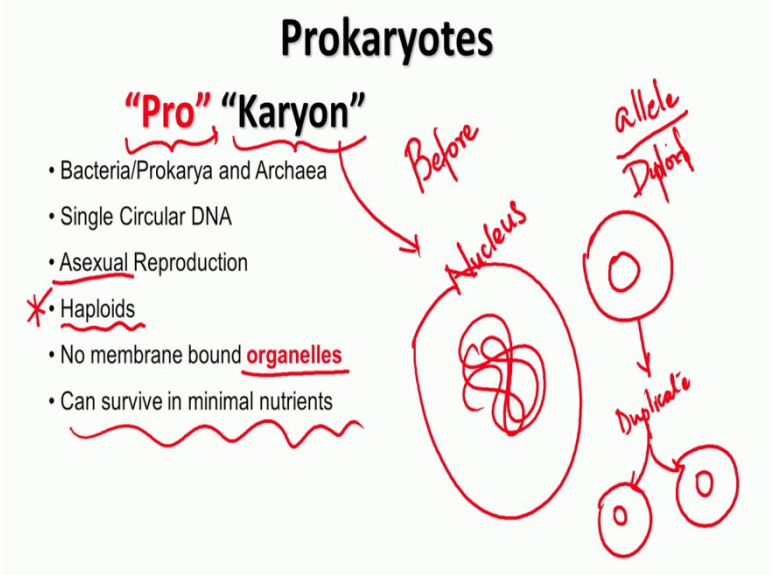


So, what are prokaryotes? The term prokaryotes is derived from 2 words, pro and Karyon. Pro means before, and karyon means nucleus. So this group of organisms basically most primitive ones as we think and the simplest of the living organisms, consist of their genetic material which is inside the cell membrane but is not in confined to a specific subpart which is called as the organelle.

Now, what are organelles? Now, organelles are small structures that you observe within a cell which themselves are bounded by membranes. So this feature you do not see in case of prokaryotes. The other thing that you observe in prokaryotes is that for most of them genetic material exists as a single circular DNA.

And, these organisms or prokaryotes divide through the process of asexual reproduction. Now what do you mean by asexual reproduction? Unlike higher organisms where there is fusion of the male gamete with the female gamete to form an egg or the zygote say for example in humans you have part of the gene set coming from your father and a part of the gene set is coming from the mother, that is what you call as sexual reproduction, that does not happen in these group of organisms.

Here each cell will first duplicate its DNA in the hope that it is duplicating it exactly with as many minimal errors as possible and then divide into 2 daughter cells directly without undergoing the process of cell fusion between 2 independent gametes a sperm and an egg, that process does not happen in case of prokaryotes. So essentially every parent cell will more or less pass on same features to the daughter cells and the other term that I want you to note is

that term called haploids and this will again become important when we talk about cell division, mitosis, meiosis and all. Now, what is haploids?

As I said, in organisms like human beings, where there is a process of sexual reproduction taking place, we always get 2 copies of a gene, say for example if for your eye colour you will have a gene coming from your father and a gene coming from your mother. So each gene, though both of the genes are coding for the eye colour; one version is coming from the father and the other version is coming from the mother which is what you call as an allele.

Now, this situation where, for every gene, you have 2 copies one from father and one from mother is called as a diploid situation. But since the prokaryotes do not exhibit that, for every gene they have only one copy, now that is interesting. One, they do not undergo sexual reproduction and for every character they have one gene.

But this gives enough scope, because if at all this single copy undergoes any sort of mutation at any point of time it becomes a variation and then it is much more easier to accumulate these variations because if it was a diploid organism, the variation may become dominant or may not become dominant and this is something that you will study in your Mendelian genetics, I am not going to cover it here but I want to bring home the same point that prokaryotes are much simpler because they undergo asexual reproduction and they have one copy of a given gene and hence are called as haploids.

And the other most intriguing feature of prokaryotes are they can survive in any form of minimal nutrients. You do not have to grow them with complex carbohydrate sources, you give them basic carbon source and they are able to survive very well.

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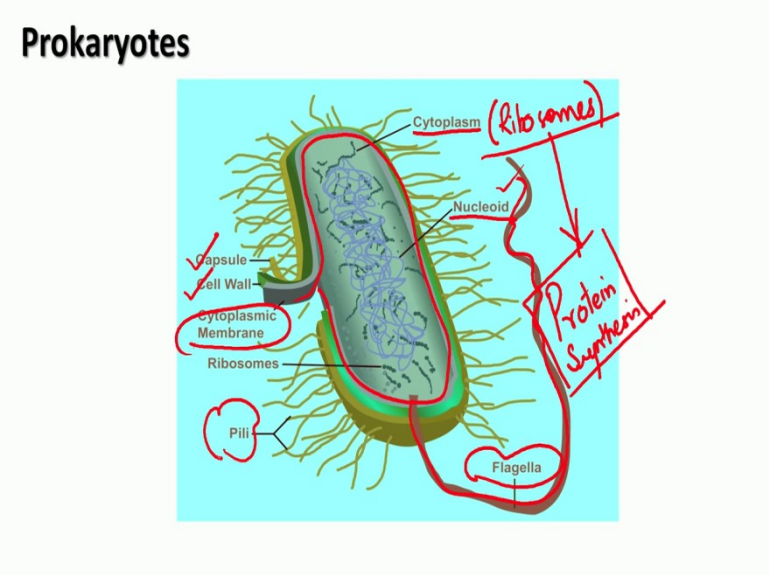


So let us look at, now here is a diagram which kind of recapsulates what I just said that the DNA in case of prokaryotes is a loosely circular, in this case it is not looking exactly circular but this is a single piece of DNA which is running around and it is kind of centred within the cytoplasm and it is not surrounded by any kind of a membrane itself. Such a structure is what you call as the nucleoid.

In addition, this DNA is floating in this jellylike substance which is what you call as a cytoplasm and the cytoplasm consists of tiny little components which are called as ribosomes. Now these ribosomes become important because they are the basic machinery which is required for protein synthesis and I will come back to this later in other videos when we are talking about protein synthesis.

It is a very indispensable component of a living world. So the cytoplasm is like this jellylike fluid which has the small tiny organelles called ribosomes though they are not membrane bound and these ribosomes play a very important role in the process of protein synthesis and then you have the genetic material which is kind of aggregated loosely in the cytoplasm not surrounded by a membrane and this structure is what you call as the nucleoid.

All this is finally enclosed in almost all the organisms by what you call as the cell membrane. So this is what it is, this grey one, right? Now this has to be there in all the organisms, but within prokaryotes you can have categories where in the cell membrane may be further protected by a cell wall or a capsule.

Now these 2 features are not mandatory but they may or may not be present. Another interesting feature which the prokaryotes have is a long hair like or projection which is what you call as the flagella. This is literally like the propeller. So if a bacteria has to move forward and it is residing in an aquatic environment for example, this particular filament, the way you have it in sperm cells, this particular filament allows the prokaryote to move forward.

In addition to this, there are small tiny hair like projections in some of the prokaryotes which is what you call as the Pili. So in terms of organisation and structure, the prokaryotes are the simpler ones where the genetic material is not enclosed exclusively by a membrane itself, in fact it exists as a nucleoid body and DNA is fairly simple.

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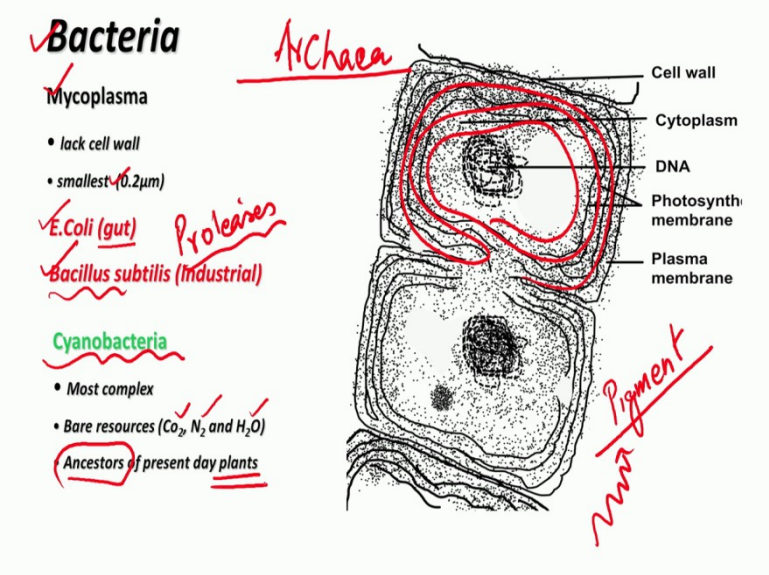


So what all comes under prokaryotes? Prokaryotes is a group which consists of 2 major domains, one is bacteria the other one is Archaea. This is something I told you even my previous class. So it has 2 major groups of organisms, the bacteria and the Archaea.

Let us look at bacteria first. Now bacteria again is a huge class of these organisms and some of them are for example Mycoplasmas which are the smallest known bacteria and they do not have a cell wall. Other common examples which you are possibly aware of already are the E. coli that you find in your gut and another very important and industrially a very important microbe which is the Bacillus subtilis. In fact most of the industrial enzymes and detergent companies for example which are used to make detergents, make use of certain products of these Bacillus, particularly a group of enzymes called proteases and a few other.

So bacteria is one of the largest classes and even within the bacteria somewhere across the course of evolution you come across a very unique organism which is called as the cyanobacteria. You have fossil records of cyanobacterium which are seen even today and you find as in this picture, as I show you in this slide, it is a little more complex.

If you notice in this cyanobacterium what you observe is the same cell membrane keeps on refolding into multiple layers and so it is not just the cell membrane which is just a single boundary, you find that the cell membrane keeps on folding and it is in these cell membrane that the organism houses a very important pigment which has a potential to absorb solar energy or sunlight. So in what is now known today is that the cyanobacterium although one of the more complex ones, can survive on very bare resources like carbon dioxide, nitrogen and water.

And it is these group of bacteria, because of their ability to capture sunlight and convert that solar energy into chemical energy which is what you call as the glucose and sugars are believed to be the ancestors of the present day plants. So you find that though it is a very simple organism the DNA is not really as complex. It has still evolved during the course of Earth's history and it has come to a situation where it has developed a capacity to on its own utilise solar energy and convert that into chemical energy, and hence are believed to be the ancestors of present day plants.

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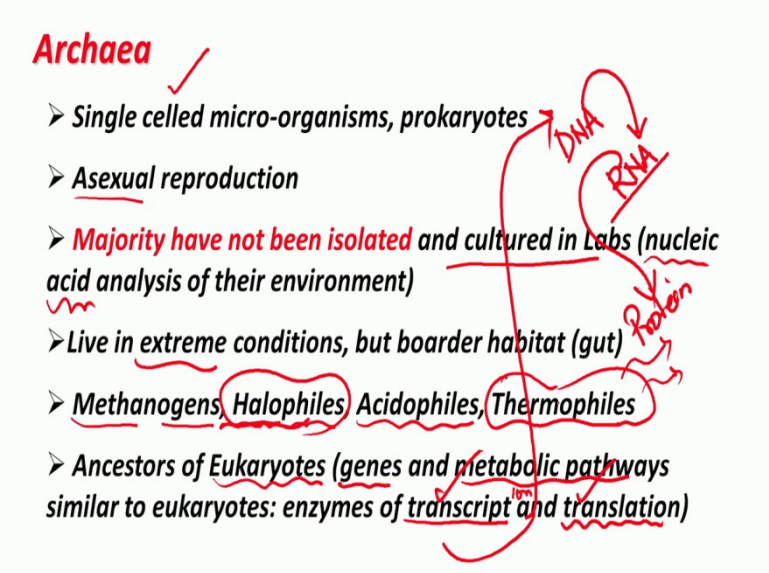


More about Archaea; now Archaea is another unique group of prokaryotes and like bacteria, they are single celled, they do show asexual reproduction. But what has been challenging

with this particular domain of living organisms is that they all are found in very extreme habitats, such as the hot water springs, the volcanic eruptions, deep down in ocean beds and or an highly acidic or a high salt content lakes. And you find that although you see them in these natural environments, when you try to isolate and culture them in the lab and you try to then study it, it has not been very successful.

And hence we do not know much about them but there are a few things which you have still figured out about them and that's thanks to our ability to at least isolate their nucleic acids from the environment in which they grow. So if you take a hot water spring water and try to isolate nucleic acids which is DNA and RNA from there, and study it, you kind of get an insight into how these organisms are and what they are capable of.

What we know about these Archaea is that they are different from prokaryotes because they grow in extreme environments and hence have managed to survive and they show abilities like ability to produce methane, hence they are called as methanogens. They have an ability to survive in high salt, halo means salt, phile means loving. This is what is called as the salt loving organisms. They can grow in lakes and ponds which have very high salt content. There are acidophiles, which love more of an acidic pH and they survive only under acidic conditions and then you have those microbes which are growing in thermal vents and hence are called as Thermophiles.

Now why they are important, I mean, it's no one's guess that if you are having an industrial setup and you are looking for a certain chemical conversion process, you will be astonished that a many a times a lot of catalysts or enzymes which are being used in some of these industrial processes, if they need to be heat resistant are usually derived from these group of organisms like the thermophiles.

So given that these groups of organisms grow under extreme conditions it is possible to isolate important enzymes and reagents from them and use it for the human benefit. What is another interesting feature and we now have sufficient proof about Archaea, is that when you try to compare certain genes and certain metabolic pathways, the metabolic certain pathways and genes which are found in Archaea are found to be very close to the eukaryotes, the other higher end organisms.

So for example if you are going to look at the enzymes which play an important role in transcription; now this is a process wherein this is a process wherein the genetic information

which is coded in DNA gets converted to an intermediary step or RNA and then even the enzymes which are involved in the process of translation which is a next subsequent step wherein information then gets passed on and gets decoded from RNA into protein. You find that the enzymes involved in these 2 processes in Archaea are very similar to the present day eukaryotes the higher end organisms.

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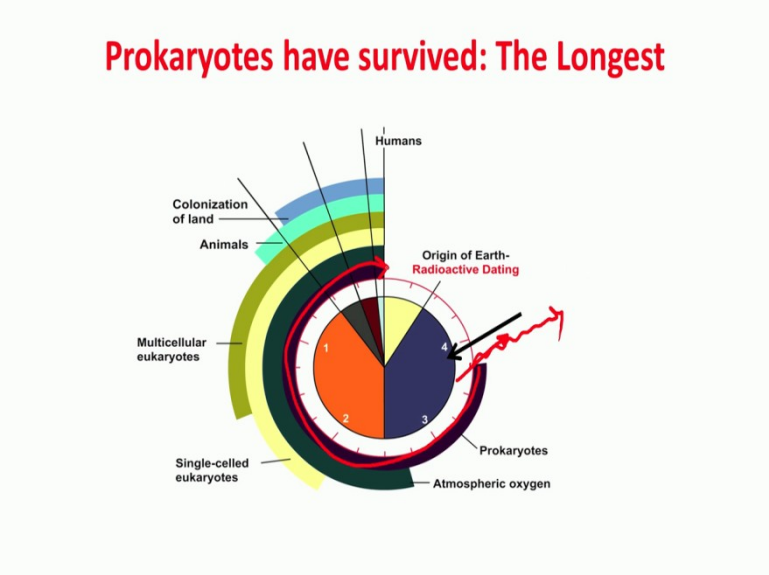


So let us look at this other interesting aspect. I had mentioned earlier that the life evolved somewhere around this stage about 3.8 billion years ago, because that is where we see the earliest fossil records. And what is intriguing and rather perplexing is to note that though prokaryotes evolved and were the first set of organisms as of our understanding today to evolve, they have continued to survive till the present day today.

So though we call it to have a very simple structure because they just have a single circular DNA, they do reproduce asexually yet this group of organisms you find have survived beyond slots which the earth must have experienced in last 3 billion or years and has still continue to survive and thrive. Now how is that possible? Now that is a very interesting thing to look at and the reason we think it is possible is because though these organisms do not reproduce sexually they do have a potential to acquire variation and they do this by the process called as horizontal gene transfer.

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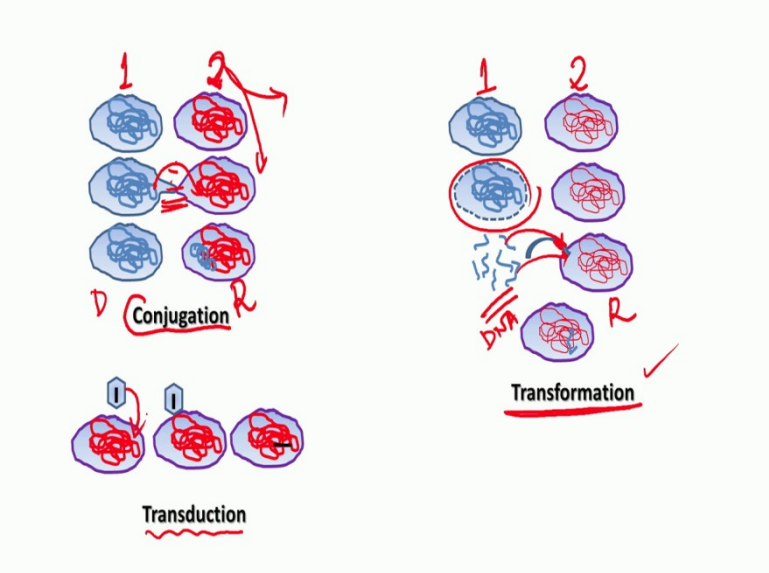


So what happens; let us take this example. There are 3 major forms of this horizontal gene transfer in prokaryotes; the process of conjugation, wherein let us assume this is one bacteria and this is another bacteria. And the second bacteria requires certain favourable features of the first bacteria.

So what happens is the 2 bacterias come together and connect through their membranes and I will tell you later why they are able to connect through membranes because that is the specific property of biological membranes, we will come to it a little later. And they form this connection and then the favourable features are passed on, mind you, not all the features, the required features can be passed on from the donor cell to the recipient cell.

So this process is what you call as conjugation. And that favourable character will then be acquired by the bacteria 2 and as the bacteria 2 divides; it is going to pass on these favourable characters to its daughter cells. The other form of horizontal gene transfer, which accounts for this variation, is the process of transformation. Now assume again that you have 2 bacteria living in neighbourhood, bacteria 1 and bacteria 2.

Now bacteria 2 is needs to survive and for some strange reason, the bacteria 1 has either died and as its dies all its material is disintegrating and then its DNA gets fragmented. The bacteria 2 finds there are a few useful pieces of DNA which if it acquires will give it a survival advantage, it ends up picking a few of those favourable DNA fragments; mind you here, this bacteria has already died unlike in the process of conjugation.

Now, in such a case the recipient again ends up receiving favourable characters from the extra DNA or rather the DNA which is floating around in its environment. This process is what you call as transformation. And a third form is transduction wherein there is a genetic information happening between a virus and a bacteria.

So although these group of organisms reproduce asexually and they try to, in their all complete honesty, pass on the complete set of genes as close to themselves the daughter cells, if there is a need in the environment to acquire variation they do undergo the process of horizontal gene transfer, either through the process of conjugation, transformation or transduction and hence these group of organisms have managed to survive for such a long time because they are far more amiable to variations than probably a diploid organism.

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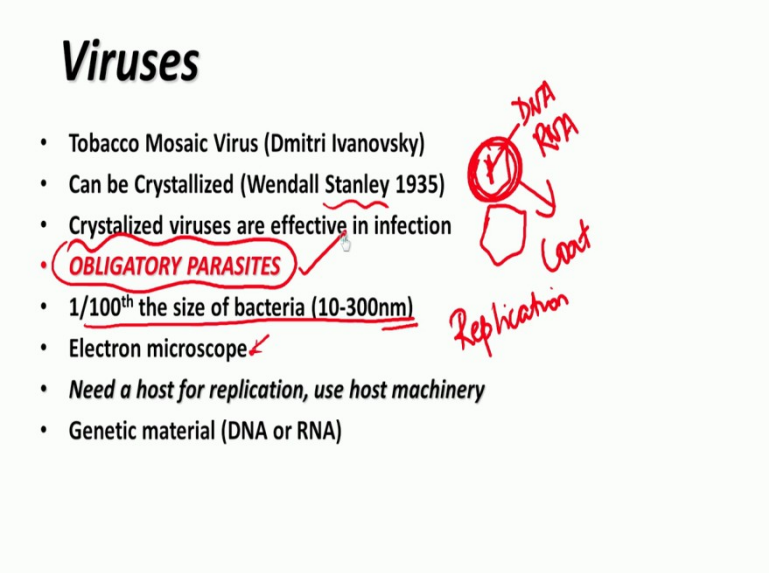


So then let us come to one last group which is the viruses. Now by the end of 19$^{th}$century and thanks the discovery of penicillin, more or less people or scientists thought that bacteria are the smallest possible organisms on Earth. But then there were a few diseases which could not be explained by bacteria, especially the tobacco mosaic disease in tobacco plants.

Now it was the effort of this Russian scientist called Dmitri Ivanovsky, who was trying to understand what causes this tobacco mosaic disease in tobacco plants and what he did was that he took the sap of an infected plant, passed it through multiple filters, such that the smallest of the known bacteria at that point of time will not go pass through the filter, collected the so-called bacteria free filtrate and put it on an uninfected plant and what he

found is that despite getting rid of all the known bacterial forms, the filtrate was still able to infect the new plant.

Later it was realised by Wendall Stanley, when he tried to crystallise what was here, he found that these are some protein like particles which are capable of crystallisation and these protein like particles, on their own, cannot replicate. So in a sense they are not completely living but when they infect a living organism, they then can initiate the process of their genetic replication. So in other words just for them to propagate they need to infect another living organism and hence are called as the obligatory parasites. So these viruses are much smaller than bacteria, in fact in the nano meter ranges and hence for a very long time they were not discovered till the advent of electron microscopy.

And as I mentioned unlike bacteria and higher organisms, though these particles retain a genetic material which is surrounded by a protein coat, for example if this is a DNA or it can be RNA, it is just encapsulated in a protein coat. But, on its own, this cannot replicate unless it infects a living organism. So you can in a sense say that these are a group of, we cannot call them as organisms completely because they still need to depend on another living organism, but they are kind of obligatory parasites.

So what have we learnt in this video? We have talked about what is, what are prokaryotes, and we have seen that they do not have a well-defined nuclear structure. So prokaryotes are a group of organisms where the nuclear material or the what you call as the genetic material is organised as a nucleoid body, it is not surrounded by any kind of a membrane and the whole organism consists of cytoplasm and a cell membrane. In addition to cell membrane the prokaryotes may or may not have a cell wall and they all undergo asexual mode of reproduction.

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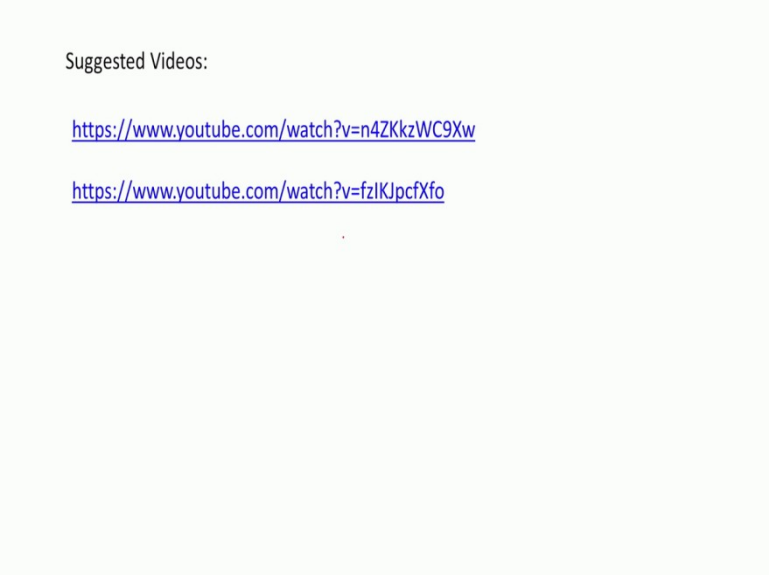


In contrast the more complex organism which involves the plants, the animals, the fungi and the protists, all belong to the class of eukaryotes where the entire genetic material is very well organised within a special membrane bound structure called as the nucleus. We will talk about the eukaryotes in our next class, but for those who are interested to know more about prokaryotes and how the asexual reproduction in prokaryotes really happens, I would suggest you to go through the 2 suggested video links and with that we conclude this video and we will meet again in the next one, thank you.

**Biology for Engineers and Other Non-Biologists**
**Prof. Madhulika Dixit**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Week- 02**
**Lecture - 11**
**Cell structure and function: Eukaryotes**

Hi. Welcome back and today we are going to talk about the second category of cells which are the eukaryotes. Now in my last class, I spoke about prokaryotes and what I mentioned to you last time was that prokaryotes; we spoke about 2 types of cells which are the prokaryotes and the eukaryotes and we spoke that the difference between these 2 categories is based on how the genetic material is really organised. Now if one were to get a fair idea about the size difference between the prokaryotes and eukaryotes.

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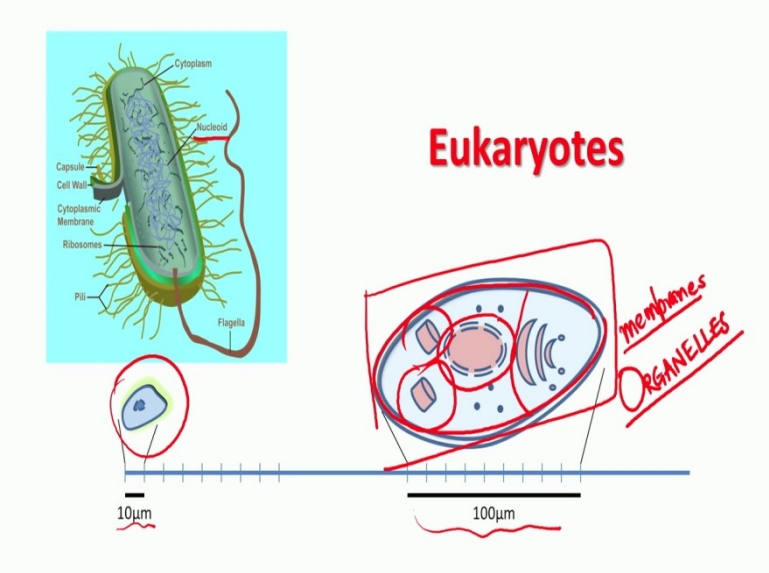


You find that prokaryotes are fairly smaller in size compared to eukaryotes which are much larger. They have a much simpler structure as you can see it in this cartoon while the eukaryotes have a much more complexity where the cell gets divided into multiple sections such as here the nucleus and the other components.

Now each of these sections in a eukaryotic cell is surrounded by the membraned itself. So it is like an addition to the cell membrane which is the outermost membrane. All these components or subdivisions in turn themselves are surrounded by membranes and hence they are called as organelles, which is organ like. So what you had seen in prokaryotes was DNA

was loosely arranged in a nucleoid body but what you find in eukaryotes for the first time in evolution that the DNA is very well organised and it is very well protected.

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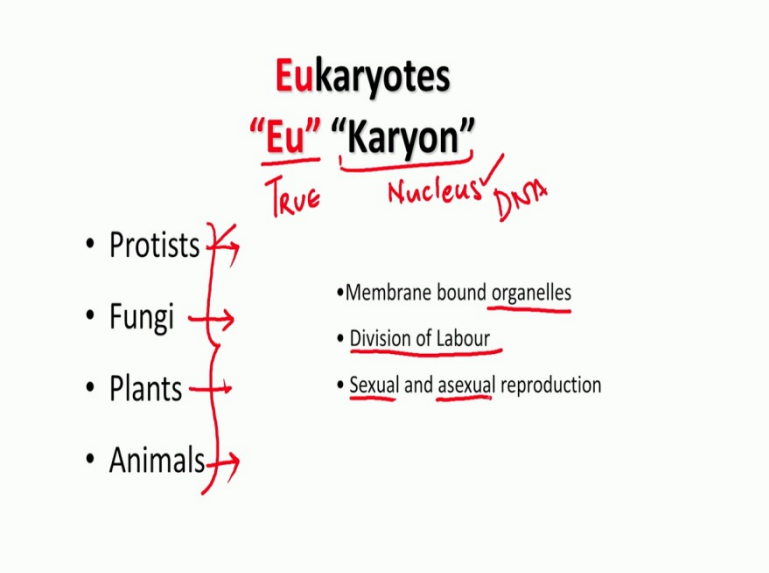


So what does eukaryote really mean, I mean if you were to split the word, "Eu" means true while karyon as I told you last time, it means nucleus. So in this group of organisms, you for the first time see a very well-defined structure which is called as the nucleus and it is this nucleus which houses the genetic material which can be DNA in most of them. Now eukaryota or eukarya as a domain consists of 4 major group of organisms which are the protists, this is where you will come across amoeba and other organisms like paramecium, if you studied in your high school classes, it also consists of the fungal domain which includes mushrooms, all kinds of flowering and non-flowering plants which will come under the eukaryotic domain as well as all the animal cells.

So the eukaryotes unlike the prokaryotes have membrane bound organelles, and the purpose is this allows the cell to have a division of labour. And as we had seen last time prokaryotes mainly reproduce asexually, but in contrast to prokaryotes, eukaryotes exhibit both sexual and asexual mode of reproduction. So let us start with some of the basic structures of a eukaryotic cell and what you find is the outermost covering of the eukaryotic cell is what you call as the plasma membrane.

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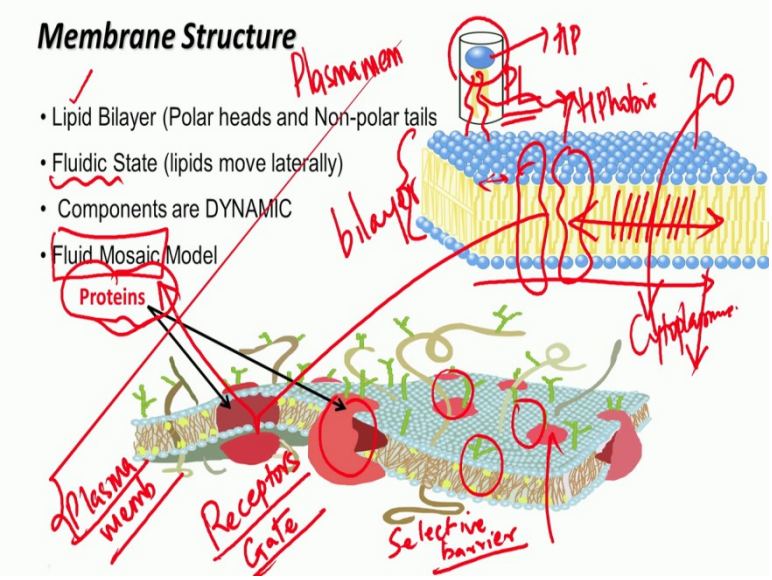


Now as I told you this is a feature which is common both between the prokaryotes and the eukaryotes and it is this outermost layer which segregates the interior of a cell from the exterior of a cell. Now each plasma membrane is made up of monomeric units which are made, called as phospholipids with; they have a polar head and the nonpolar tails.

And what you find is that these phospho-individual units arrange in tandem and they form a polymeric complex which is what you call as the plasma membrane. And what is interesting is to note that it is not just made up of lipids that is the phospholipids, on a routine basis if you were to look at the structure of the plasma membrane you find that, it kind of get interspersed in a very random fashion by proteins here and there, it is almost like the mosaic tiles that you see in your homes classically which has no specific pattern, you just have a mosaic arrangement of these proteins.

So there are 2 features to these membranes; one, a mosaic arrangement of proteins and these proteins can be anything, these proteins can be the receptors which can sense what is happening outside and then relay the information inside, these proteins can be the gatekeepers which will allow only specific material to enter from outside to inside. So the whole and sole purpose of the plasma membrane is to provide that selective barrier and act as a gatekeeper, it will make sure that only what is allowed to move in or what is allowed to move outside of a cell goes through this barrier, otherwise no.

So there are 2 major aspects one has to remember about membrane and membrane structure is that not only are they made up of lipid and units called phospholipids, these lipids are

highly fluidic in nature. Each of these monomers, it is like they are arranged in tandem and they can literally sway either which way, and that fluidity is the crux for the ability of a cell to change its shape, to move one point to the other, so even though it retains that selective barrier property for example if you have an amoeba sitting, and if the amoeba wants to change its shape and move forward this change in shape is possible because the membrane can contract and can easily rearrange because these individual phospholipid monomers can transition, so they are really fluidic in nature.

And despite being fluidic in nature they maintain selective barrier properties because of multiple proteins which are present, interspersed in the membrane structure. Hence you find that the membranes have what is called as a fluid mosaic structure and these plasma membranes are very important because the polar heads are hydrophilic, so the outer edges, the heads are hydrophilic while the tails which are the long fatty acid chains are hydrophobic. So it is almost like when they are sitting in an aqueous environment they end up forming 2 layers, that is why you call it as a bilayer.

With the hydrophilic ends exposed to the aqueous environment either outside the cell or inside the cell towards the cytoplasm, while the hydrophobic ends come together. So this forms a very nice barrier in terms of, if you look at the thermodynamics of any material to pass through you find that if at all there is a hydrophilic molecule and it has to gain its access say from outside to inside, unless it crosses this barrier of hydrophobic zone, it cannot go through. And the only possible way it will go through is that if there is a gate somewhere around and this gate is made up of proteins as I mentioned earlier. So the outermost covering of any eukaryotic or prokaryotic cell is the plasma membrane.

But then the whole plasma membrane cannot hold itself, it will end up collapsing unless there is something to hold it together. And this plasma membrane essentially sits on a scaffold of various, I would say, fibrous proteins or scaffold its you can literally visualize it like a meshwork of wires on which this membrane is sitting, and this forms what we call as the cytoskeleton.

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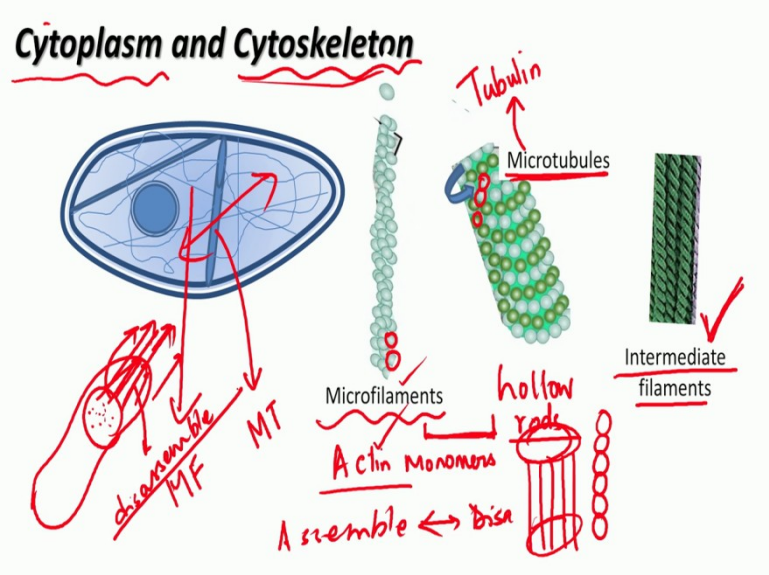


So what you have is the interior of the cell which consists of a fluidic arrangement and that is what we had seen in prokaryotes also which is a cytoplasm, and then the plasma membrane kind of a rests on this scaffold of various fibres and what you call as the cytoskeleton. Now there are 3 different kinds of cytoskeletal elements, and that is interesting to note because each of these elements have different tensile strength and each of them will provide a different rigidity to the cell. So you will have something called as microfilaments which are helical small extensions of polymers of small proteins called as Actin.

So these Actin monomers, so here each circle is an actin monomer, they arrange in an helical arrangement and they form this highly flexible fibres within the cell. So if a cell has experienced some sort of an external pressure from outside and the cell needs to contract for example this contraction would be possible, thanks to the actin fibres. In addition to microfilaments, there are slightly more thicker filaments which are called as microtubules. These are almost hollow rods and each rod in turn is made up of smaller units where each unit arranges in a cylindrical fashion, so it is like a hollow cylindrical rod which is much more stiffer than the microfilaments. So it also helps so in this diagram for example you will find that these straight thin lines are the microfilaments while these rod like structures are the microtubules.

Now the beauty is, just like actin monomers, just like microfilaments the microtubules are made up of another protein called as tubulin and each circle here shows you a tubulin. So how do these tubulins arrange, what is important here is to understand that each tubulin arranges in a linear fashion, like a string of beads. So I want you to imagine each tubulin to

be a bead and if you connect them linearly to each other it is like a string of pearls or a string of beads.

Now take such 13 such strings and arrange them in the form of a cylinder, a hollow cylinder. That is what is a microtubule. So compared to microfilament these are a little more rigid, but then microfilaments are far more flexible. What is again interesting and important to note is that because they are made up of these individual monomers it is very easy for these 2 groups of filaments to assemble and disassemble at short notice.

You know you can literally visualize this as a set of Lego toys where each small piece of Lego can come together to form a structure and when it want to disarray they just go, you can just separate them. What better when you have these small molecules as small beads coming together if they need to form a filament and then dissociate if there is no more need. And it is this cytoskeleton along with the fluidic ability of the plasma membrane which allows the cell to contract and relax.

And the third category of the fibres are the intermediary filaments. Now intermediary filaments unlike microfilaments and microtubules are more like ropes, you can visualize them as nylon ropes which are twisted and they are much more having a much more higher tensile strength than the microfilaments or the microtubules. So in regions of the cell where there has to be much more rigidity required you will find that the cell consists of intermediary filaments.

So let me rephrase it. The cytoskeleton is the scaffold or the structure on which the plasma membrane rests and it holds the entire shape of the cell together. It has 3 different kinds of components; the microfilaments which are the most flexible ones, followed by the microtubules which are slightly more rigid but they are hollow tubes, hollow cylindrical tubes, and then the most resistant to mechanical changes are the intermediary filaments.

So these 3 group of fibres kind of hold the cell together and because of their ability to easily assemble and dissemble, particularly for microfilaments and microtubules, it is possible for a cell to quickly assemble or change its shape and reassemble. Let me take for example if you have an amoeba sitting here, and the amoeba needs to move forward, what will happen is all these monomers of actin will then start arranging and start pushing towards the leading edge of the amoeba.

So what happens is though they were existing as individual monomeric units, if the need be they immediately organise as rods and push the cell forward. So this is possible because of this property of cytoskeleton. And once it has moved forward and the work is done, the same filaments will disassemble back to the monomeric units and the shape will change. So it is possible easily because of the cytoskeletal elements for the cells to change their shape.

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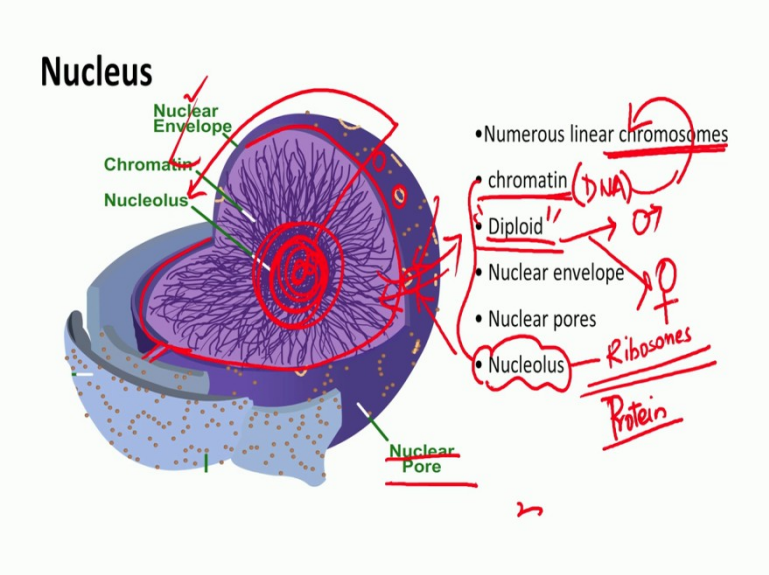


Now let us come to one of the most distinguishing features between the prokaryotes and the eukaryotes and that is the nucleus. Now the nucleus in case of eukaryotes is much more well defined because the DNA is very well organised. I mean, you will be surprised to know that if you were to actually measure from end-to-end, the length of extended DNA molecules let us say in humans, it will be at least 2 metres long and yet you find that this fibre which is supposedly 2 metres long and has your entire information coded in it is compacted and stored inside a cell which is about 10 to 20 microns in diameter.

Now how is that possible? Now that is possible because the DNA gets reorganised and refolded it is like you take a thread of wool and then you start winding it to form a ball of wool and then further compact it with the help of a class of proteins which is what you call as a chromatin.

So the DNA in case of eukaryotes is organised into chromatins, and this chromatin will loosen up to form chromosomes at the time of cell division. We will talk about cell division later. So unlike prokaryotes which have single circular DNA, here in case of eukaryotes the DNA is much more complex and hence the DNA is much better organised and it is organised

with the help of protein DNA complex which is what you call as the chromatin. And this chromatin is very well organised inside the nucleus and the nucleus and the entire genetic material then gets surrounded by a membrane double membrane structure which is what you call as the nuclear envelope.

Then the other thing which is observed is that this nucleus does not exist in isolation. This nucleus has to communicate with the rest of the cell and it does so because the entire nucleus has numerous openings which is what you call as the nuclear pores. So these are the gatekeepers and these are the channels through which the material can move in or move out of the nucleus.

In addition to this you find that the chromatin in the nucleus is condensed and it gets condensed into multiple structures and forms and then at the centre you have this highly compacted chromatin material which is what you call as the nucleolus. And it is here in the nucleolus that the ribosomes, we spoke about ribosomes in the last video as well where I told you that these are the machineries which actually do the work of synthesising proteins. So what you find is that this protein synthesising machinery is actually produced and assembled in the nucleolus.

So this has been the major difference between the prokaryote and the eukaryote whether DNA is far more compact and the reason it is far more compact is because unlike the prokaryotes which are haploid here you are getting genes from both set of parents, the father as well as the mother. So you find that all the eukaryotes are diploid for every gene you have 2 copies one coming from the father and the other coming from the mother. So this is the basic difference between the arrangement of genetic material between prokaryotes and eukaryotes.

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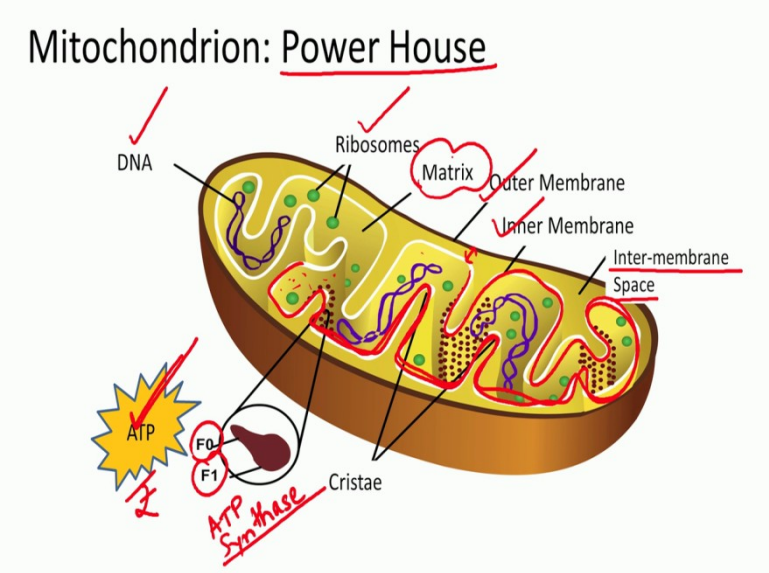


Now let us come to another very important organelle which is present in eukaryotic cell and that is the mitochondria. Now mitochondria is also called as the powerhouse of the cell because as I mentioned earlier also that when you want to do any life activity, the life activity requires energy and there has to be production of energy and that energy or the currency in case of a living world is this molecule called ATP.

Now this energy currency has to be constantly synthesised and supplied to the rest of the cell for other processes to happen. So this actually happens, this process of energy generation happens in the mitochondria and it is again a very specialised organelle. It is again a double membraned organelle, it has an outer membrane, an inner membrane and what you notice intriguingly for the first time is that the inner membrane has multiple foldings and it is not without a purpose, there is a reason for this folding and I will come back to it.

You find that the inner membrane unlike the outer membrane has far more invaginations and it is studded with a set of particles which is called as the F0, F1 particles. It is this particle or F0, F1 it is nothing but the ATP synthesising enzyme, also called as ATP Synthase. And you find that when you have so much of invagination of the inner membrane and every nook and corner of this inner membrane gets studded by this particle, it increases the surface area and it provides sufficient positioning of this ATP Synthase to work.

I will not get into the machinery as to how it functions; if you are interested you can always look at topics of mitochondrial respiration, it will become evident how this ATP Synthase work. But I will insist on one thing; the inner membrane is highly impermeable compared to

the outer membrane of the mitochondria. And the space which is present between these 2 membranes play a very important role in ATP synthesis and this space is called as the inter membrane space.

What you also find is that the inner part of the mitochondria like cytoplasm in mitochondria has a fluidic arrangement which has a lot of ribosomes, it has its own DNA so mitochondria consists of its own DNA and it has its own protein synthesising machinery, you find it present or suspended in section of mitochondria which is called as the matrix. So mitochondria, its sole purpose is the generation of energy that is ATP and it has very interesting structure because it has its own DNA, it also has its own ribosomes. And I will come back to this again when we talk about origin of eukaryotes.

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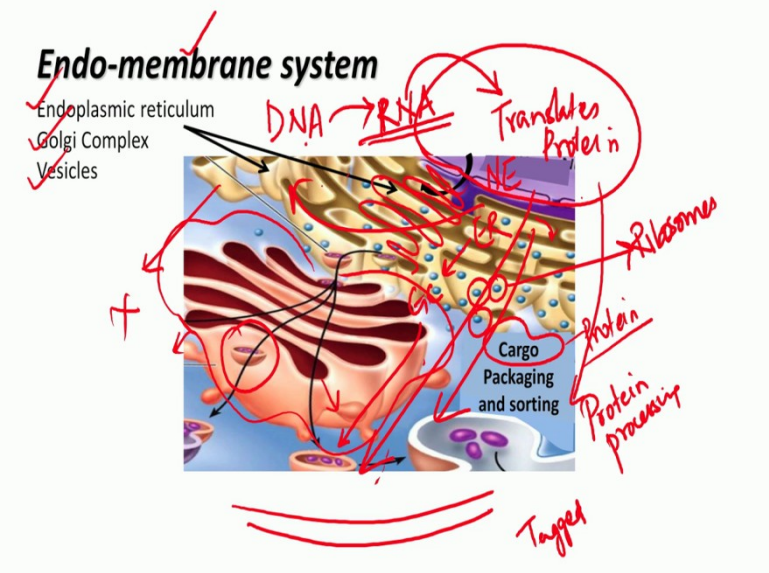


Then we look at the other parts of the cells. So this is the interior of a cell in this slide and what you can see here is that starting from, so if this were the nucleus, from the nuclear boundary moving outwards you see a whole channel of membranes extending all the way up to the plasma membrane, so plasma membrane would be somewhere here. And what you find is that this entire meshwork of these membranes is called as the Endo-membrane system, and it consists of subsections, it has right next to the nuclear envelope, 2 kinds of membrane bags, a set of bags which are studded on their outer surface with ribosomes, another set of bags which do not have ribosomes.

And what is observed, this set of bags or this set of network of channels is what you call as the endoplasmic reticulum. And this endoplasmic reticulum plays a very important role in

protein processing. So we did talk about the central thematic that is DNA is read by and translated DNA is converted to RNA where kind of decodes the information from DNA and having decoded it, it then passes it on and translates it into a product which is what we call as the protein.

Now once the proteins have been synthesised, the proteins can get further modified and they can even be tagged. For example how will a cell know that a protein which is synthesised here needs to be secreted or a protein needs to be put into the mitochondria. So that tagging process, the postal tagging happens in these organelles.

So that happens partly in the endoplasmic reticulum and then the further fine processing happens in the Golgi complex. Not only does the processing happen in these 2 organelles, the endoplasmic reticulum and the Golgi, at the terminal end of the Golgi you start observing that once a product, which is what we call as let us say a cargo has been synthesised, now in this case the cargo is nothing but the protein and a processed protein.

Once the protein has been synthesised, processed, it kind of starts getting packaged, it is almost like an assembly line where things kind of get packaged and then they need to be sorted and sent to the respective destinations. That kind of sorting of proteins and sending the proteins to the respective destinations within a cell, it can be a plasma membrane, it can be a mitochondria, it can be any other organelle, that happens at the Golgi complex because from the terminal ends of Golgi complex the cargo gets packaged into small structures which is what you call as the vesicles, and it is these vesicles which are like the ferries which will then ferry the cargo to various parts of the cell.

So I can kind of summarize Endo-membrane system as that part of the cell, which has not only the assembly line for synthesising and processing the proteins, checking the quality control, but is also the section or the postal section, where the addresses are marked on the proteins having marked the proteins it ends up sorting the proteins and it is also the delivery system. And as a result what you find is all the way from the nuclear envelope to the endoplasmic reticulum to the Golgi complex to the vesicle there is a continuity and there is a continuity and the flow of material happening to all different directions of the cell.So that is the importance of the Endo-membrane system.

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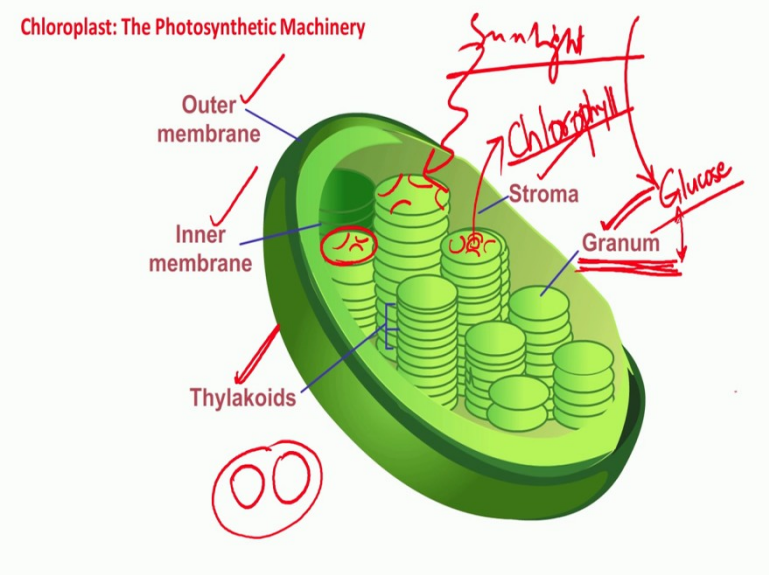


Then if you talk about plants which unlike animals have developed this remarkable activity and ability to convert solar energy into what you call as the chemical energy which is the glucose, you have an additional cell organelle which is called as the chloroplast. Chloroplast similar to mitochondria has 2 membranes, an outer membrane, an inner membrane, a matrix which is called as the stroma. And then you find that this chloroplast has this arrangement of coin like structures which are called as the Granum.

Each coin you can visualize a set of let us say 1 rupee coin and you start putting them one above the other. Each coin will be called as the Thylakoid and then the entire stack of Thylakoid will be called as the Granum. So what is the purpose of this Thylakoid, now these are disc like membranes which essentially harbour the light harvesting complex or let me say that each of these discs have these antenna like complexes which are suitably oriented so that they can capture the sunlight.

Now that is the beauty. Here you find that not only are the membranes flattened and are studded with all these antenna like molecules which is nothing but a set of pigments, the most common one for which the plants are green in colour is the chlorophyll. So all these chlorophyll molecules, they beautifully arrange like antennas, light harvesting antennas in these flattened discs called Thylakoid, and to accommodate more and more harvesting potential the Thylakoid membranes exist in stacks. And it is here that you start seeing in this organelle the conversion of sunlight into glucose. So this is something which you again find in chloroplast and chloroplast also has its own DNA and ribosomes.

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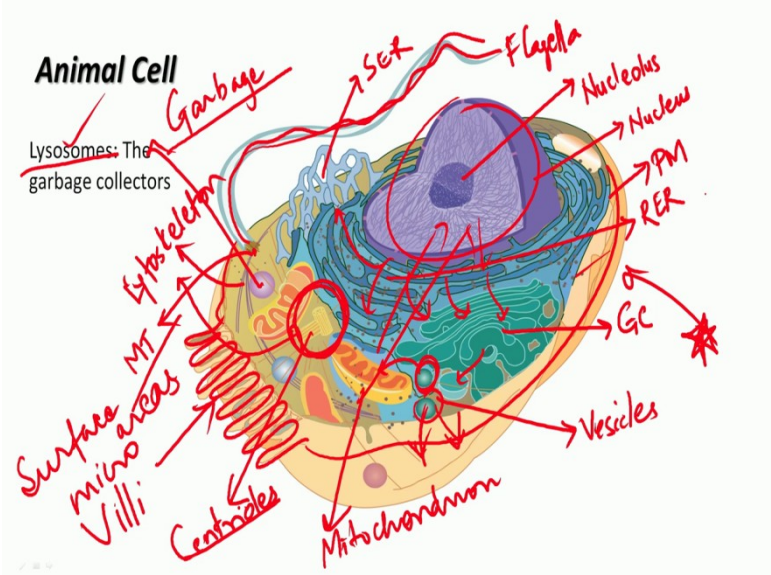


So let us look at the animal cell and what all it will contain and in this exercise you can see the central most part here is what you call as is the nucleolus, this entire structure is what you call as the nucleus. The nucleus keeps on extending into Endo membrane system which consists of rough endoplasmic reticulum, the smooth endoplasmic reticulum and the Golgi complex. And you find that it is in these networks of channels that the sorting, processing, packaging and delivery of the proteins and other constituents of the cells happen.

The Golgi complex then ends up packaging its content the content starts getting delivered either outside or to the mitochondria, so this is the mitochondria; starts getting delivered to the mitochondria and or to the outside if the need be and these structures is what you call as the vesicles. The whole entity of a cell is then surrounded by the outermost membrane which is what you call as the plasma membrane and the whole cell is held in shape because it consists of these fibre which are the cytoskeleton.

You also find the cylindrical objects which is the part of the cytoskeleton which is the microtubules and particularly in the animal cells at one polar end you find a pair of these microtubule like structures which are called as the Centrioles. In addition to all this you also find that animal cells have some other special organelles which are called as the lysosomes. Now what are these? These are nothing but the garbage collectors. So if a cell is doing so much of a function, obviously it is going to produce a lot of waste matter which needs to be collected and appropriately processed and discarded and that happens in lysosomes.

Animal cells for example sperm will propel further with the help of its propeller which is nothing but the flagella. This flagella extends out of the plasma membrane and internally it consists of motors which are made up of microtubules. And then the plasma membrane also consists of, in some cells it keeps on folding itself to enhance the surface area, these are called as Villi or microvilli. Especially these are seen in cells for example which are lining your intestinal tract where these foldings will help allow a cell, a greater surface area for more and more absorption of let us say nutrients.

So when there has to be a need of the, for the absorption of nutrients then this can happen in intestinal cells. So this is how the animal cell look like, so though it looks fairly complex. I again want to reiterate that each of these components of the cells are in crosstalk with each other. Not only are they in crosstalk with each other and there is a dynamic interaction happening at all given points of time, the cell is also sensing things from outside and accordingly responding.

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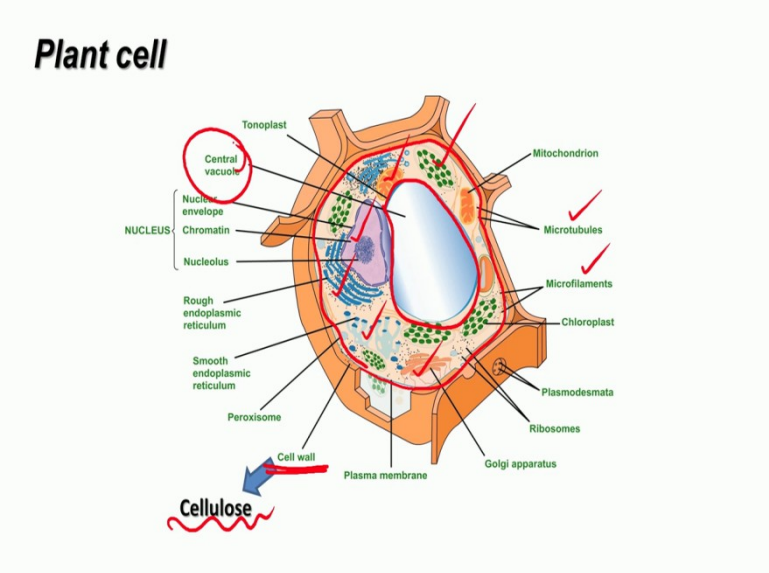


Now these are the common features which you will see and lot of these organelles you will see them again in a plant cell like the nucleus, the endoplasmic reticulum, the smooth endoplasmic reticulum, the Golgi complex, the mitochondria, in addition to that since the photosynthetic machinery you will find the chloroplast. In addition to that there are 2 additional features which you see in plants, one-way by which the plant cells kind of hold on to the ground and resist any kind of attack on them is by having an extra layer outside the plasma membrane which is called as the cell wall. And this cell wall is made up of one of the largest polymers available on the surface of the earth which is the cellulose.

And this cellulose is something that you and I use on a day-to-day basis because all our cotton clothes are nothing but made up of cellulose fibres. So like animal cells, the plant cells also has microfilaments, microtubules and all the other organelles plus they end up having a central large organelle which is called as the vacuole. It is this organelle which ends up storing a whole lot of water in its inner content.

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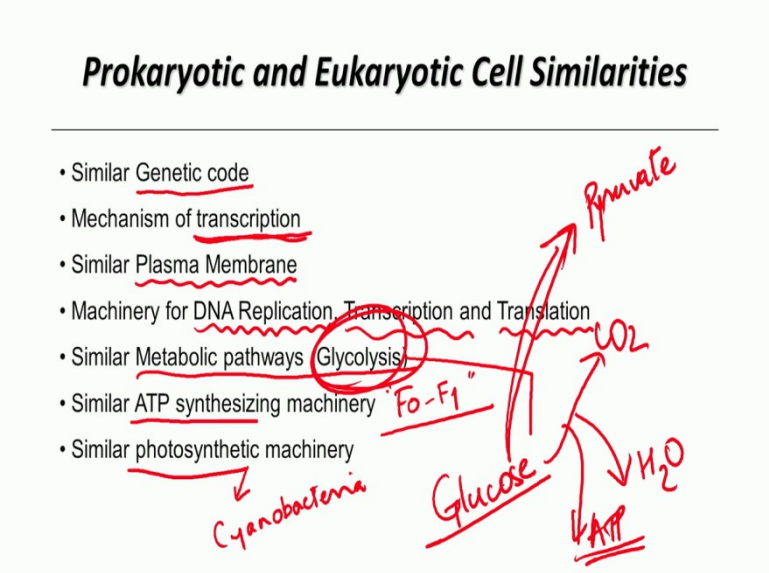


So let us go back and look at what are the similarity between prokaryotic and the eukaryotic cells. I said all this while I spoke about the diversity, the diversity was in terms of the presence or absence of nucleus, the presence or absence of membrane bound organelles. Now let us look at the unifying feature because as I said there are common features across life and what as that unifying features. And the unifying features are these; the way in which the information is coded which is what you call as genetic code is similar between prokaryotes and eukaryotes.

The way in which this genetic code is deciphered or read and interpreted is done through the mechanism of transcription is fairly conserved. The structure and the biological activity of the plasma membrane, the outermost boundary of these cells is similar. Even the machinery which is used by these cells to replicate their DNA before they can pass it on to the daughter cells or the decoding process by transcription and its conversion into the actual product of protein which is what you call as translation is fairly conserved right from bacteria to humans. Then the ATP synthesising machinery; remember we spoke about the F0-F1 particle in the mitochondria, its architecture and its mode of action is fairly conserved across evolution.

Even the machinery for photosynthesis, in bacteria we spoke about the cyanobacteria, right? In case of plants you have the chloroplast, you find there is a huge amount of similarity. And then some of the reactions which very commonly occur in life, the most easy one is the reaction where the glucose which is your stored energy is kind of broken down into carbon dioxide and water, this happens in cytoplasm and mitochondria for the release of ATP. This process where the glucose first gets converted to pyruvate and this first step happens in the cytoplasm. This is what you call as glycolysis. This machinery in cytoplasm which breaks down glucose intermediate, to its intermediate molecule called pyruvate is conserved across prokaryotes to eukaryotes.

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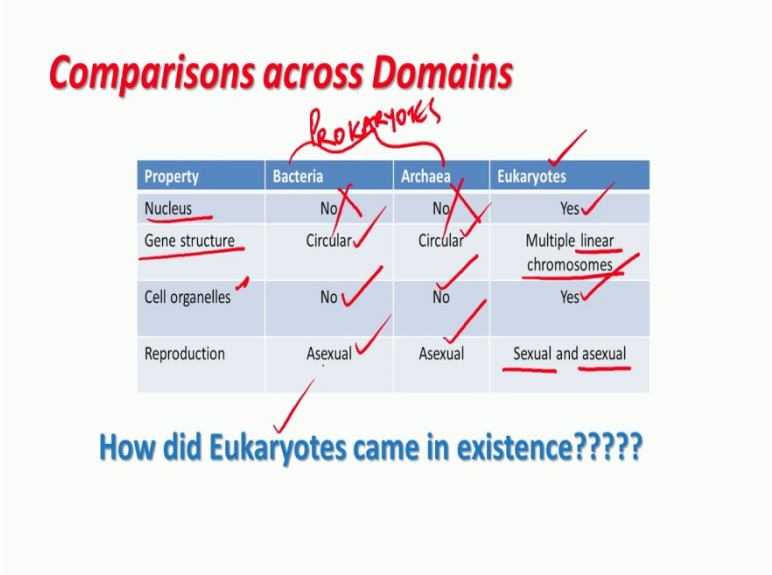


So let us look at the comparison against bacteria, Archaea and eukaryotes. As I said bacteria and Archaea belong to prokaryotes, right? And then you have the eukaryotes. If you were to look at the nucleus, it is not present in bacteria, it is not present in Archaea. Both of them are prokaryotes. But it is very well-defined in the eukaryotes. If you were to look at DNA or the gene structure, the DNA is circular in bacteria and Archaea, but in case of eukaryotes is far more complex and with the help of chromatin is organised inside the nucleus and the can at the time of cell division convert to linear chromosomes. There is no evidence of any division of labour in bacteria and Archaea so you do not see any cell organelles, but you see very well-defined cell organelles in eukaryotes.

If you look at the mode of reproduction in case of bacteria and Archaea the mode of reproduction is asexual, but eukaryotes exhibit both sexual and asexual mode of reproduction. So there are, in my previous slide I said, there were plenty of similarities yet there are

differences. So how is it that the eukaryotes came into existence, I mean this has been a puzzling question and it is partially answered by the available evidences that we have today. But before I get to that, I need to explain you a few terms.

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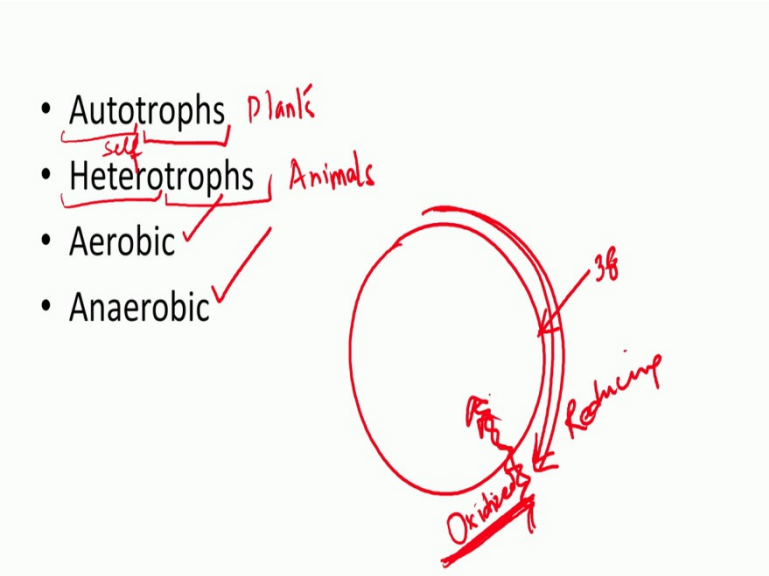


Autotrophs, “auto” means self, “Trophic” means synthesising, food synthesising. Organisms which can synthesise their own food like plants are called as autotrophs. Organisms which depend on others, like animals which depend on plants, are called as heterotrophic. So this (autotrophs) will have plants, this (heterotrophs) will have cyanobacteria while this will have animals, amoeba, so on and so forth.

Now if you remember we were talking about the origin of the earth and the origin of life I had talked about that the life originated somewhere around here about 3.8 billion years ago, and then for a very long time the earth’s atmosphere was the reducing one. Then suddenly due to the advent of photosynthesis, the atmosphere started getting oxidised, right?

Now, a set of organisms which can manage to break down their glucose in absence of oxygen are called as the anaerobic organisms. That means these guys are able to still obtain energy from glucose by breaking it down, glucose into lactic acid or ethanol, while those organisms which later ones the environment became oxidised they should have developed some more mechanism to now utilise that atmospheric oxygen and still break down glucose. So, those organisms which break down glucose with the aid of oxygen are called as the aerobic organisms.

So if you were to look at the history of earth, this becomes very important; the initial phase is reducing, suddenly there is a pressure created which is again what I will call as natural selection in terms of Darwin's theory, you suddenly have a pressure created because the atmosphere becomes highly oxidised.

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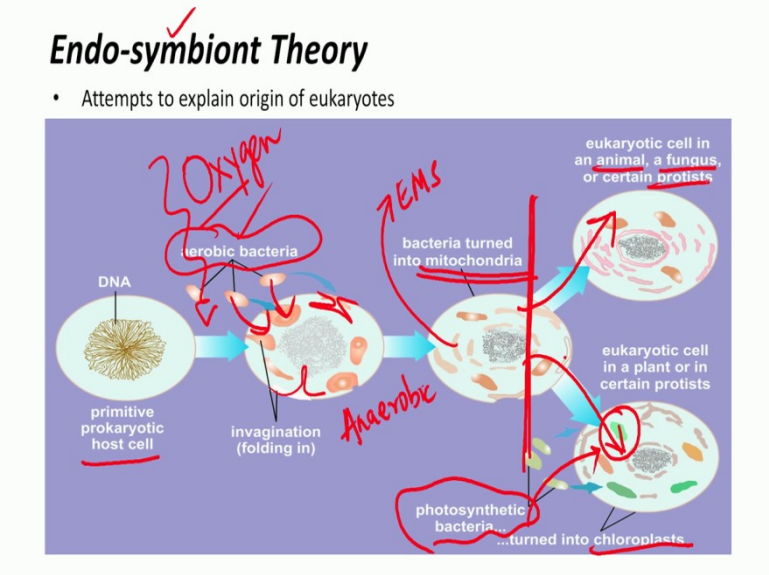


That means the organism should have evolved a process by which despite having oxygen around it should be able to survive. So with this background let us look at the theory which explains the origin of eukaryotes and that is called as the Endo-symbiont theory.

What it says is the life started as a prokaryotic cell with a very simple DNA, but somewhere around the time when there became a need, because of the atmosphere having high amounts of oxygen, some bacteria must have developed the ability to survive in such an oxidising conditions, and they were able to utilise atmospheric oxygen and hence were aerobic bacteria. So somewhere during the course of evolution, an anaerobic bacteria must have engulfed this

aerobic bacteria to survive, right? And this aerobic bacteria eventually ended up becoming what we call today's mitochondria.

Simultaneously theere must have happened, simultaneously or even later around the same time the plasma membranes must have started invaginating, and this must have led to what we see today as our Endo membrane system. And again if there were scarcity of nutrients, some of these organisms must have realised that they cannot survive on their own unless they develop the ability to synthesise their own food and some set of bacteria must have developed that ability of photosynthesis so this organism might have even engulfed a photosynthetic bacteria which later ended up becoming today's chloroplast.

So it is at this junction the branching must have happened where the plants must have evolved while the ones which could not become autotrophic continued to become the animal, a fungal or the protist group. So this is what the Endo-symbiont theory says, it says that in order to survive an anaerobic bacteria during the course of evolution must have engulfed an aerobic bacteria and this must have led to some sort of a symbiosis which is mutually beneficial relationship which will have then lead to final evolution of the mitochondria and similarly in case of plants there must have been an engulfment of an early photosynthetic bacteria which would have then ended up evolving into chloroplast.

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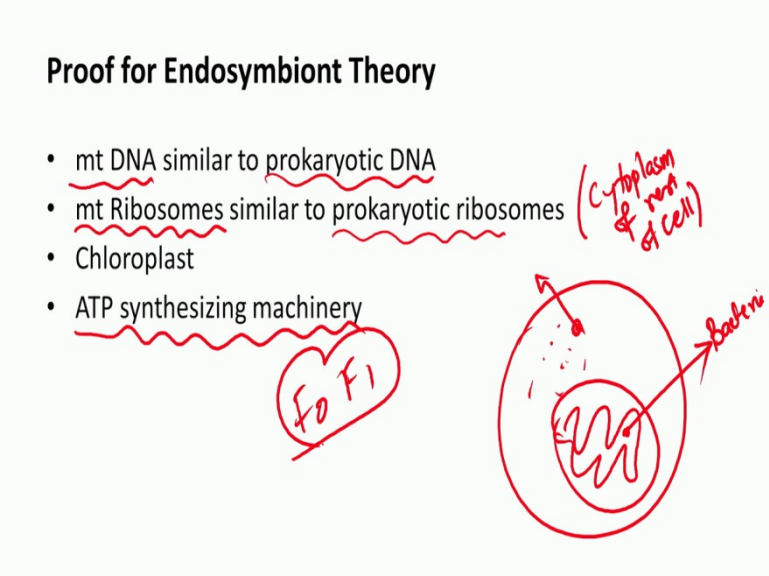


But theories are theories, do you have evidence? And we do, to some extent. If you were to look at the mitochondrial DNA of today's eukaryote you will be surprised to know that it is very similar to prokaryotic DNA. Not just that if you are to look at the mitochondrial

ribosomes you find in terms of the chemical structure, in terms of their size they are very similar to prokaryotic ribosomes. And these mitochondrial ribosomes are, mind you, are different from the ribosomes which will be seen in the cytoplasm of the rest of the cell.

So if you take an animal cell, the animal cell in the cytoplasm will also have ribosomes, will have a mitochondria and within the mitochondria it will also have a ribosome. But this ribosome in mitochondria is similar to the ribosome of bacteria while this ribosome is different. So clearly there are similarities. Similarly you find the same sort of analogy in case of chloroplast and even the machinery which I spoke to you, the structure of F0-F1 particle, the mechanism by which the F0-F1 particle functions, has remained very similar to that of prokaryotes.

Thus we believe that today's eukaryotes have actually evolved from the prokaryotes. So I would like to end this video by saying, eukaryotes have been far more evolved than the prokaryotes, they seem to have come out of prokaryotes and they seem to have a very well-defined nucleus and a very good division of labour, which provides complexity for sure, but it also enhances the efficiency of the organism which may not have been in case of prokaryotes. Some may argue that despite this, the prokaryotes are probably the superior forms, given the fact that they have managed to survive all kinds of onslaughts on this earth for the last 3.8 billion years.

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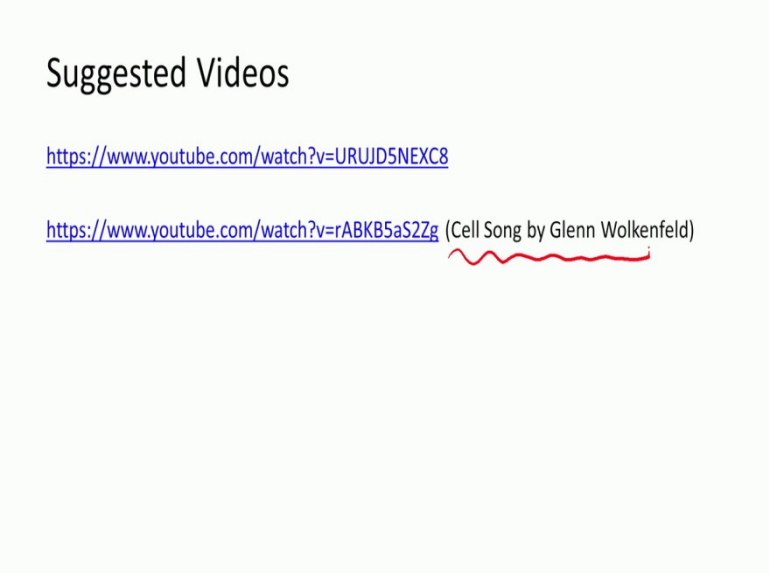


Well I leave that decision for you to make but I would like to suggest a few videos, and there is a very interesting the ones who are musically inclined to just look at this fun song on cell

and cell structure by Glenn Wolkenfeld. And hopefully I will see you again in the next video where we will talk about cell cycle, and other aspects of cell division. Thank you.

**Biology for Engineers and other Non-Biologists**
**Professor Madhulika Dixit**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Lecture Number 12**
**Cell Cycle**

So hello again and in today's class we are going to talk about the process of cell cycle. Now before I get into what is cell cycle and what are the different features of cell cycle, let's start looking at how we start our lives. Now we all know that we start our life as a single celled organism that is the zygote and this happens after the fertilization of sperm with the ova.

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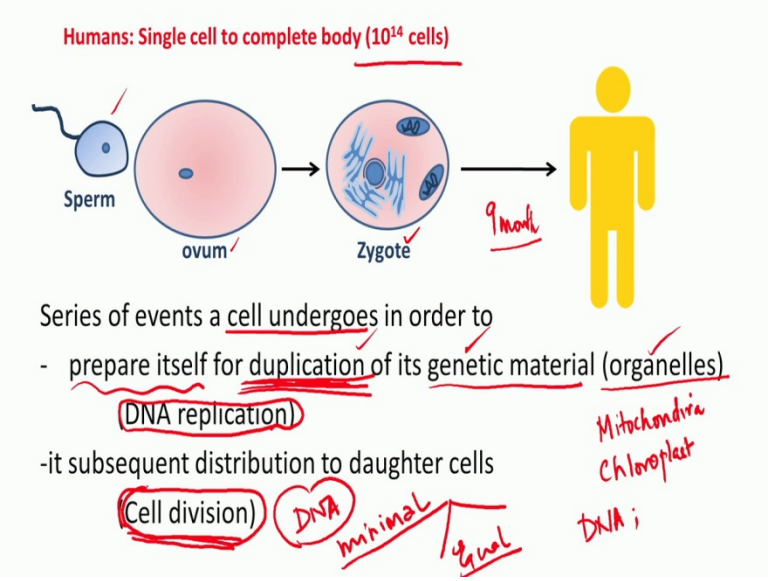


What's interesting is to note that though we all start our life's journey as a single cell in our mother's womb, in about nine months' time, we are born, and then later as we grow and become an adult, our body is made up of roughly $10^{14}$ cells. Now how is that possible?

Now this is possible because every living cell has one basic function to do, and that is to replicate its DNA and then pass it on to the daughter cells. So cell cycle basically talks about how a cell prepares itself to duplicate its DNA and then, to divide into two daughter cells. So, this is something very interesting. Now, how will you define cell cycle? I would say it's a series of events that a cell undergoes, and the reason it undergoes all these events is because it tries to prepare itself, and tries to generate enough raw materials in the cell for duplication. Now this is

important. For the cell to pass on the information, the duplication of the genetic material is very critical point. So it first duplicates its genetic material, it also tries to have sufficient copies of its various cell organelles. For example, the cell has mitochondria, the cell; in case of plants will have a chloroplast.

So, if a cell has to divide into two daughter cells, it has to pass on the same amount of genetic material, which is DNA. Also, it has to divide all the other cell organelles, so that the new daughter cell which is formed, can do all its functions. So the first thing that a cell cycle involves is the preparation for duplication of the genetic material and this involves DNA replication, we will cover this section of DNA replication in a separate class. Today we'll only focus on the various steps of cell cycle.

So it has to duplicate it's genetic material, it also has to duplicate and give more copies of different cell organelles, and the second stage of cell cycle is the actual process of cell division. So once the DNA has been duplicated, how is it that the same amount of DNA with minimal errors. Now this is important to know that every round of cell cycle, the DNA gets duplicated with minimal errors, and this DNA then gets equally divided into the daughter cells.

Now this division is what you call as the cell division. So cell cycle consists of multiple steps and before I get into the different steps of cell cycle, I just want you to ponder over a few things. Now, it's not necessary that each and every cell of your body will undergo cell cycle at the same rate. And there are in fact, few exceptions where certain set of cells, after they have been formed and they have acquired high level of efficiency, they do not divide, and some of them are the nerve cells.

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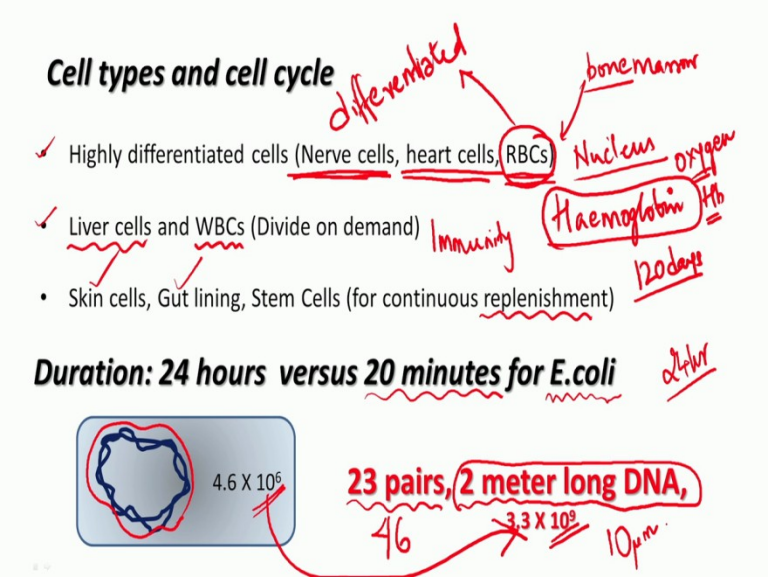


The neurons, once they have been completely formed, except for the few early years of our development, and once they have been totally formed and they have become highly efficient in their function, they do not divide. They, in fact do not undergo the process of cell cycle. I'll come to it as to why, and the reason in fact I would say that they don't divide is because they are too busy doing their function and they are highly specialized, in biological terms we call it as, they are already 'differentiated'.

Similarly, if you look at the cells which form the walls of our heart, the cardiomyocytes, they do not divide after they have differentiated. The red blood cells, these cells periodically come out of the bone marrow, they are formed from bone marrow cells and you find, you must have studied in your school textbooks that this is one cell type which once it has differentiated, once it has reached its differentiated ability, it loses its nucleus, and that makes sense because the primary function of the red blood cells is to carry haemoglobin. Now haemoglobin is the main pigment which absorbs and carries oxygen in our blood, and that's its major sole purpose. So if it wants to accommodate more and more amount of haemoglobin, it has to get rid of the nucleus.

So upon differentiation, the RBCs do not have nucleus, and as a result they do not undergo cell division once they have differentiated, and because they do not undergo cell division, each RBC which periodically keeps coming out of our bone marrow pool has a life span of about one twenty days. So, there are certain cells which will not undergo cell cycle. But then, there are

certain cells which undergo cell cycle or cell division only if there’s a demand. And one good example is the white blood cells. Now we all know, that every time we get some sort of an infection, our body has an ability to fight it out, which is what you call as ‘immunity’.

Now this immunity is possible because every time the white blood cells are sensing the invaders, there is a whole series of events which are taking place in white blood cells, and it allows and triggers now the white blood cells and sends a message to the individual white blood cells that we need to multiply, we need to divide, have enough soldiers ready so that they now kill the invading pathogen or the invading bacteria. So, there are cells in our body which will undergo cell cycle, if the need arises. Same example, similarly you find that the liver cells, they regenerate, they divide only when the need is.

In contrast to these highly specialized cells, I won't say the other group of cells are not specialized, but then their function is to maintain the body parts. For example our skin. Now if you have any kind of wounds taking place, the skin has to heal itself and it has to repair itself. Now these kind of cells, which are usually the epithelial cells, the lining cells, the lining of the skin, the lining of the gut, they have to keep on dividing and replenishing the lost cells. So these are the most frequent cells which undergo the process of cell cycle. So, depending upon what is the sole purpose of a given type of cell, lot of cells may choose not to undergo cell cycle unless pushed to do so; while certain cells have to routinely divide and give rise to daughter cells.

And one point to be noted, is that every time they divide and give rise to the daughter cell, they have to pass on the exact information to the daughter cell. Now that is a lot of job and it requires a lot of precision. Before I go again further, I want to again highlight another important point. Now each eukaryotic cell will have obviously different lengths of cell cycle. But if we were to compare an average cell cycle for an average human cell which is anywhere between sixteen to twenty four hours, you find prokaryotes like E.coli multiply every twenty minutes. Now that's interesting because this should also tell you why once you have a bacterial infection, it just spreads so quickly because every twenty minutes, the microbe is actually giving rise to two daughter cells.

But, unlike humans, and I’ll take the examples of humans for simplicity, you find that the bacterial structure is much more simpler, it just has a single molecule of circular DNA, which is

made up of about four million base pairs. Now compare this to humans where would have about twenty-three pairs of chromosomes, in total we have forty six chromosomes, and if we were to unwind these chromosomes, or the DNA and put it together end to end in, let's say, a lab, you will find that the actual length of DNA from a single cell in humans is probably two meters long. And not just long, if you look at the number of base pairs it has, it's about thousand fold bigger than the prokaryotes.

So our architecture, the architecture of human DNA, is far more complex, and it's interesting to note that our two meter long piece of DNA fits into a cell which has a diameter of about 10 micrometers. Now how is that possible? Now, this is possible and it's important for you to understand this, for understanding both, cell cycle and mitosis and meiosis, so I will cover it right away, is how are these DNA arranged, and they are arranged into chromosomes is all what we know, but there's an architecture which is very different in case of eukaryotes than in prokaryotes.

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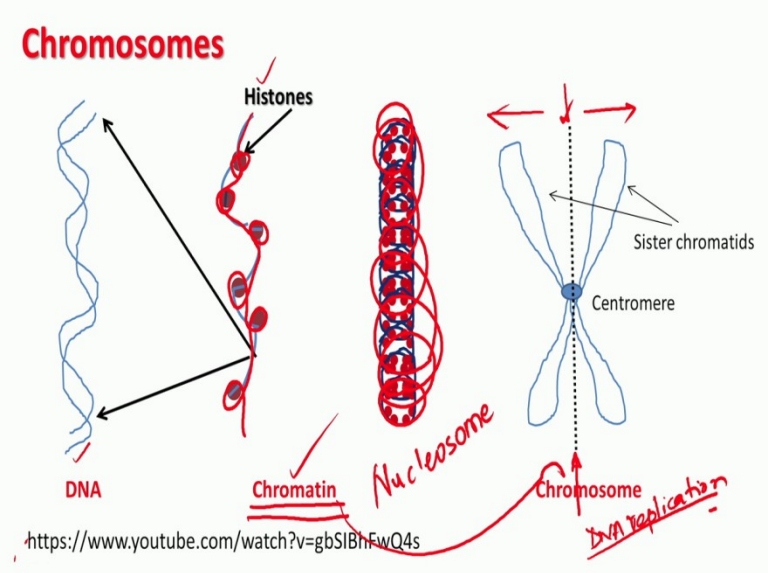


So a DNA which is, in this case, is this molecule. When you want to package it, when the cell wants to package this DNA into a compact structure, this DNA, here it's represented as a string, actually winds around a set of proteins called as the 'Histones'.

So it's like, you have a string of beads, where each bead around that bead, the DNA strand wraps, and those proteins are called as the Histones. Now that's the first order of organization, and then

this string of beads will further coil in a helical fashion and pack over each other and they will form nucleosomes, and so in this case this is one Nucleosome, then it coils again, and the next coiling and so on. Now each of these Nucleosomes get further condensed, and that structure is what you call as the ‘chromatin’. So in humans or in eukaryotes, the DNA being more complex, much more bigger in size is packaged through the help of histones, the proteins which help in their packaging and help them in holding in structures called as chromatin.

And each one of us, as I said, have twenty-three pairs of chromosomes, and each chromosome is made up of chromatids, and what you find is after the process of DNA replication in cell cycle, we will cover this separately; after the process of DNA replication has taken place, each chromatid will give rise to the sister-chromatid. These are sister-chromatids, but each one of them is actually a chromosome. And, these sister-chromatids, right after DNA replication will be held together at a central point by a structure called as ‘centromere’.

Now this is a very important structure, we will refer this to, we’ll come back to this when we are talking about cell division and why it becomes very important. Now it is here, that the two, at the time of cell division, the chromosomes will separate, and I will come to this when we talk about mitosis. So, you have to appreciate that, unlike prokaryotes, which have much simpler DNA, the DNA architecture is a little more complex in humans, and almost all the eukaryotes and that is because we need to compact all these large amount of information into a tiny cell which has a diameter of about ten microns, and within that, it has to packet into a nucleus which will have a diameter of about two to five microns.

So, that is what, that is how the DNA in our body is organized. Now it is not that throughout the cell cycle, you will find that a DNA always exists as a chromosome. That's not true. Mostly it is organized as a loose set of fragments of chromatin, but right before a cell is ready to divide, and divide its genetic material, the chromatin gets condensed into chromosomes. So we’ll look at all this in our class on cell division. So what is cell cycle? Let’s get back to cell cycle.

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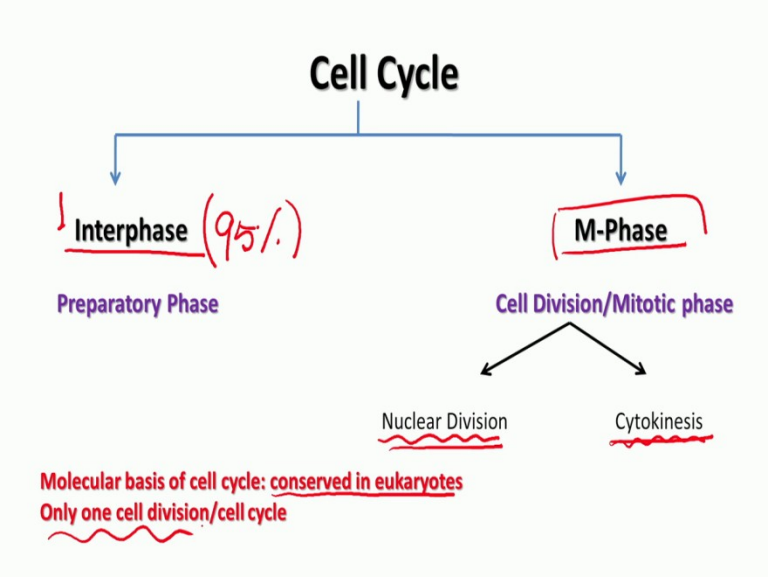


As I told you, the purpose of cell cycle is to ensure that it passes on its exact information, to the two daughter cells, and in the process of doing it, it has to first duplicate it's DNA, and then distribute it to the two daughter cells, it also needs to duplicate it's organelles and distribute it to the daughter cells.

Now, so, this cell cycle consists of two major phases; the first phase is the ‘interphase’. Now interphase occupies almost ninety-five percent of the time of a cell cycle, while ‘M-phase’ is much short lived, and it's in the M-phase that first the genetic material, after being duplicated, the nucleus divides into two nuclei and then the cytoplasm divides and gives rise to two daughter cells. So you have nuclear division, you have cytoplasmic division. Now, one thing which is intriguing and surprising rather, is that if you were to look at the players, the molecular players which govern this interphase, mitotic phase and DNA replication, you find that they are highly conserved in eukaryotes.

So, that's interesting. The second thing is in every cell cycle, the cell undergoes cell division or division of the genetic material only once. So every cell prepares itself, generates enough raw material, gets ready to duplicate its DNA, having generated equal copies of its genetic material it then divides. So, in one cell cycle, the cell division or the nuclear division and the DNA replication happens once. So let us go to the cell cycle. This is a little busy slide, so let me just walk you through this. So let us say, this is the cell which has decided to divide into two daughter

cells and pass on the information to its daughter cells. So, the major part as I told you is the preparatory phase, so all the way from the beginning till here is the interphase. Alright?

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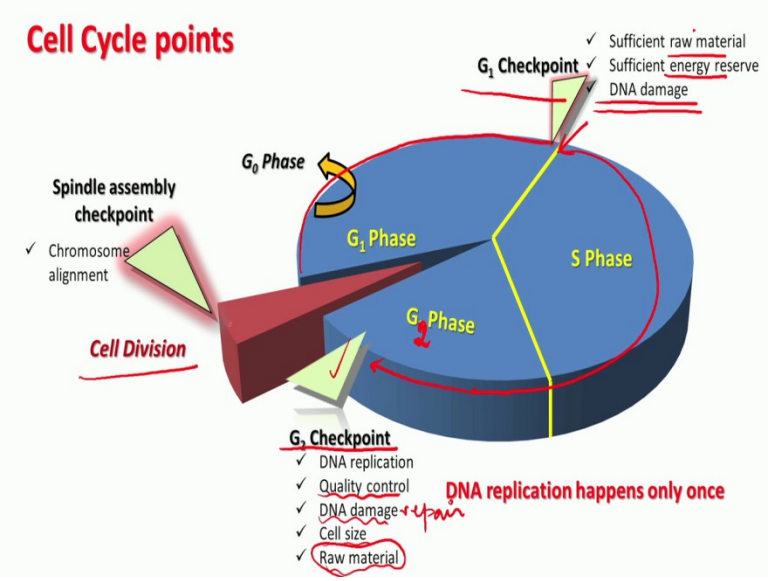


Now in this interphase, we again have three stages, the first one is called as the G1 phase. And, it's also called as the growth phase one. Now it is, it lasts anywhere about eight to ten hours and in this phase, the very first phase of the cell cycle, the cell basically starts growing in size, it starts accumulating enough currency, because it will need all that energy to do the subsequent processes, so it starts generating a large amount of ATP, it starts duplicating it's organelles because eventually the organelles have to be passed on to the daughter cells, and it generates enough resources.

Now one of the major resources which have to be generated in bulk quantity are the histones, because of the DNA is going to get duplicated, the new DNA has to be organized as a chromatin structure, and for that, lots of histones are required. So the building blocks, a lot of these proteins which are necessary for the process of DNA replication, also for compacting the newly synthesized DNA, for the packaging of the newly synthesized DNA, which is the histones are getting synthesized in the G1 phase. And, before the cell actually commits and starts doing the process of DNA replication, it has to make sure that there is no DNA damage whatsoever. A cell cannot afford to allow a faulty piece of DNA to undergo DNA replication because then, it will

enhance the chances of passing on the non-favourable characters to the daughter cells, which is not accepted.

So, in G1 phase, the cell is essentially preparing itself, generating raw materials, generating energy, and, so that now the cell is ready to move on to the phase of DNA replication. So the second phase of cell cycle is the S-phase, where the actual duplication of DNA takes place, each chromosome duplicates itself, and it lasts anywhere between six to eight hours in humans, and we'll cover this topic of DNA replication separately, because it involves a little bit of understanding of how the DNA actually makes sure, that it is passing on the exact information. So I'll cover that in a separate class. But, for the time being, you remember that the second phase of interphase is the S-phase.

Now once the DNA has been religiously and cautiously duplicated, the interphase has the third phase, which is, I am sorry it should be a G2 phase here. The G2 phase. So you have the G1 phase, the S-phase and the G2 phase. Now in the G2 phase, again, remember, the cell has still not divided, it has only duplicated its DNA, and it's ready to divide. But, for it to divide, and it has to, for it to ensure that the chromosomes get properly distributed, It needs a whole lot of new machinery, which is the machinery required for mitosis. We'll again cover cell division, there are different types of it, but here in this case it is mitosis.

And for mitosis to happen, it has to make sure that the sufficient raw materials which are required to carry out the process of cell division are getting synthesized. Again, there is sufficient energy available with the cell to commit itself to the next step, and a very important part is that it will make sure at this stage that the process of DNA replication has been foolproof. It has to make sure that there has been no errors incorporated due to the process of DNA replication. So a quality control exercise to make sure that all that newly synthesized DNA is intact, consists of correct information, is packaged correctly happens in the G2 phase.

Once that is all proper, the cell is now ready with its duplicated DNA, its duplicated organelles to be divided into two daughter cells, and that is the M-phase. So this is the M-phase, which will last for about an hour. Now the M-phase again has two steps; the first step is where the nucleus divides into two daughter nuclei, that is called as karyokinesis. 'Karyon' means nucleus, 'kinesis' means generation, so generation of two daughter nuclei happens in the first step, which is

mitosis. Once the chromosomes have been now successfully divided into two daughter nuclei, and there is no error happening there, then the cytoplasm actually divides, and that is called as the ‘cytokinesis’.

So what happens is, after the mitosis or the M-phase, you find that the single cell becomes two exact copies as the daughter cells. So, let me again tell you this. So the cell cycle has the major part which is the interphase. Interphase inturn has the G1 phase, the first preparatory phase, the S-phase, when the actual DNA replication happens, and then the second preparatory step, where it is called as the G2 phase. And in this G2 phase, the cells prepare itself to divide, and then the actual process of cell division, which is called as the M-phase. Now, as I mentioned in the beginning, I said some of these cells do not choose to undergo cell cycle like the neurons.

Now these cells perpetually stay in a state of quiescence, or a resting phase. So they do not even enter the G1 phase, they just exit the cell cycle and they stay in what is called as the G0 phase. This is also called as the phase of cell arrest. Now it doesn't mean that in this phase the cells are not living, the cells are living. They are doing the routine job, they are doing their function, but, they are not committing themselves, they are just so busy that they just don’t have time to undergo the process of cell cycle in simple terms. So, this is the G0 phase.

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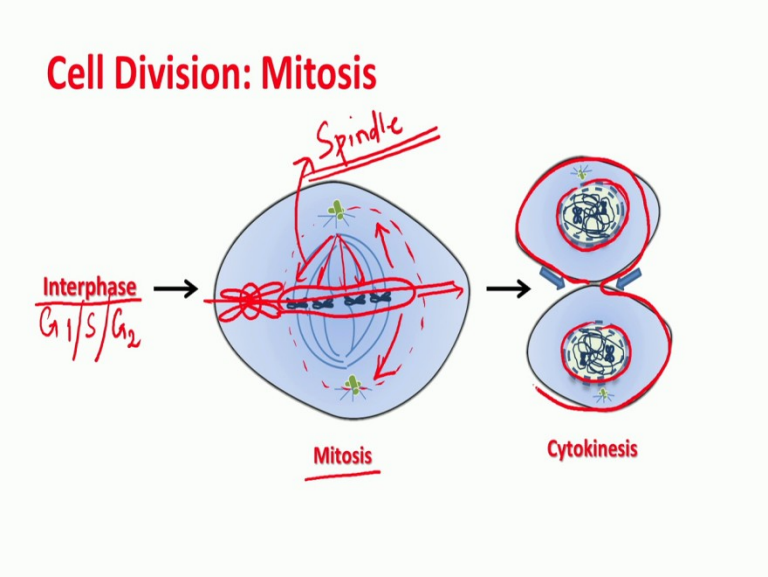


But then, so let me just look, pictorially also depict how the cell division happens. So this is where the interphase happens, there was a G1 phase, the preparatory phase, the S-phase of actual

DNA replication, the second preparatory phase of G2, and then, we had all the chromosomes ready and duplicated. So you can see that each chromosome had given rise to its new copy, and then you had the centromere. Now all these chromosomes, during mitosis or cell division, will align to the equatorial plate of the cell, and you observe here that the nuclear envelope has disappeared. The parent nuclear membrane has disappeared.

And then, there is a special structure formation taking place called as the spindle fibres. Now I'll come to all this when we talk about cell division, but for simplicity in this class, I just want you to understand that after the DNA has duplicated, the chromosomes divide from the equatorial plane towards the two separate poles of the cell, and it is possible because of the spindle structure. These are literally like threads, which are pulling the chromosomes apart. Like it will like you have these chromosomes arranged like puppets on the equatorial plane, and then there is a tug-of-war, and then the chromosomes get separated.

And then, once they are separated, the nuclear envelope restarts to appear. So you start getting daughter nuclei formed, and then, the cytoplasm or the plasma membrane pinches and separates the two daughter cells, which is what you call as the cytokinesis. So, this is what happens in cell cycle, but then, as I said, a cell cannot afford to pass on wrong information, so there has to be very good checkpoints, there has to be some sort of toll gates, which make sure, that as the cell moves from one phase to the next phase, no wrongdoings have been done. So in cell cycle, there are three major checkpoints; the first check point is right after the G1 phase.

Before the cell commits to enter into S-phase, and in this G1 checkpoint, it will make sure that there is sufficient raw material, there is sufficient energy and most importantly, the cell will make sure that the initial DNA that it's going to duplicate. So the starting material, the starting DNA has no DNA damage. Then the S-phase happens, the DNA gets duplicated, passes on, goes to the G2 phase, right? And, before the cell commits itself to the actual cell division, you have the second check point, which is the G2 check point. Now in this checkpoint, there is a quality control which takes place. This mechanisms make sure that no unnecessary mutations, or no unnecessary errors have happened while copying of the parent DNA. If at all some damage has happened, then the repair mechanism, the DNA repair mechanism happens.

The cell size is appropriate because now, it has not only divide the nucleus, it has divide the cytoplasm. It has to make sure the organelles are sufficient, that there is a sufficient space and volume available for the parent cell to divide. So that is also taken care of in the G2 checkpoint. And of course, it makes sure that there is a sufficient amount of raw material available for the actual process of cell division. The third checkpoint is actually in the process of mitosis.

I told you, let me go back to the back slide, that the chromosomes arrange at the equatorial plane. Now if these chromosomes, each forty six pair; each forty six chromosome and its copy. If it is not properly arranged in the equatorial plane, the distribution is not going to be equal. So, that checkpoint that the chromosomes are appropriately aligned, they are appropriately connected to the spindle machinery, happens at the M-phase and it is called as the spindle assembly checkpoint. So, you find that there are enough checkpoints available in the process of cell cycle.

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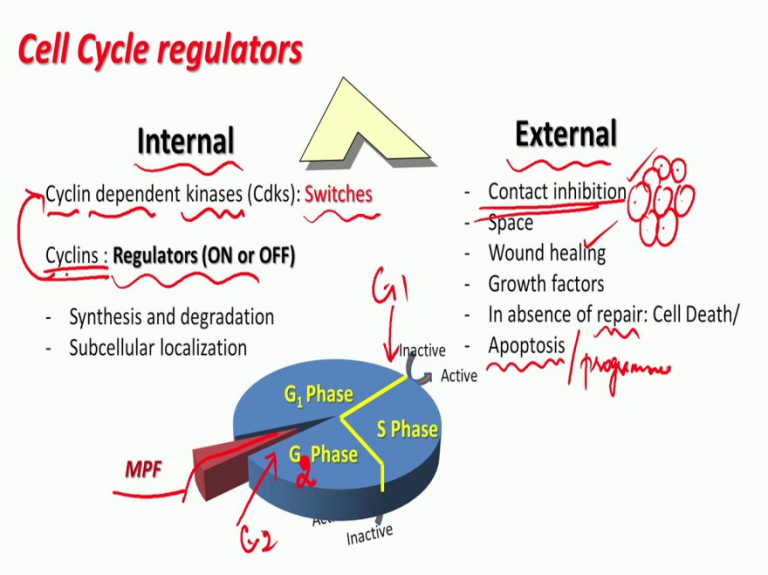


But how are these checkpoints brought about, there are internal and external regulators, to make a more complex information simpler, I just want you to remember that there are enough switches at each of these checkpoints, at the G1 checkpoint, at, I am sorry with the slides, this is a mistake, this is a G2, G2 checkpoint, and at the mitotic checkpoint. There are these switches, which can be turned on or turned off, and these are turned on or turned off by regulators which are called as ‘cyclins’.

So these cyclins, in turn regulate the activity of switches which are called as the ‘cyclin-dependent kinases’. These are a group of enzymes which keep adding phosphate to proteins, I will not get into details here, but for simplicity, just take it like this, the checkpoints has sufficient regulators in place, and their relative distribution within a cell, their rate of synthesis determines how a cell cycle is regulated.

The external features is, for example if you have a wound created. Now one cell will go on dividing to give multiple cells, but it starts growing as long as the wound is closed. So once the wound has been completely closed, there will not be any further cell division, and this is called as ‘contact inhibition’, which is very often seen in wound healing.

Now, many a times, despite all the checks and balances in place, a situation may happen when the cell is not able to repair the DNA, it's not able to repair the damages done. In such a case, the cell itself takes a call of self-destruction and that is called as ‘apoptosis’, it undergoes cell death, or it is also called as programmed cell death. So it's interesting to see that the cell regulates its own cell cycle, and it also has machinery in place to decide if there are damages happening beyond repair, it's no point passing it on, just self-destruct, and that happens through the process of apoptosis.

So, we saw today that cell cycle is a process by which cell prepares itself to divide and in the process of doing it, it duplicates its DNA first, it duplicates its cell organelles, and then it actually divides to the process of mitosis.

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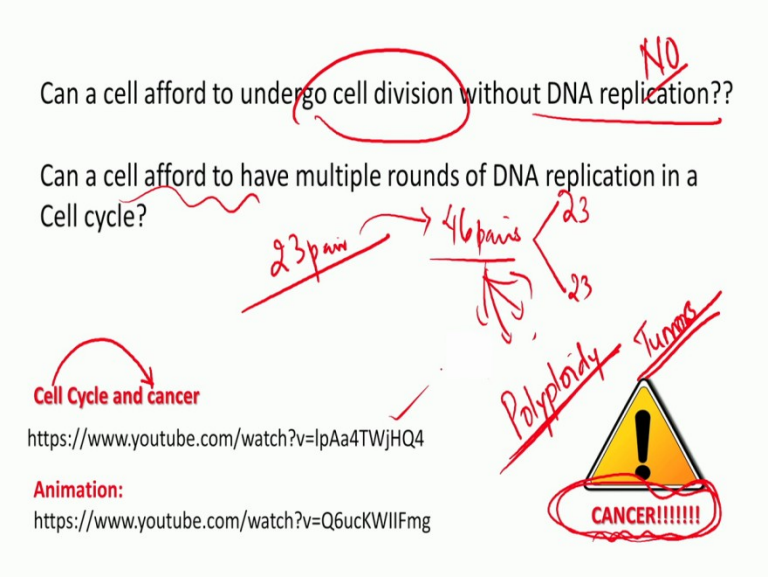


Now one thing I want you to remember is that in every cell cycle, the DNA replication or the DNA duplication happens once, and, I will pose this question to you, that can cells afford to undergo a cell division, without undergoing DNA replication?. And the answer is ‘no, that is not possible’. And it is very obvious because if a cell without even duplicating its DNA is going to divide, it is just going to pass on the chromosomes in a very arbitrary fashion and the two daughter cells will not receive all the necessary information to do the body functions or the organelle function.

So it is not possible for a cell to divide without the process of DNA replication. Similarly, you will find that a cell cannot afford to undergo multiple DNA replications and then divide. Then what will happen? If you are starting with twenty-three pairs of chromosome, you are duplicating it, so you end up having fourty-six pairs, right before the cell division, and then each daughter cell again ends up getting twenty-three pairs. But, if it goes on doing these rounds of DNA replications, number goes on increasing, and then when you divide, what will happen is, the daughter cells will not retain the same number of pairs of chromosomes, and that will lead to ‘polyploidy’.

Now this is again not good for the body. So naturally, if the cells are not able to regulate their cell cycle properly, we will have a lot of diseases happening in our body and one classic example is ‘cancer’. Now these are cells which just lose that control of restraining itself if damages have

happened and destruct themselves. They just lose that ability, they don't listen to the restraining mechanism which asks the cells to die if things are not fine. And as a result, what happens is these defective cells, they keep on accumulating, they become highly aggressive, and you start having a mass of uncontrolled cells, which is what you call as the 'tumors'.

So if you are interested to know exactly how cell cycle plays a very vital role in progression and initiation of cancer, I invite you to see this video, a very nice animated video in a very simple language. And if you really want to visualize the whole process of cell cycle in an animation, I will recommend you to go through this video. I think with that, we have covered cell cycle, and in our next class, we will actually talk about the process of cell division, that is how exact number of chromosomes get passed on to the daughter cells through mitosis, and then another form of cell division, which is meiosis. So thank you again, and do write back if you have any doubts, you will be given access to our weblink, and we'll see you again next time. Thank you.

**Biology for Engineers and other Non-Biologists**
**Professor Madhulika Dixit**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Lecture Number 13**
**Cell Division - Mitosis**

Hello and welcome back. In our previous video, we spoke about cell cycle and we did talk about how much time a cell spends in preparing itself for cell division. In today's video, we are going to talk about cell division, and there are two different kinds of cell divisions, and one is mitosis and the other is meiosis, or 'Myosis' as some people call it. So let me start by talking about our own origin.

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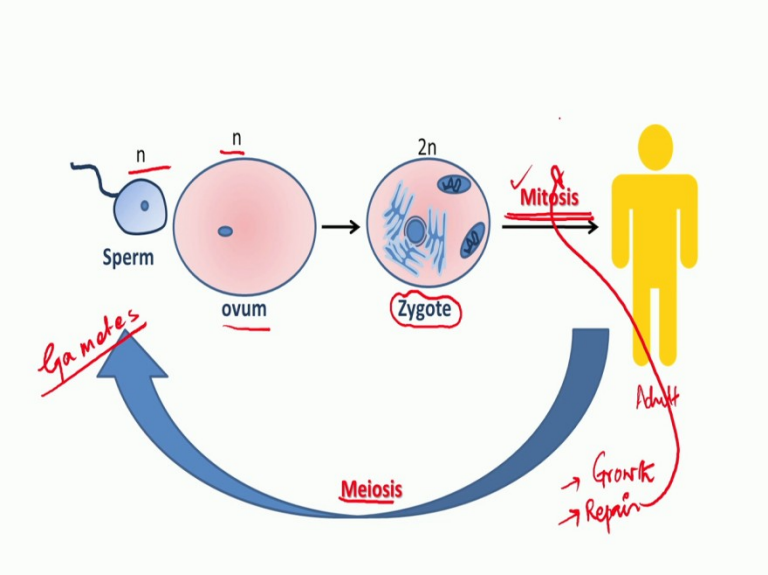


Now you would notice and we all know this that we start our life through the process of sexual reproduction, wherein there is fertilization of the egg, which is also what you call as the ovum by the sperm cells, and it is after fertilization that there is a formation of a zygote.

Now we all start our life as a single celled zygote, which then over the period of next nine months undergoes multiple duplications and divisions and gives rise to the full born baby, and then eventually we end up developing into a full adult. Now, what is intriguing is to note that we all start our life as a single cell, but we end up becoming a whole individual with more than billions of cells in our body. Now how is that possible? Now that process by which one daughter

cell, one cell gives rise to two identical daughter cells is what you call as the mitosis and we will study about this today.

And the other one is the process of meiosis. Now in this process, in the process of meiosis which happens only in the reproductive organs, the cells which are in the reproductive organs basically undergo reduction division and the give rise to gametes, that is, the formation of sperm and the ova, which has half the number of chromosomes. We will talk about meiosis in a separate video. Today, let us talk about mitosis. Now what is the importance of mitosis? As I just mentioned, it not only helps in the growth of an organism, here I have taken an example of human beings, but the same applies to almost all the other eukaryotic organisms.

Then, we also find that if there is a need for repair, for example, if you have injured yourself and there is a wound on your skin that needs to be repaired, what you find is after a few days, the wound gets repaired and a fresh skin reappears. Now this kind of a repair mechanism is possible because of mitosis. Similarly, if you look at some animals in our regular household, for example lizard, you will find that they easily regrow their clipped tails; and that is possible because the cells in the tail undergo the rapid process of cell division and that is again through the process of mitosis.

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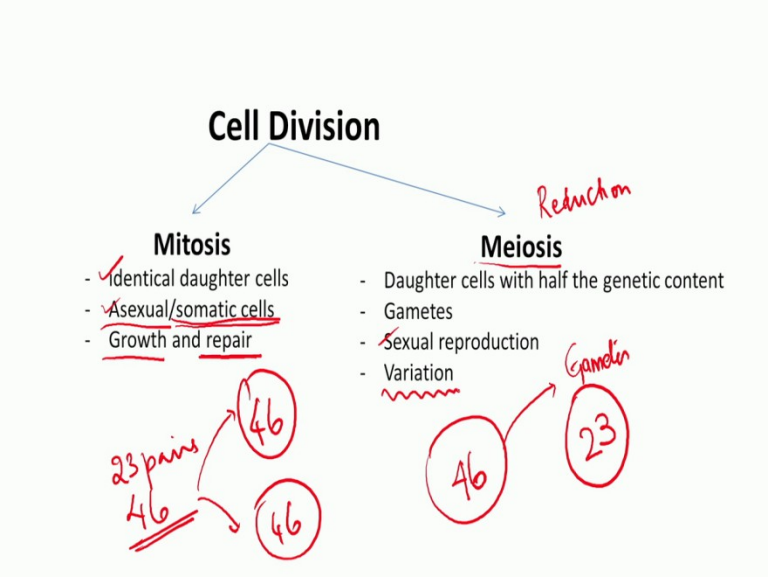


So mitosis is basically the cell division which plays an important role in repairing the damaged parts of the body and also in the growth of the organism. The difference between mitosis and

meiosis in cell division is that mitosis always gives rise to identical daughter cells with same number of chromosomes; for example in humans, we all know that we have twenty-three pairs of chromosomes, or in other words we have forty six chromosomes. So in mitosis, by the end of every round of mitosis, you will have two daughter cells, each one of them containing forty six chromosomes.

So mitosis is usually seen in somatic cells of our body, the cells which are actually not involved in the process of reproduction, and mitosis in lower eukaryotes is also responsible for the process of asexual reproduction. In contrast to that, meiosis is different. Meiosis is again a form of cell division, but it is also called as a reduction division. In this process, when a cell undergoes meiosis, what happens is that it gives rise to sex cells, which are also called as gametes with half the number of chromosomes. So, if the germ cell of the ovary is starting, it will form, it will start with forty six chromosomes but after the end of meiosis, will give rise to an egg cell with half the number of chromosomes.

So this is the difference between mitosis and meiosis and when we talk about meiosis, I will also emphasize that it has a very crucial role to play, not only in sexual reproduction, but in inducing variations; and we will come to it when we talk about meiosis in another video. So let us come back to mitosis and before I get into the actual process of mitosis, it is very important to understand how our chromosomes are arranged.

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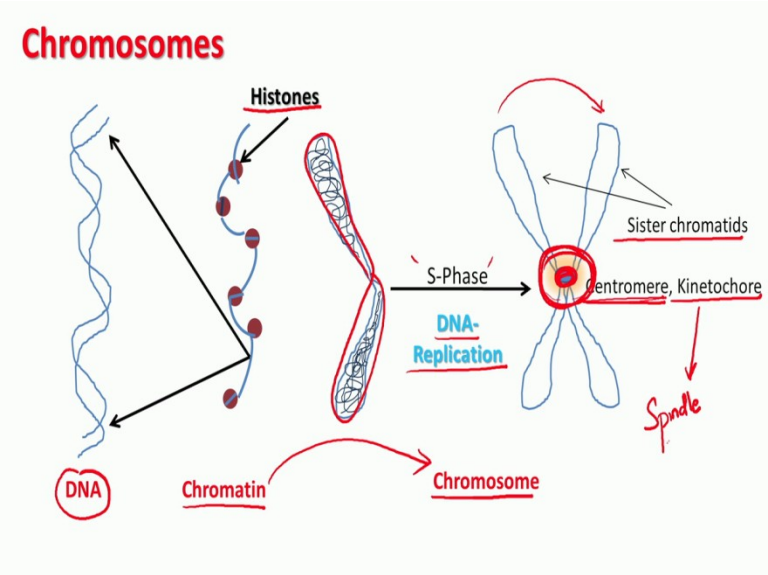

And we all know that our genetic material is encoded in DNA, but then a single strand, a single stretch DNA is way too long and it kind of gets package through multiple folding and wrappings around a set of proteins called as histones. So you have the DNA which wraps around this histones to form what is called as chromatin. Now each chromatin further twists and folds to form a very compact structure and that is what you call as chromosome. Now normally, in a cell which is undergoing interphase and DNA synthesis, the chromatin is relatively loosely arranged.

But right before it is ready to undergo cell division, the chromatin further condenses into chromosome. Now, there are two terms which are always confusing, so let me clarify that. So each chromosome is nothing but a set of condensed chromatin and after the chromosome has undergone DNA replication which usually happens during the S-phase of cell cycle, each chromosome gives rise to a copy of itself, right, because that is the purpose of DNA duplication or DNA replication.

So now this duplicated copy is also attached to the parent chromosome by a central structure which is called as the centromere and each of these are called as sister chromatids. So, these sister chromatids are nothing but the same duplicated DNA after the process of DNA replication in the S-phase, and they still remain connected at a central point which is called as the centromere.

Now the centromere is also surrounded by a certain class of proteins, which is depicted as this halo around a centromere, which is what you call as the kinetochore. Now I will come back to this a little later but for the time being, just remember that it is usually surrounding the centromere and it is the protein complex which helps in attaching the chromosomes to a very important structure during cell division, which is called as the ‘spindle fibres’.

Now what are these ‘spindle fibres’? The spindle fibres are basically a set of structures which are formed by what is called as the centrosome. Now, in case of animal cells, what you find is each cell at one of its polar end has this structure called as the centrosome which actually is made up of centrioles. Now these centrioles are structures which are placed perpendicular to each other, and it is from this centrosome that you start having extension of microtubules. Remember we spoke about microtubules when we were talking about cell structure.

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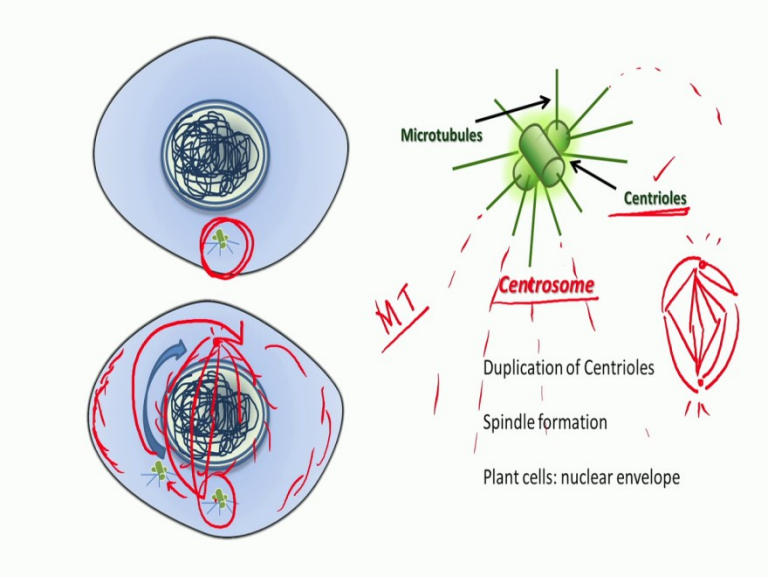


So each of these gives an extended structure which is called as the microtubules which then forms a spindle shaped, or a cage like structure of these microtubule fibres.

And I will come back to this when we are talking about how the chromosomes actually get separated. So in animal cells, there are centrioles, plants do not have it, but that does not mean that the plant cells do not form a spindle; they still form a spindle which is made up of microtubules, and that is because of some of the microtubules, filaments which extend outside of the nuclear envelope and also some of the filaments which originate from structures called as cortex, cortical actin rather form the region in and around the plasma membrane.

So what these tubules do is that they basically, during the process of cell division, the centrosome first replicates, and one of them goes and occupies the other end of the cell, and then, once it has organized to the other end of the cell, they also form fibres, or microtubules, this centrosome also forms microtubules and they form a cage or a mesh-work, I would say a cage like mesh-work of micro tubule fibres, and we will come back to this when we are talking about how the chromosomes get separated.

So two things I want you to bear in mind; one, that after interphase, or during the process of interphase, there is a very important step which happens which is the process of DNA replication, and once the DNA replication has taken place, the chromosome duplicates itself and

the duplicated chromosome is attached with the first one through a center point called as the centromere, and each of these chromosomes are called as the sister chromatids.

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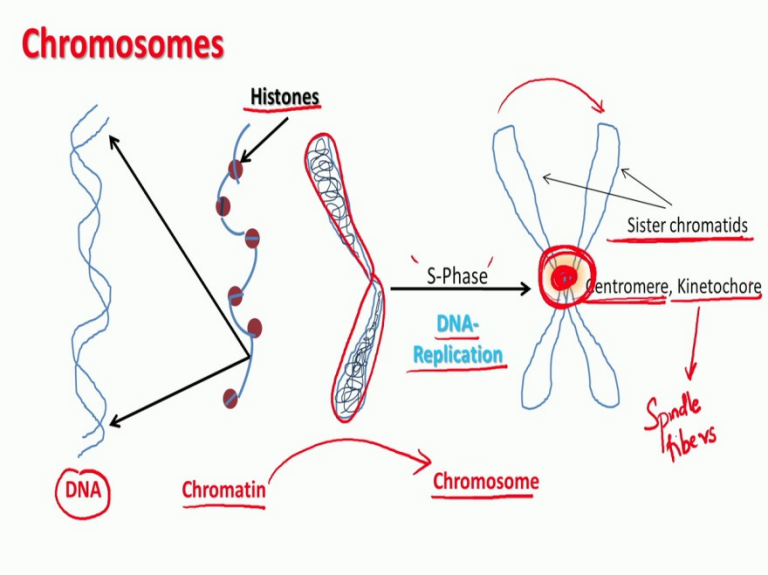


The centromere is also surrounded by a cloud of proteins, it is like a coat of proteins which is called as the kinetochore, and this kinetochore is very important when a chromosome has to attach itself to the spindle fibres. How are the spindle fibres made? In animal cells, they are made, thanks to the presence of centrioles, and it is organized in a structure called centrosome. So, these spindle fibres which are made up of microtubules, extend from one pole to the other pole of the cell like a cage like structure, and we will come back to this when we are actually looking at the separation of chromosomes.

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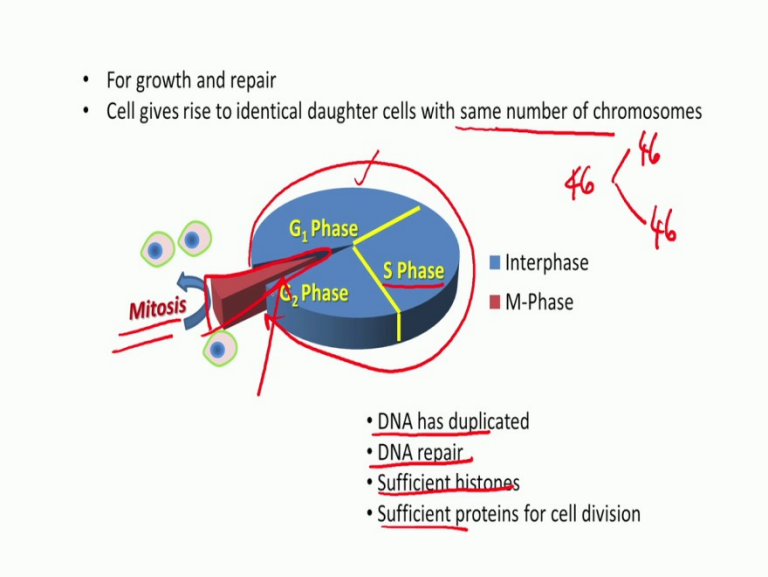


So what is mitosis? As I said, it is a process in which one cell gives rise to two exact daughter cells, it is a process of cell copying itself with exact number of chromosomes and it is important for growth and repair and what you notice is that, it gives rise to daughter cells with same number of chromosomes. So if a cell starts with forty six chromosomes, each daughter cell will end up having forty six chromosomes.

So, when we go back to cell cycle, I told you that majority of the time, a cell exists in the interphase; and the interphase is of very huge importance because it is during this stage that the cell grows in size, duplicates its organelles, it also ends up duplicating its DNA, which happens during the S-phase, and then during the G2-phase, the cell ensures that the DNA has been copied exactly, there are no errors in DNA replication, if at all there are any errors in, errors in DNA replication, the process of DNA repair will take place.

The cell will also ensure that there is a sufficient histone proteins which are available for the process of cell division to take place. So by the end of G2-phase, before a cell actually enters the process of mitosis which is in this part of the pie-chart, the cell ensures that the entire machinery is ready and is willing to commit itself to the actual process of mitosis. So, what is mitosis and how is it divided?

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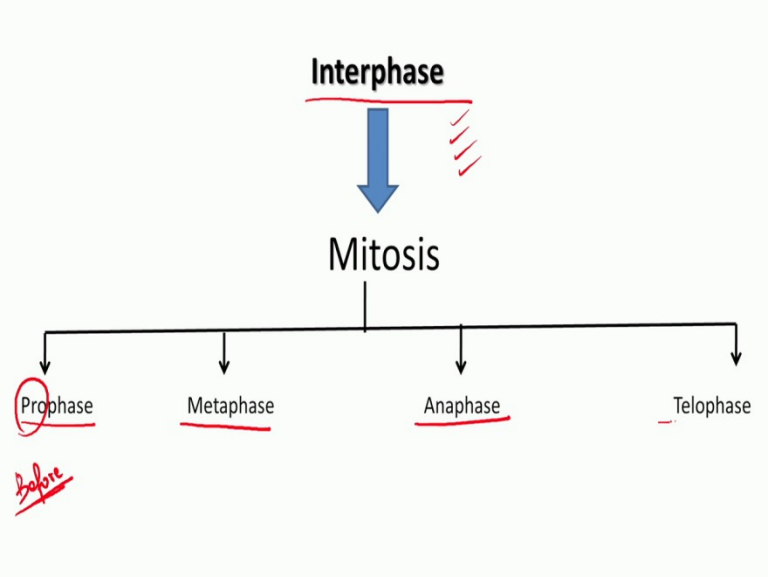


Mitosis follows right after a interphase, provided all the processes have been checked and the cell is convinced that everything is in order to undergo the process of mitosis. The mitosis is divided into four major phases; prophase, pro as I told you means before, so you can take this as the first step, the prophase. The metaphase, the anaphase and the telophase. So, let me show you in one figure exactly how it happens.

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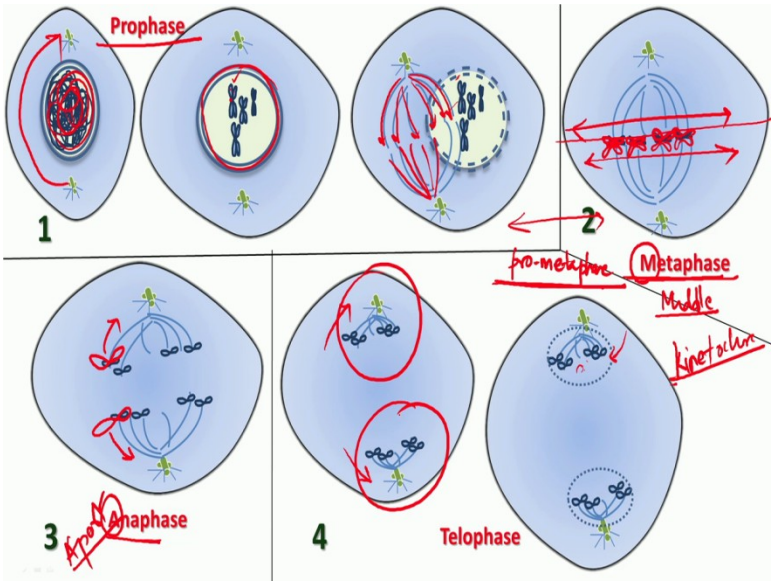

So what happens in prophase? Now the interphase is over, the DNA has duplicated itself, there are sufficient histone proteins available and now the nucleus is ready to divide. So what happens in the first step is that as I mentioned, the centrosome has already duplicated and the other centrosome has gone to the other pole of the cell. You also find that the chromatin, right, this is a duplicated DNA, starts condensing itself into a much better organized structure, which is what you call as the chromosomes, and you will notice that each chromosome is now consisting of two sister chromatids, which are held together at the centromere.

Also, during the prophase, you find that the duplicated centromere starts forming a spindle fibre which is with the help of these microtubule filaments which are extending from one end to the other. At the same time, the nuclear envelope, right, starts disappearing, even the nucleolus, which is the section where the nucleus prepares a large amount of ribosomes is also disappearing, so it is like the nuclear envelope disappears and the entire spindle formation happens within the cytoplasm nuclear mix, and you find that the chromosomes have condensed and they are now ready for the next step. And what is the next step?

At the next step, you find that the chromosomes start arranging in the spindle and this happens somewhere in between prophase and metaphase, and there is an intermediary step which is also called as the pro-metaphase. So in the pro-metaphase stage, what happens is that the chromosomes have now started arranging themselves in a fashion that they start connecting themselves and attaching themselves to the spindle fibres, and now how do they attach themselves to the spindle fibres? As I told you, they do that through this structure called as the kinetochore. So this is the kinetochore structure with which the chromosomes will now attach themselves to the spindle fibres.

Now during the metaphase, these chromosomes start arranging right at the equatorial plane of the cell. So I would say you remember metaphase as it is a point where the chromosome starts meeting in the middle of a cell, M for middle. So, you find that in metaphase, the condensed chromosomes start arranging themselves along the equatorial plane, and now each of these chromosomes are connected to the spindle fibres through the kinetochore. Then comes the third step which is the anaphase. Now it is at the anaphase that these attached chromosomes, which are sitting on a spindle network and you have the chromosomes sitting at the equatorial plane, that you now start seeing that this, the microtubules of the spindle fibres start pulling apart.

It is almost like a tug-of-war which is happening between the two ends of the spindle, and as a result, the sister chromatids now start getting separated. So one set goes here, the next set is starting to get pulled apart at the other end, thanks to the contraction of this spindle network. So in anaphase, the actual separation of these chromosomes or sister chromatids which were attached all this while starts getting segregated. And then by the time you come to telophase, the chromosomes have separated, they have reached the other two ends of the cell, and at this point of time, you also start seeing the reappearance of the nuclear envelope and the nucleolus.

So, you start in prophase where for the single cell, you find that the first, there is a movement of the centrioles to the other end, there is a disappearance of the nuclear envelope, there is the condensation of the chromosomes and there is the formation of the spindle fibre. The prophase is followed by the prometaphase, at which, these chromosomes start attaching to the spindle fibres, and by the time the cell reaches the metaphase, all these chromosomes are arranged along the equatorial plane and they all are attached to the spindle fibre through this structure which is with the help of a kinetochore.

Then comes the anaphase and at the stage of anaphase, the spindle starts contracting and the chromosome starts getting pulled apart. So you can say that ‘A’ for chromosomes moving 'apart'. And then finally, by the end of it, the chromosomes have reached the other two ends and as a result, you find that these separated chromosomes now start getting enclosed in the newly formed nuclear envelope.

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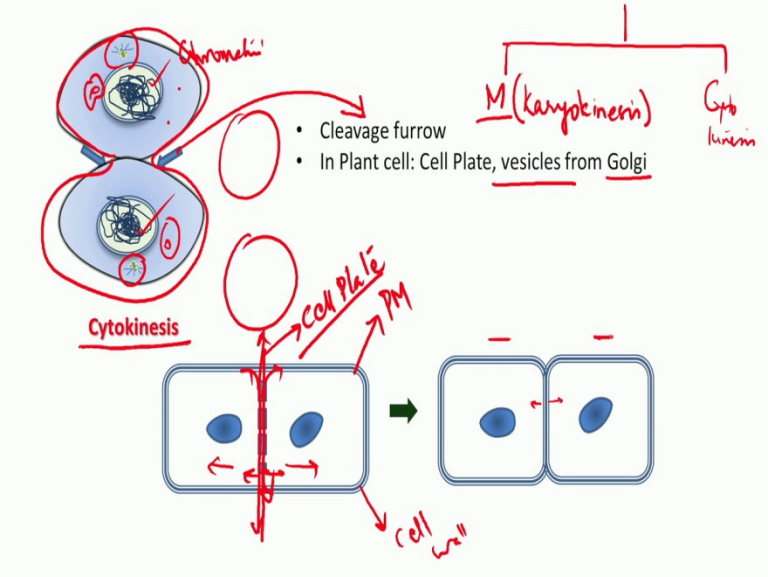


So by the end of telophase, what happens is that you end up having almost two daughter nuclei and these two daughter nuclei now have equal amount of chromosome, and the chromosomes have now started to rewind and unwind into chromatin. The cell organelles, it is not shown in this cartoon, but all the cell organelles like the mitochondria, the Golgi complex also gets equally distributed. One set of centrioles go to one side, then the other set of centriole goes to the other side, and now you have a cell which has got two nuclei, or two daughter nuclei, and now the cell actually needs to divide.

Now, this tag, where, once a daughter nuclei have been formed, the cell actually starts constricting at the centre, right, and this constriction happens, thanks to the fluidic nature of the plasma membrane, this is what you also call as the cleavage furrow. You find that once the daughter nuclei have reached the other two ends, there is a formation of cleavage furrow, the cytoplasm divides and then, the cell pinches off into two daughter cells. This process, where the cytoplasm also divides, is what you call as the cytokinesis. So, the cell division consists of basically two things; it consists of the mitotic phase, and the cytokinesis.

Now mitosis is nothing but also a stage which we also call as karyokinesis, right? And it is in mitosis that the DNA gets separated and two daughter nuclei are formed, and once the two daughter nuclei has been formed, the cytoplasm divides, and that leads to cytokinesis. Now, there

is a issue when it comes to plant cells because the plant cells are also surrounded by this rigid structure which is what we call as the cell wall.

So in addition to a plasma membrane, what happens in plant cells is they have this outer covering made up of cellulose, which is what you call as the cell wall. So for a plant cell, it is not easy to undergo this cleavage furrow; instead what happens in case of plant cells is once the daughter nuclei have separated and being pulled apart right at the centre of the cell, there is a new foundation laid for a newer cell wall which is called as the cell plate.

Now this cell plate, the components of the cell plate comes from various organelles like Golgi and the vesicles found within the plant cell. So in case of plant cells, you do not see the classical cytokinesis as you see in animal cells. Instead, what you see is once the daughter nuclei have segregated, there is a formation of cell plate right at the centre of the cell and then the cell plate starts growing in a fashion that it starts basically moving in this direction and as it goes on growing, it ends up meeting the original cell's cell wall and ten you end up having two daughter cells, again, each daughter cell having the same number of chromosomes as what it started with in the parent cell.

So, what did we study today, what we observed today is that mitosis is that form of cell division which plays a very important role in cell growth and cell repair. In this form of cell division, the number of chromosomes which are passed on to the daughter cells remain the same. So if you start with forty six chromosomes as in case of human beings, and in our somatic cells, the daughter cells will also have forty six chromosomes, and, the cells in mitosis, there are four different stages; prophase, metaphase, anaphase and telophase, and the reason it is able to segregate the chromosomes equally is because the chromosomes align at the equatorial plane and that is because the chromosomes can attach to the spindle fibres with the help of kinetochore and then there is a tug-of-war between the two ends of the spindle fibre.

As a result, the chromosome starts getting pulled apart, and the sister chromatids move to the two sides, and then there is a formation of daughter nuclei, and the daughter formation of daughter nuclei is then followed by the process of cytokinesis when the cytoplasm divides, while in case of plants, you end up having formation of a cell plate.

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So if for those who are interested to see certain animations which can explain this whole thing in a better fashion, I would recommend you to go through this video on youtube, and there is also a very nice fun rap song by Glenn Wolkenfeld, I would invite you to see that as well. Thank you and I will see you later in another video. We will talk about meiosis.

**Biology for Engineers and other Non-Biologists**
**Professor Madhulika Dixit**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Lecture Number 14**
**Cell Division - Meiosis**

So hi! Now today we are going to talk about the process of meiosis, and as I had mentioned in my previous video, we all know that we start our life as a single cell, and this single cell arises because of the fertilization of egg by a sperm.

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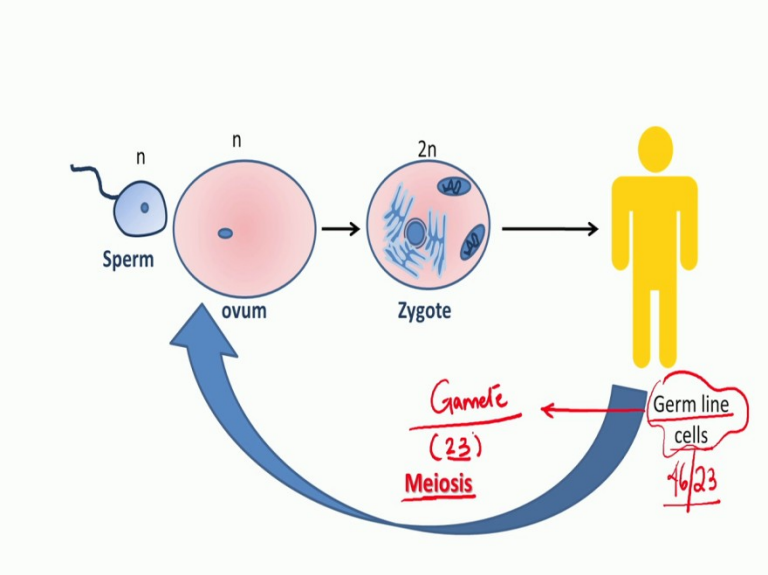


Now, what we have to remember is that when we start our life, we always get a set of chromosomes from our mother, and a set of chromosomes from our father. So if we have twenty three pairs; for each pair we are getting one chromosome from the mother, and one chromosome from the father. So then, we are formed as a child and then as an adult, we end up having forty six chromosomes.

But, once one attains puberty and is now ready for formation of gametes, we find that certain specialized cells, called as the germ line cells, are capable of forming these gametes, so that we can pass on our characteristics to our children. So these germ line cells will contain forty six chromosomes or twenty-three pairs and then through the process of meiosis, these germ line cells divide and they give rise to gametes, right, with each gamete having twenty three chromosomes,

wherein each chromosome is now represented only once. So, how does this process of meiosis takes place is what we are going to study in today's video.

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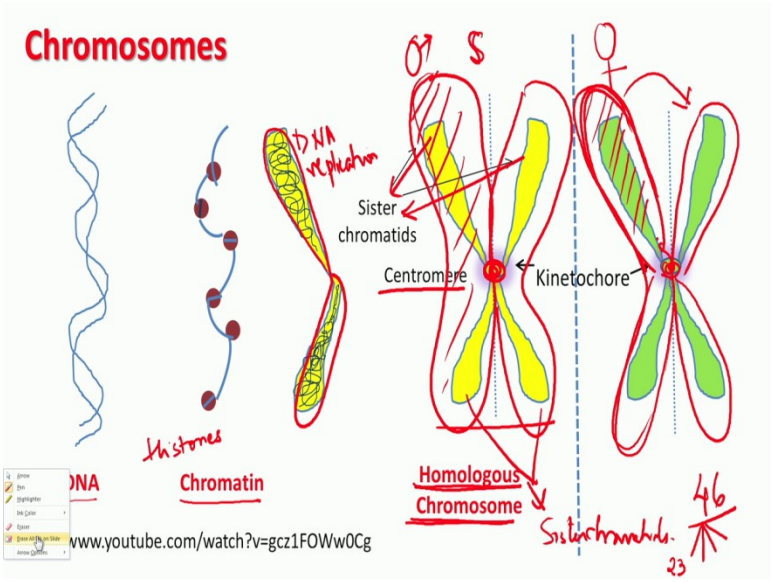


But before I get there, I again want to re-emphasize the arrangement of chromosomes. As I had mentioned during my mitosis video, the DNA normally condenses itself into chromatin with the help of proteins which we call as histones. And then, each chromosome consists of a further condensation of this chromatin. And every time a chromosome undergoes duplication through the process of DNA replication, the duplicated chromosome is attached at the center with the help of a structure called as 'centromere', where each of these then called as 'sister-chromatids'.

Now what we are going to introduce today is one more term which is called as the homologous chromosome. Now homologous chromosome is nothing but the pair. So let us assume this yellow chromosome is what this part, right, this part of the yellow chromosome before the cell entered S-phase was received from the father. Okay? Now after the process of DNA replication has happened, the cell has undergone the S-phase, this chromosome that we had received from our father got duplicated and you had the sister-chromatid form which is now attached with the centromere.

Now the same chromosome, not identical though, but a chromosome which is coding from similar features we'll also receive it from our mother, right, so let us say that this green coloured chromosome was a chromosome that we had received from our mother. So both this one, right,

this part, is from the father, while this part we had received from our mother, and when this green coloured chromosome which we had received from our mother underwent DNA duplication, it again gave rise to sister-chromatid which is now the duplicated chromosome attached at the centromere.

So such chromosomes, which are basically this chromosome from father, and then this chromosome from the mother is called as the homologous chromosomes, because these chromosomes contain a set of genes which code for similar characters, say for example hair colour, where you will have a gene, having a chromosome having a hair colour gene from father, and a chromosome having a hair colour gene from the mother. So this set is what you call as the homologous chromosome, and then each of the homologous chromosome will then duplicate itself during the S-phase of the cell cycle and as a result, each homologous chromosome will have its own sister-chromatids.

So this is important to know. So what happens in meiosis, is that we are going to separate these homologous chromosomes and we are going to each cell, each reproductive cell; mind you this process of meiosis does not happen in all the cells of the body, it happens in those cells which are capable of undergoing formation of gametes which are the cells found in testes and ovaries in human beings or animals. So, these cells will undergo the process of reduction division and the homologous chromosomes will get segregated into the gametes.

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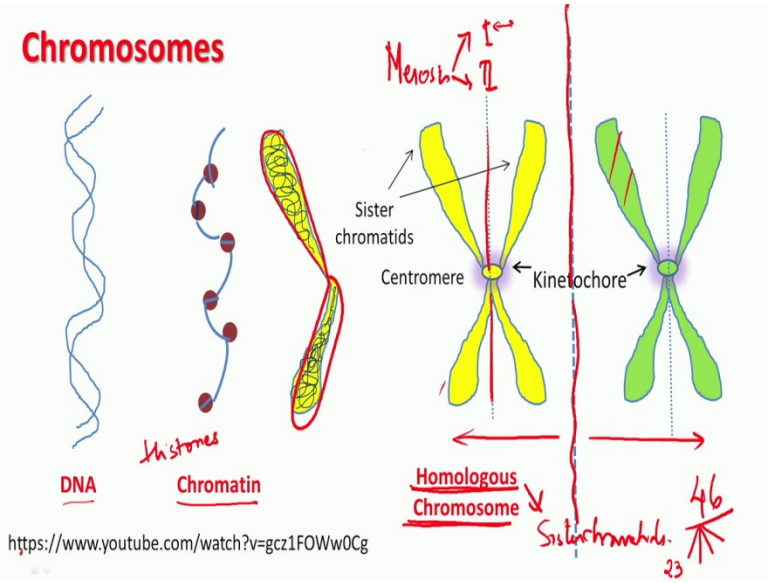


So the idea is, if you start with forty six chromosomes by the end of meiosis, you will have daughter cells, you will have four daughter cells with each daughter cell containing twenty-three chromosomes. So this is what happens in meiosis and we have to remember the terms, mainly the homologous chromosomes and sister-chromatids.

So let me just erase this outer bit again, and then come back to what happens in meiosis a little more in clarity, so that it clarifies your confusion again. Now, so the homologous chromosomes are the chromosomes that we are receiving each set, one set receiving from mother, and one set receiving from father. Now in meiosis, what happens is, there are, as I said, it is a reduction division. So in what we are going to study in meiosis is that, meiosis itself has got two parts; it is divided into meiosis one and meiosis two.

Now in meiosis one, the first part, the homologous chromosomes will get separated. So after meiosis one, you will have daughter cells where these chromosomes would have segregated. And then, in meiosis two, you will find that there are sister-chromatids which will get separated. So we will come back to this a little from now.

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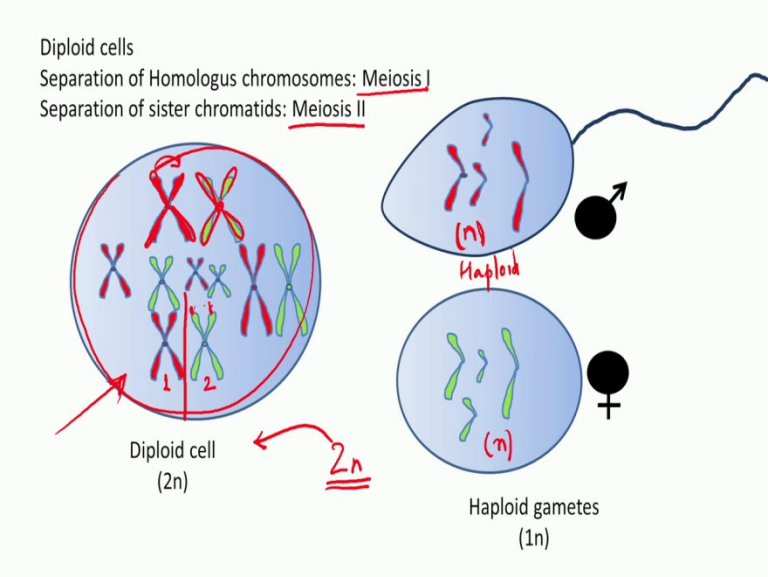


So let us go back to what is a diploid cell. As I mentioned, we all start our life through the process of fertilization where each gamete has one set of chromosomes. So such a cell is called as a haploid cell. Right?

So a sperm coming from father will have only twenty-three chromosomes, so it is a haploid cell. The sperm will then end up fertilizing an egg which again has a single set of chromosomes, and again it is a haploid cell. After fertilization, when these two cells fuse, you end up getting a diploid cell. So these will get mixed up, right? And then when this diploid cell undergoes DNA replication at the S-phase of cell cycle, each of the chromosome will then give rise to its copy, that is a sister-chromatid, it has attached at the centromere. Right? So, this is, this is going to be a starting point, right?

So after the process of cell cycle has happened, the process of DNA replication has taken place, we will be starting our process of meiosis from here. Okay? Now, so again meiosis has two processes; first is meiosis one; in meiosis one, these homologous chromosomes, for example you take this pair and this pair, they will first get separated and then you will have meiosis two, in which each of the sister-chromatids will get separated. So it is a reduction division.

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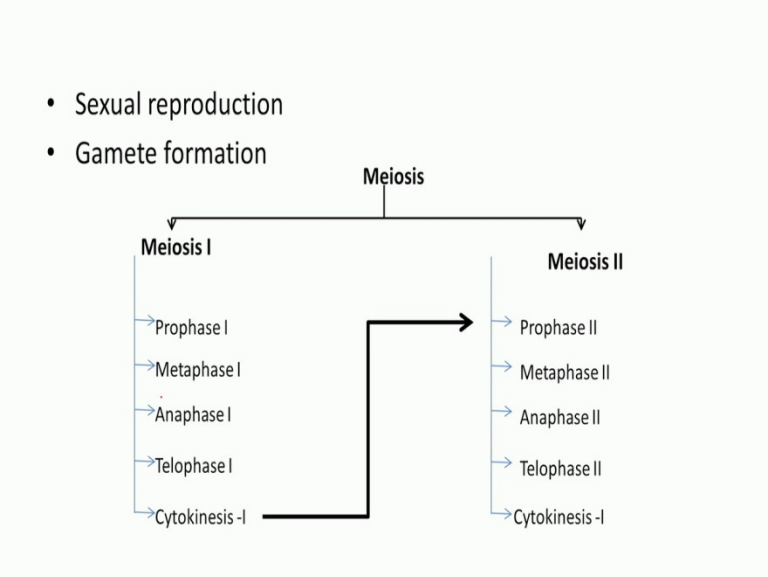


So, what is the importance of meiosis? See it is very important to maintain the chromosome number, you know if this process of meiosis does not happen, then in the next generation, what will happen is the mother cell will give rise to forty six chromosome and then the father cells, let us say have forty six chromosomes and they are fused together, you end up getting ninety two chromosomes.

Now that is almost increasing the entire characteristic by two-fold and in the subsequent generation further increases, now that cannot be allowed right? So it has to maintain the same genetic information, the same number of chromosomes and for that, it is important that before an organism undergoes a process of sexual reproduction, its copy numbers are reduced to half, and then, after the process of sexual reproduction, it is restored back. So meiosis plays a very important role in sexual reproduction and it is this division which is responsible for gamete formation.

Now, similar to mitosis, in meiosis, each step of meiosis one, or meiosis two is divided into prophase, metaphase, anaphase, telophase, followed by cytokinesis one; so if once the cell undergoes meiosis one, let us say it starts with one cell; at the end of meiosis one, it will have two cells, alright? Then, each of these cells will then further undergo meiosis too, which again has its stages of prophase, metaphase, anaphase, telophase, followed by cytokinesis, and an each cell in turn give rise to two daughter cells. Same thing happens here. So at the end of meiosis,

unlike mitosis, you have four daughter cells with half the number of chromosomes. This is what happens in the process of meiosis. So let us see how it happens.

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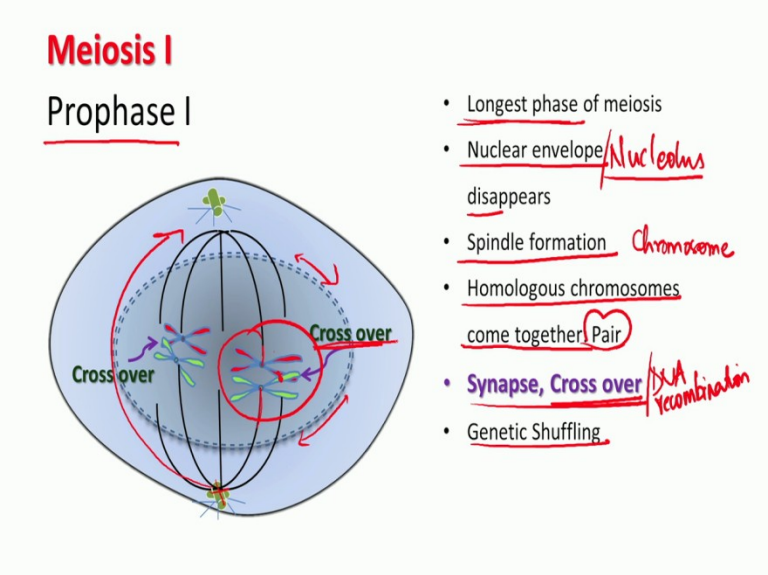


Now the first step of meiosis one which is prophase one is the longest phase of meiosis. Now in this phase, just like in mitosis, the nuclear envelope first disappears, that is why I have drawn this dashed line representing disappearance of the nuclear envelope. Even the nucleolus, remember nucleolus is the part of the nucleus which consists of ribosomes and other machinery also starts disappearing, and then, you find that the pair of centrioles have duplicated and one of them has reached the other end of the cell and then, these are leading to formation of spindles.

And these spindle formation is important because it will allow and help the chromosomes to attach. So the chromosomes will start now condensing, as it happened in mitosis, the chromatin will condense into chromosomes, and the homologous chromosomes, remember one each coming from mother and one from the father, the pair, the homologous pairs will start coming together during the process of prophase one. And as they come together, they, so here if you look at this one, you find that one of them is from one parent and the green one is from the other parent.

They they start coming together, at the same time, they also, the homologous chromosomes start attaching themselves to the spindle formation. Here they are still not arranged at the equatorial plane, and that happens in the metaphase; but in the case of prophase, there is one another

important thing which happens, which does not happen in mitosis, is that, when the homologous pair of chromosomes come together. So assume this is one chromosome, and this is another chromosome, when they come together, they tend to undergo a process called as synapse, or cross over.

So you have a chromosome coming from father, and a chromosome coming from mother, and they are sitting here together. So this is two sister-chromatids, there are two sister-chromatids, they are coming together, and when they come together at the stage of synapse, there is an exchange of genetic material between these chromosomes, and this process of exchange of material is what is called as the cross over. Now that is important, because remember, I told you one important reason why there was advantage of sexual reproduction during evolution, is to bring in variation.

So what happens is because of this cross over, there is a genetic shuffling of some part of the mother's chromosome will exchange information with the father's chromosome, and there is an interchange of material; this process is also called as DNA recombination. Now what happens is, because of this, what will happen is the new daughter cells, which will have these chromosomes will not be an exact copy of the parent cells. So now because of the cross over, some characters of the father's chromosome have been exchanged with the mother's chromosome and vice-versa. So that kind of a shuffling of characters have happened, and that process is called as the cross over.

So prophase one involves few things; disappearance of the nuclear envelope, disappearance of the nucleolus, condensation of the chromatin into chromosome, homologous chromosomes coming together, attaching to the spindle fibre, and as they come together, they undergo the process of cross over. Now that is the most important difference from mitosis, because there the homologous chromosomes are not pairing together for cross over, it happens only in meiosis one. And thanks to this crossing over, new genetic shuffling takes place and as a result, these chromosomes have exchanged information in material with each other.

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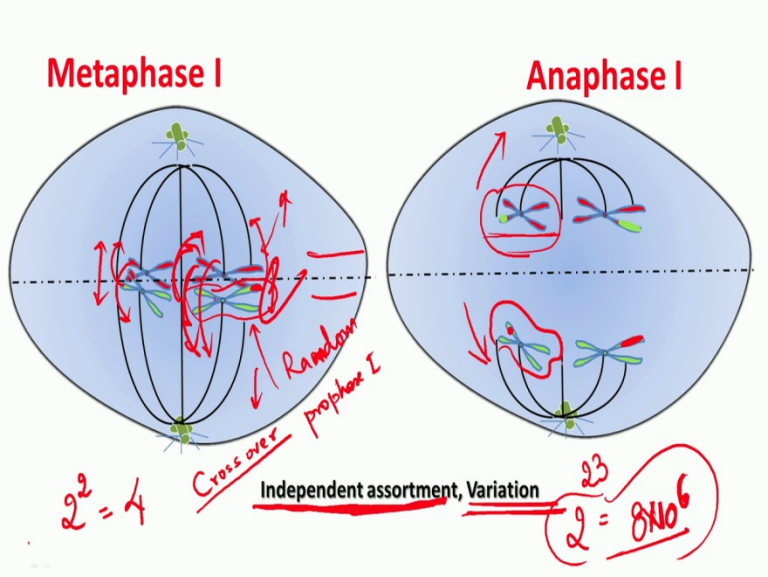


Then you come to metaphase. Now in metaphase, that each of these homologous pairs, they arrange at the equatorial plane, and you would find that by this time the cross over has already happened, the material has been already exchanged. So for example, this one chromosome is actually, it has already exchanged material with the other chromosome, and it has received some features of the other chromosome. So this arrangement happens. Now it is not necessary that every time the chromosomes for one parent will appear at one, will arrange at one half of the cell, and the other set of chromosome, let us say coming from mother will arrange at the other half.

It can, it is a random arrangement. So right now, though I have shown in this slide, green chromosomes on this side and the red chromosomes on that side, it is not necessary. It can just be that this pair can be flipping over. so if this pair flips over, so this side can flip over and the red chromosome can be at this end and the green chromosome can be at the other end, it does not matter, but this arrangement is a random arrangement, and thanks to this random arrangement, you end up getting independent assortment.

Now let me give this to you with an example, now in this case, we have taken two pairs of chromosomes; so, each pair of, so you have two chromosomes, so there are $2^2$ possibilities by which these four chromosomes can arrange themselves. There are four different ways in which these chromosomes can arrange themselves with two red chromosomes on this end, two green

chromosomes on this end, or, this green chromosome arranges here, and the red chromosome arranges here, while this remains this way, or, it is this green chromosome going up, and then this red chromosome going down, it does not matter.

The point is, these four chromosomes can arrange themselves in permutations and that itself is called as independent assortment. Now imagine this we are talking with just two pairs of chromosomes, if you have twenty-three pairs of chromosomes, you have $2^{23}$ possibilities in which, these chromosomes can arrange at the equatorial plane, and this is close to about eight million different combinations, right?

Now that is interesting, and I will come back to this a little later. You have ten to the power six, eight into ten to power six different possibilities in which after the cross over has taken place, the homologous pairs can arrange themselves. So that happens in metaphase. Having done that, and this independent assortment and this multiple combinations is what leads to variations, alright?

And so there are two things which leads to variation; one, as I mentioned, is the cross over, and this cross over happens during prophase one of meiosis one, and the second one is the independent assortment of the homologous chromosomes. That in turn gives rise to about eight million different possibilities in which these chromosomes will arrange themselves along the equatorial plane. So that happens in metaphase.

After they have arranged themselves at the equatorial plane, just like in mitosis, in anaphase, these homologous pairs start moving apart. Now you will notice that this chromosome, thanks to the crossing over has changed, it is not the complete red chromosome, what it was in the beginning. Similarly, this chromosome has received some information due to the cross over. So you find that this is introducing variations.

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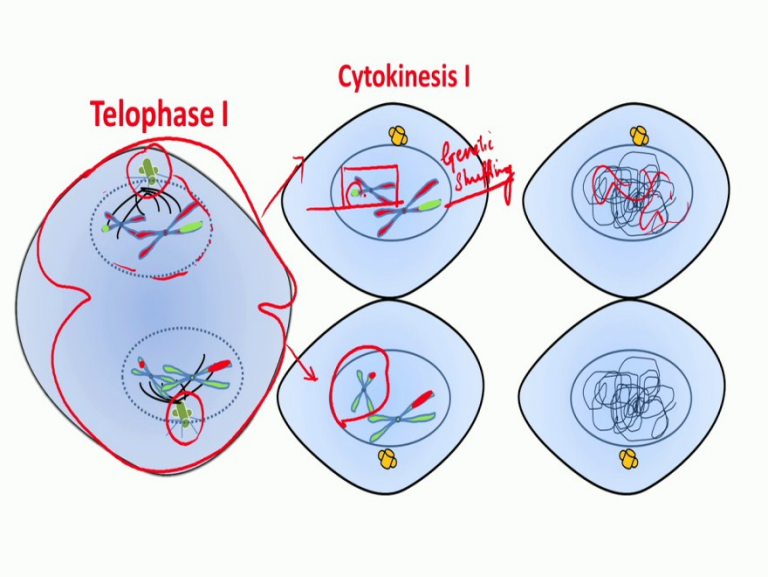


So during anaphase, the chromosomes will move apart, and by the time they come to telophase, you start seeing that the nuclear envelope starts reappearing, and, the daughter nuclei are getting formed, and one pair of centrioles on one end, another pair of centrioles at the other end, and this would be followed by formation of a cleavage furrow after telophase one, leading to cytokinesis and two daughter cells.

Now what you will notice is that in these daughter cells, you have received slightly newer version of the chromosomes, thanks to the process of genetic shuffling or the synapse. So, the meiosis one ends with the separation of homologous chromosomes, so this is one part, this is another; so this is one homologue, this is the another homologue, and mind you again, each of these homologues are slightly different from their starting material because of the cross over.

Now after cytokinesis one, there is a brief period before which the cell again goes back to meiosis two, and then again, it just loosens up the DNA, which is the chromatin, and then, immediately, there is no more further DNA replication now. Whatever DNA replication had to happen has already happened during the S-phase of cell cycle, it is not happening anymore.

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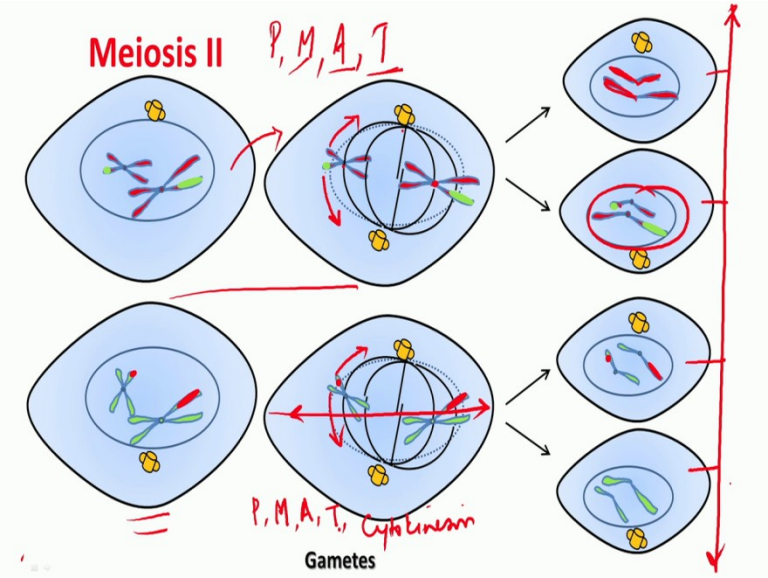


Now, after cytokinesis one, the cell then undergoes the process of meiosis two, and meiosis two again, as I said, contains prophase, metaphase, anaphase and telophase. Now each of these cells, so you will notice, here the homologous pairs are separated, Now in this, meiosis again, this cell again undergoes the process of prophase, where the nuclear envelope disappears each chromosome, still attached to its centromere. It is attaching to the spindle fibre and it aligns to the equatorial plane during the metaphase.

So, you find that in prophase, the chromosome start attaching to the spindle fibre through the centromere, they will arrange at the equatorial plane at the metaphase, and then at the anaphase, they start separating apart. And, by the telophase, and then, followed by cytokinesis, which have not shown in this cartoon, but it is like a continuous process. After the metaphase, you have the anaphase taking place, then you find that the two chromatids have separated.

So this chromatid, so what has happened after the anaphase has taken place, after the telophase has taken place; this chromatid has gone onto this side, and this chromatid has gone onto the other side, and then the telophase happens, cytokinesis takes place, and now you have the new daughter cell with the chromosomes.

Now you will notice that the chromosomes are different; for example this daughter cell has received a different version of the chromosome than the starting material. So, you find, same thing happens with the other cell. This cell also undergoes the process of prophase, where the

nuclear envelope disappears, spindle formation takes place, the chromosomes with the sister-chromatids is attaching to the spindle fibre; then the metaphase happens where each of these chromosomes try to arrange at the equatorial plane, followed by anaphase, at which each of these chromatids will separate, they will reach the polar ends in the telophase, and this will be then followed by cytokinesis.

So what has happened is after meiosis two, you end up getting four different cells. With half the number of chromosomes, and most importantly, none of them, none of these gametes are an exact copy of each other. And why it is so? The reason they are not an exact copy of each other is thanks to the crossing over which has taken place in prophase one of meiosis one.

And this is one of the reasons why, though we have characters which are similar to our parents, we are still not an exact carbon copy of our parents. We may receive some features from our parents, and some features from our mother, and even the features that we receive from our mother is not an exact carbon copy, it is still a slight variation, because of the process of crossing over.

So what have we learnt in today's video is that meiosis is a process which allows for reduction of chromosome, it happens only in the reproductive cells which are responsible for formation of gametes, and meiosis is divided into two stages, meiosis one and meiosis two. In meiosis one, the homologous chromosomes get separated, while in meiosis two, the sister-chromatids get separated.

In meiosis one, prophase one is the longest step and it is one of the most critical step which sets it apart from mitosis because it is in prophase one that the homologous pairs come together, they undergo a process of crossing over, and because of the process of crossing over, you introduce new variations. And that is the reason why none of us are an exact copy of our parents, though we have characters which are similar to our parents.

The other important thing to note is that in meiosis one, during the process of metaphase, there is various, $2^{23}$ possibilities by which these homologous chromosomes can arrange themselves. Now that is a huge combination of independent assortment, which further tells you why two different, two siblings of a given parents are not exact copy of each other. So, meiosis is very important in the process of cell division because it is meiosis which is responsible for variation, and for

maintaining the chromosome number from one generation to another, because it is meiosis which is responsible for, for first reducing the chromosome numbers into the gametes, and then its the sexual reproduction which brings back these chromosome numbers to the constant number for a given species.

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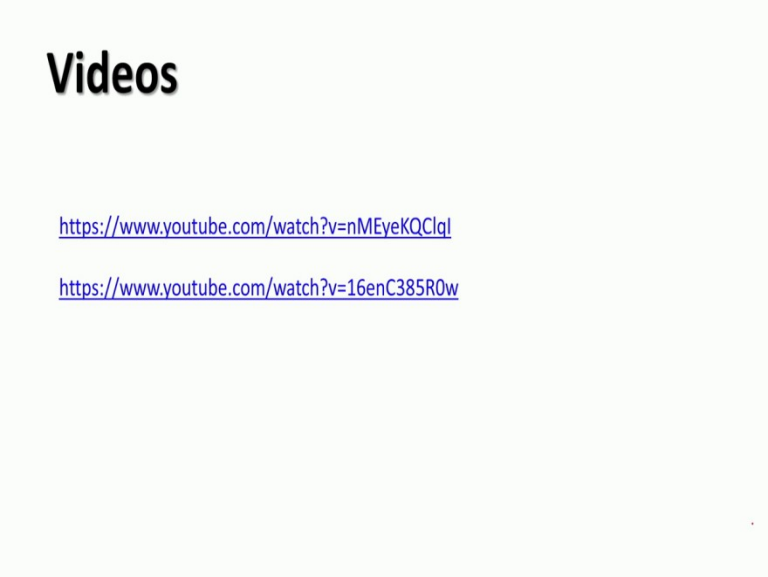


I hope this video has been helpful; I would also recommend you to go through some of the interesting videos on YouTube. Some of them have got very good animations; I would like you to go through them which beautifully explains the process of meiosis. Thank you and see you later.

**Biology for Engineers and other Non-Biologists**
**Professor G.K.Suraishkumar**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Lecture Number 15**
**Culture Growth**

Welcome to this lecture on 'Culture Growth'. It is popularly referred to as 'cell growth' and it actually represents the growth of a population of cells or a culture. So these terms will be used interchangeably; cell growth is a very 'common term', rather misleading because we are not looking at the growth of a single cell here. You already know from the previous lectures, that the cell goes through a cell cycle, during which its size changes, its volume and other things change as it goes through the cell cycle; and therefore you can probably look at it as some sort of a growth, from our perspective. But that is not what we are going to talk about in this particular lecture.

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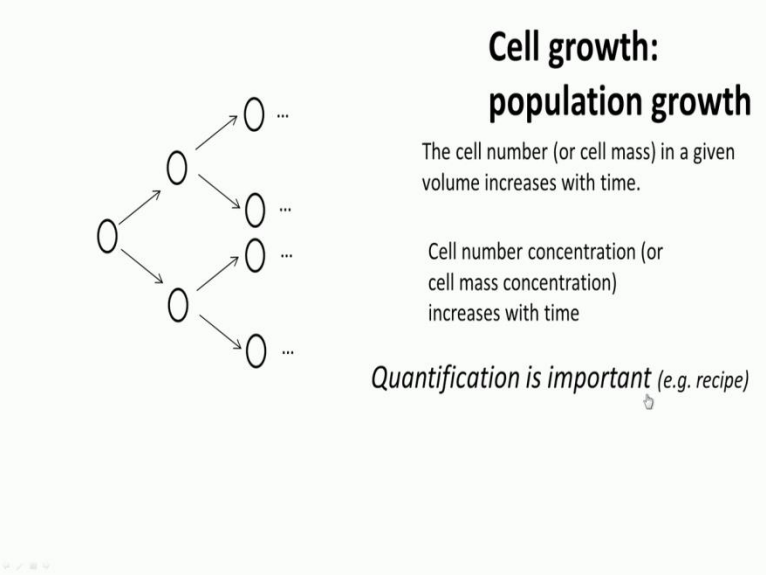


It is referred to as cell growth, but actually means the growth of a population of cells or the growth of culture. What we mean is actually this, a single cell divides to become two cells, it is either mitosis, of all the somatic cells, which yields two daughter cells or the meiosis, of germ cells, which yields two gametes. We, let us limit ourselves to somatic cells to understand the population growth here, and that is typically where it is used, predominantly in the production, from a production perspective.

And therefore let us look at mitosis of one cell giving two cells, and each one of those giving two cells and so on and so forth. It is this process that we are interested in, in this particular lecture. The cell number, or the cell mass in a given volume, right, that is, what we are normally interested in, the cell number, number of cells in a given volume, or conversely, the cell mass or alternatively, the cell mass in a given volume increases in time because of this multiplication process. In other words, the cell number concentration or the cell mass concentration increases with time. When you normalize it with respect to volume, we call it concentration usually.

Cell number concentration or cell mass concentration increases with time and to know how it increases in a precise fashion is important. In other words, quantification is important; quantification is important in biology also. More and more, that is being realized more and more even by classical biologists nowadays.

I will give you a very simple example to understand why quantification is important. Suppose you are making a dish, cooking a dish, okay? There is always a recipe that we average people follow, we are not, let us assume that we are not great chefs who have an, an inherent feel for what they cook, and therefore they do not need all these things. That is inside them.

Let us say that we are average people, we are following a recipe to cook a dish. The, if the recipe says you add rice, you add milk, you add sugar, and so on and so forth, we will be completely lost, right? We need to know, okay let us make it a little more specific. Let us say that we are making cup of tea. If it says boil water, how much water do you boil? If it says add tea leaves or tea dust, how much tea dust do you add? Do you add an entire box, to make one cup of tea? All these are very common questions that come about even while making tea, right?

You need to know that you take, let us say, a cup of water, then you add, let us say, a teaspoon of tea powder, and if you are adding any masala to it, may be about a quarter teaspoon of it and so on and so forth. Therefore every single thing needs to have a certain measure associated with it for us to successfully make a dish, okay? So the quantification, one glass of water, one teaspoon of tea leaves and quarter teaspoon of chai masala and so on and so forth are very essential aspects to get the dish right. And therefore, quantification is very important in many different things, especially if you want to do things reproducibly.

In biology too, this aspect is gaining more and more importance, and here, let us see how to quantify this population growth process, cell growth. For dynamic systems, dynamic means, which change with time. Biological systems are dynamic systems, including the cell, they change with time all the all the time.

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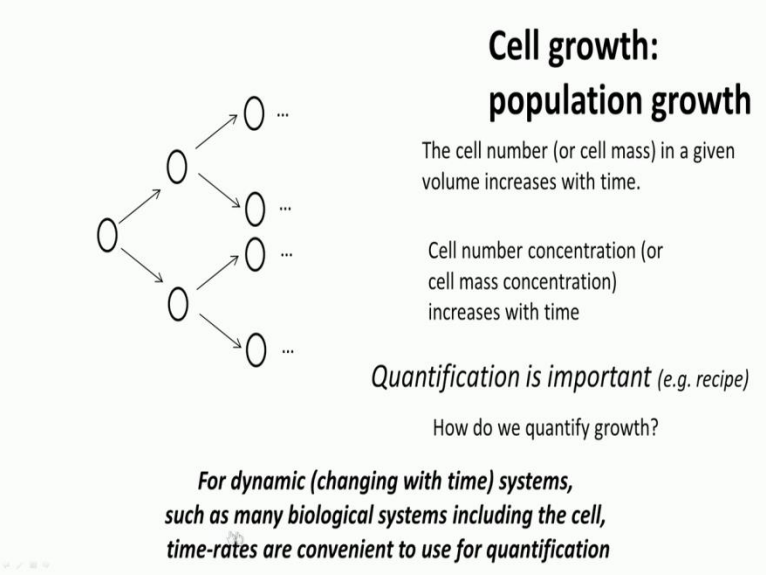


They go through life, right? So they, they are subject to changes at every single point in time. The time rates are convenient to use for quantification, this is something that has not been realized much, and I think it is best to present it during a first course in biology; that the time rates are rather important (because) I mean are very important, it is, it does not make much sense to deal with just masses in volumes and so on and so forth, when we are trying to quantify a dynamic system, such as a cell.

Only rate, time rates would provide us with easy answers to aspects that we are usually interested in, especially when we are trying to use biological systems for various different ends, as well as understand biological systems quantitatively so that it can be reproducibly used later on. To understand this, let me start at the very basics, and, so that we are all at the same level, everybody understands the need and everybody takes a certain view to that. Let us say that we are filling a water tank; water tanks are quite common in Chennai in summer, they provide water for use when water scarcity sets in. The typical volume, or let me say an average, kind of an average volume is about twelve thousand litres. These were kind of old tankers, twelve thousand

litres. Nowadays you have eight thousand litres or sixteen thousand litres, depending on the size; twelve thousand is somewhere in the middle.

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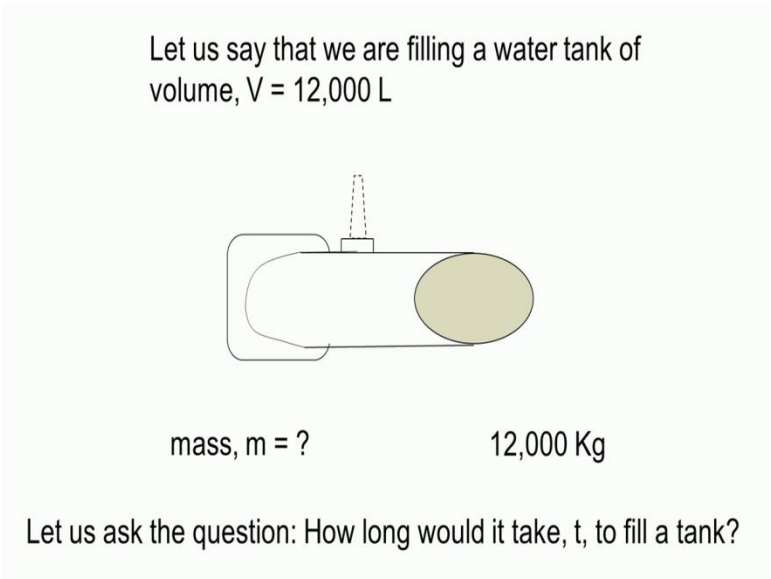


So I have chosen this for our representation. The volume here is twelve thousand litres. So what is the mass? You know that the density of water is one gram per centimeter cubed, one, one gram per ml, or ten power three kilogram per meter cubed, okay? So if you are dealing with twelve thousand litres, that is twelve meter cubed, right, and therefore, the mass is volume into density, which would be twelve thousand kilograms. This is rather straightforward for people who have some background in physics, maths and so on.

Now let us ask the question, how long would it take to fill the tank, and let us call that time t. How long would it take t to fill this tank?

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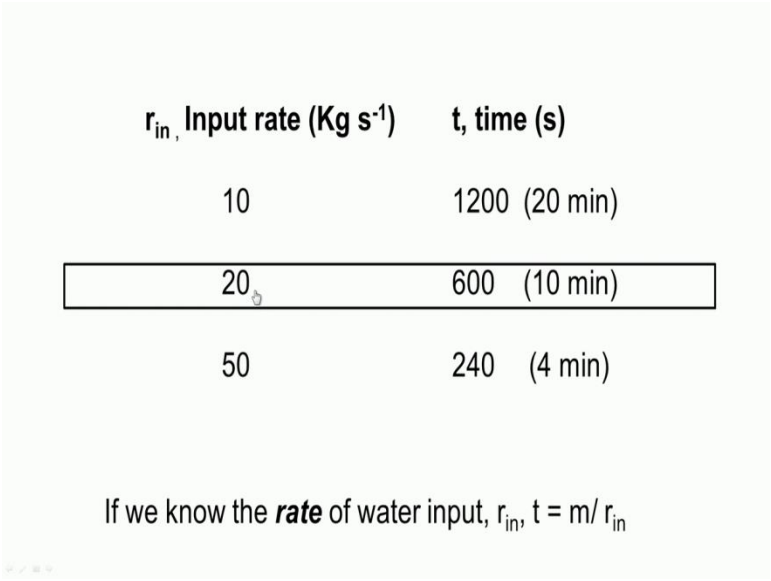


This can be easily answered if we know the input rate of water into the tank. If the input rate happens to be ten kilogram per second, the time taken would be thousand two hundred seconds, or twenty minutes. If the input rate is twenty kilogram per second, the time taken would be six hundred seconds or ten minutes. If the input rate is fifty kilogram per second, the input, the time would be 240 seconds to fill he tank, or four minutes, okay?

The, in other words, if we know the rate of water input, okay, then the time is nothing but the mass, total mass divided by the rate. It becomes very straight forward. For further discussion, let us choose this time or this rate, twenty kilogram per second; ten minutes is the usual time that it takes to fill tankers here. So that is what we are going to choose for further discussion.

Remember, input rate is twenty kilogram per second. Now, let us complicate things slightly. The first one is straight forward, it did not really need a certain different view to answer, only thing is that if you had known that view, the rate view, it was a very straight forward answer. Even otherwise you would have got into that answer by some roundabout means.

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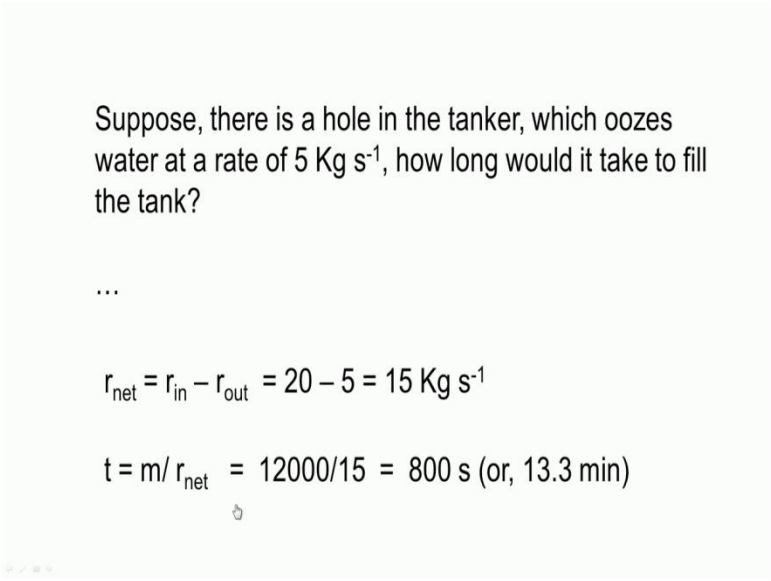


Now let us complicate the question that we are asking or the situation that we are in. Suppose, there is a hole in the tanker, which oozes water at the rate of five kilogram per second. Now, how long would it take to fill the tank, right? If we need to answer such questions, if we start looking at mass volume, there is going to be total confusion, okay you can try that. Many people do this; even engineers who have gone through courses in material balances don't internalize it, don't internalize the concept of rate and kind of intuitively get into mass and volume and so on so forth, and get confused.

Whereas, if you know the net rate, that is the rate of input minus the rate of output, water is coming in at twenty kilogram per second, going out at five kilogram per second; so the net rate is fifteen kilogram per second, and it is a one step answer to find the time, it is mass by the net rate. 12,000 kgs by 15 kg/s, that is 800 s or 13.3 min. So if you know the rate, if you focus on the rate, it is a one step answer to the kind of information that we engineers or quantifiers are interested in. Biology is no different.

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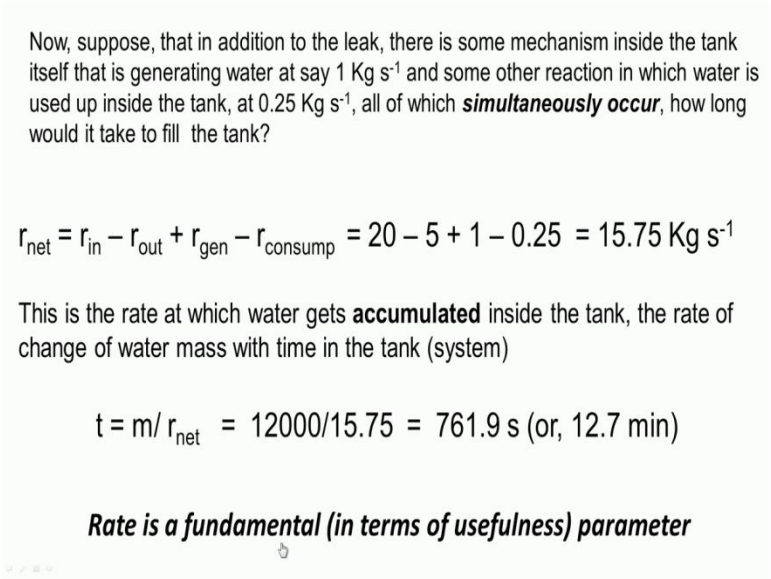


Let us complicate things even further. Now suppose that in addition to the leak, there is some mechanism inside the tank itself that is generating water at one kilogram per second, okay? Fictitious, but let us consider this. Some reaction inside the tank that is generating water, and some other reaction in which water is used up inside the tank at a rate of 0.25 kg/s, and all of them simultaneously occur, okay, which is usually the killer for any intuitive kind of an approach with mass and volume, okay? All these processes simultaneously occur, there is filling in, there is water oozing out through the hole, there is water being generated and there is water being consumed. Okay?

And all of these are simultaneously occurring. If this is the case, how long would it take to fill the tank? And this becomes very straight forward, I mean if you take the mass and if you keep thinking about the mass and volume and so on so forth; you are welcome to do it, but most likely you will probably never arrive at the answer. Whereas, if you take the rate route, the net rate is nothing but the rate of input minus the rate of output, plus the rate of generation minus the rate of consumption, okay? That happens to be fifteen point seven five kilogram per second, twenty is input, five is the output, one is the generation and 0.25 is the consumption; all rates that comes out to be 15.75 kilogram per second as the net rate.

Now, if this is the net rate, this is the rate at which water should get accumulated inside the tank, right? Or in other words, the rate of change of water mass with time inside the tank, and we are

going to call the tank as our system. Some of you would be familiar with this term system, something on which we focus our attention, and we, we are considering the the tank as our system. That is the case, it is a straightforward calculation one step, total mass is, mass that it can hold is twelve thousand, the rate, the net rate at which it is getting filled is 15.75, and therefore the time to take is 12,000/15.75 which is 761.9 s, which is 12.7 min.

So, rate is a fundamental parameter in terms of usefulness. In most engineering calculations, and that is something that needs to be internalized. This is true for all dynamic systems, the cell biological systems or all dynamic systems. So, we need to look at rates of things happening, their rates of processes happening there, for us to be able to meaningfully quantify anything to do with this cell, okay, this I would like all of you to internalize because this is pretty much the key for any meaningful analysis. I see a lot of people even after lot of experience have not internalized this particular concept.

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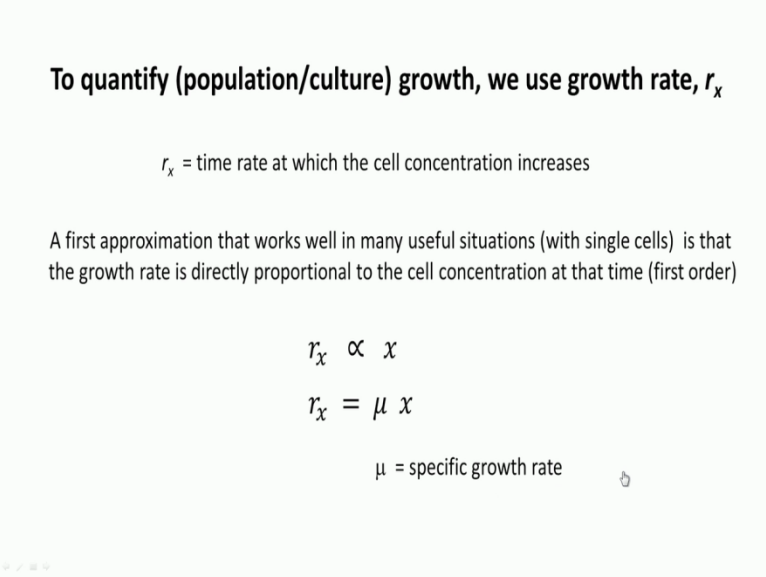


Now to quantify the population or culture growth, we are going to use something called a growth rate, which is represented as, $r_x$. The $r_x$ is nothing but the time rate at which the cell concentration increases, okay? Cell, see, talking of cell concentration makes sense, because whether you have a one litre system, or whether you have a 10,000 L system, this concentration can easily be considered the same or can be scaled up, can be considered to be equal in between these two

systems. If you are looking at total cells in the one litre, it will be something; in the ten thousand litre, it will be ten thousand times that, okay?

So it is not kind of scalable. That is the reason why we normalize the cell number or the cell mass with respect to volume, we call that cell concentration, that remains the same across scales. It is scalable. For certain purposes, even cell concentration becomes a problem, we will, we will look at it if we have a need. Our first approximation that works well in many useful situations, especially when you have single cells, is that the growth is directly proportional to the cell concentration at that time. Okay? The, in other words, the rate of growth, growth rate is directly proportional to the cell concentration at that time, and this is nothing but the standard first order representation.

Rate is proportional to the concentration, okay, to the broth. And, the constant of proportionality, let us call it as mu, it is usually represented as mu, where mu is called the 'specific growth rate', okay?

It is all it is growth rate with respect to cell concentration '$r_x$ by x', if you want to look at it that way; therefore that has been normalized with respect to cell concentration; therefore it becomes specific, and therefore, it is called specific growth rate. Let me tell you another important principle that will be useful for analysis, I will just mention it to you and then leave it there, may be we will use one aspect of it.

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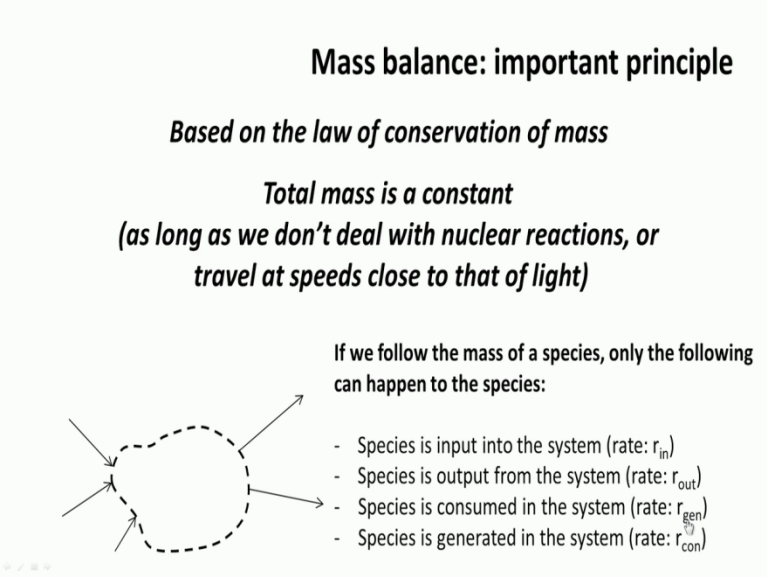


This is a very general principle. This is a principle of mass balance or material balance. As you would have guessed this based on the law of conservation of mass, which essentially says that the total mass is a constant as or the mass of a species is a constant as long as we do not deal with nuclear reactions or travel at speeds close to that of light. Right? I mean total species in in a certain formulation as a constant, we look at total mass is a constant as long as we do not deal with nuclear reactions where there is mass to energy conversion, or if we do not travel at speeds close to that of light, there is mass dilation and so on. Hence we will not consider.

As long as we are not dealing with these things; we are quite comfortable not dealing with these things for our purposes, then, if we focus our attention on something which is represented by these dotted these dashes, this could be anything, this could be a bioreactor or this could be the entire building, this could be an entire city and so on and so forth. All it says is that we are going to concentrate on that particular volume. There are inputs to this, there are output streams from this, and therefore, if we follow the mass of a species; we are looking at a species here, not total mass here; mass of a species, only the following can happen to the species.

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$$net\ rate = r_{in} - r_{out} + r_{gen} - r_{con}$$

$$net\ rate = \text{rate at which the species mass gets accumulated in the system,} \frac{dm}{dt}$$

If the species is cells ($x$)

$$r_{in} - r_{out} + r_{gen} - r_{con} = \frac{d(m_x)}{dt}$$

You can think about it, all all you want, if you can come up with something else, please let me know, you would be a genius, what I mean is, see whether you can come up with something else. This species is input into the system, may be at a rate of $r_{in}$; the species is output from the system at the rate of $r_{out}$; the species is consumed in the system at the rate of $r_{con}$, and species is generated in the system at the rate of $r_{gen}$. These have been interchanged, please write this as consumed con, and this is generated.

Therefore the net rate is rate of input minus rate of output plus the rate of generation minus the rate of consumption of that particular species. The net rate is the rate at which the species mass gets accumulated in the system, and let us represent it by ddt of m, okay? This is an accumulated mass; and if the species happens to be the cells x, then rate of input of cells minus rate of output of cells mass in terms of mass, plus the rate of generation of cells, minus the rate of consumption of cells equals the rate of accumulation of the cell mass. Remember, we are dealing with mass rates in all these cases, not concentrations.

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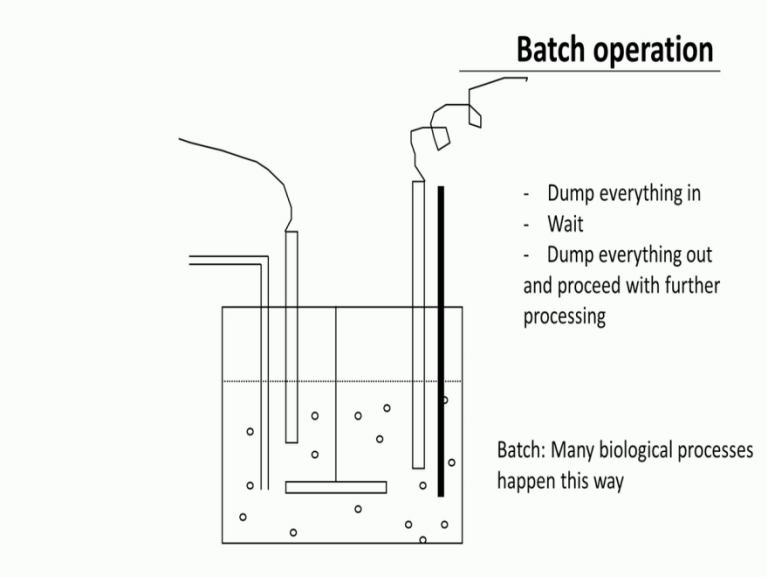


The batch operations are very common in the industry. A batch operation just means that, this is the representation of a bioreactor, you have a vessel, you have a highly controlled vessel, specialized vessel, you have a broth here with cells, you have a stirrer to keep the cells in suspension, and for other means, you have various probes here, probably the pH probe, temperature probe and may be an air inlet here to provide oxygen to the cells. This is a typical stirred reactor, stirred bioreactor. The batch operation of the bioreactor is something like this.

We dump everything in, okay? Medium, cells and so on and so forth, and at time zero, the operation starts after we have dumped everything here. Then, wait for the process to go to completion, then dump everything out and proceed with further processing. In such an operation, rather such an operation is called a batch operation. There are other kinds of operation, we will not get into that. And, very many biological processes happen this way, specially in biological and pharmaceutical industries, okay? The workhorse of a biological or a pharmaceutical industry is still a batch operation, because it is rather easy compared to the other processes to carry out, given the various constraints.

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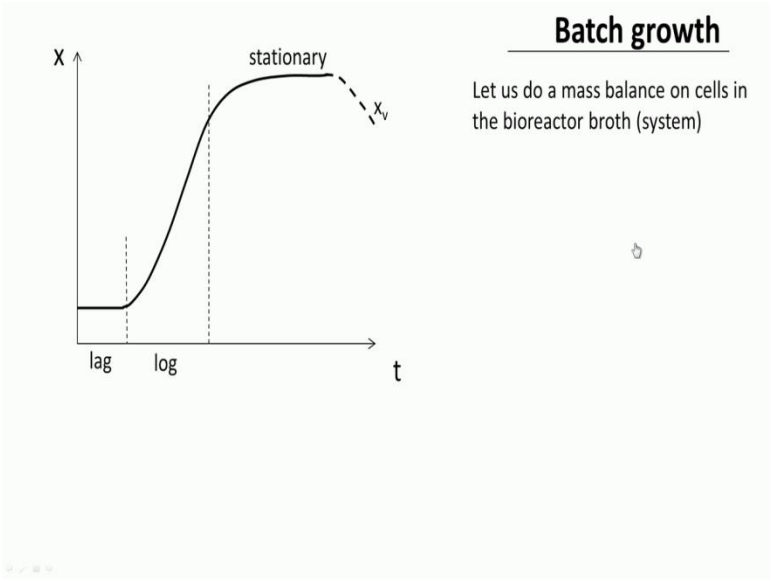


So, we are going to consider batch growth, growth in a batch culture, in a batch operation. If we plot the cell concentration with time, the way the cell concentration varies with time during growth in a batch, that is we have dumped all the cells and provided the medium and so on so forth, staggering the process and we are monitoring how the cell concentration varies with time. The variation is typically like this. You have a certain time when there is not much of an increase in cell concentration. After a certain time, there is a significant increase in cell concentration, and then there is, it kind of flattens out, there is not much of an increase in cell concentration after it reaches its maximum value.

The period during which, the initial period during which there is no change, not much of a change in cell concentration is called the 'lag phase'. The period where there is a significant increase is called the 'log phase', and the later phase where it tapers off is called the 'stationary phase'. By the way, this is a typical batch growth curve. You may not always find it. Depending on the situation, the log phase may start immediately after inoculation. Or, it may take a very long time in lag phase, and then grow very quickly and then reach stationary phase, and so on and so forth, okay?

There are variations to the theme, this is a typical theme. And after it has reached a certain stage, if you are following the total cell concentration, it will probably remain (station), remain the same for a large period of time and then probably go down depending on the situation. Whereas

if you are following the viable cell concentration, it is going to go through the same lag phase, log phase, stationary phase, and then there will be a decrease in the viable cell concentration. Now, let us do a mass balance on cells in the bioreactor broth. A bioreactor broth is what you are going to consider as a system.

The bioreactor broth is nothing but this, right? This is what we are going to take as our system, this is what we are going to concentrate on, this is what we are going to focus our attention on. If we do that, the basic material balance equation, this equation can be blindly written. We are following cells, therefore the rate of input of cells minus the rate of output of cells plus the rate of generation of cells minus the rate of consumption of cells equals the rate of accumulation of cells in the broth, bioreactor broth. Since this is a batch system, and we are going to consider only the growth now, let us not consider the death process here and so on and so forth, we will consider till somewhere here.

There is no input, because the batch operation, as I had said, we dump everything in, and start the time after everything is inside, okay? So there is no input stream into the system that we are considering. Similarly, there is no output stream. After the process is complete, we take everything and and go for further processing. And our period of interest now is the time when from start, after everything has been dumped in, to end before everything has been taken out. Therefore during that time of interest, there is no, there are no inputs, there are no outputs, and therefore the rates of input and output of cells is zero.

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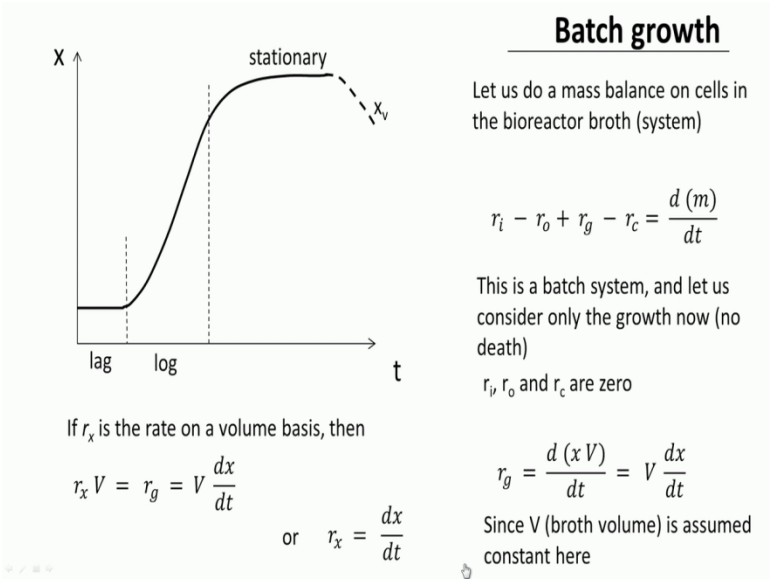


The rate of consumption of cells is also zero because the cells are not getting consumed by some means. They are only being generated. This is the case, then this, this and this go to zero, $r_g$ is the only term that remains and $r_g$= dm/dt. We can write mass as nothing but concentration times volume, because concentration is nothing but mass per volume, right? So, concentration, given as x, x into the broth volume, this gives you the mass of cells, d/dt of xV equals $r_g$. In the case of a batch, the broth volume remains a constant, and therefore it can be taken out of the derivative here, and you get V dx/dt.

If $r_x$ is the rate on a volume basis, which it usually is, then, we need to write $r_xV = r_g$. $r_g$, remember, is on a mass basis, right? It is mass per time, whereas $r_x$ is cell concentration per time. So cell concentration needs to be converted to cell mass, which can be done by multiplying it by the volume, $r_xV = r_g = V\ dx/dt$. Or, $r_x = dx/dt$. One of the most common models for growth rate that we have already seen is $r_x = \mu x$. Therefore, if you substitute $r_x = \mu x$, we get $\mu x = dx/dt$. Let us write as $dx/dt = \mu x$.

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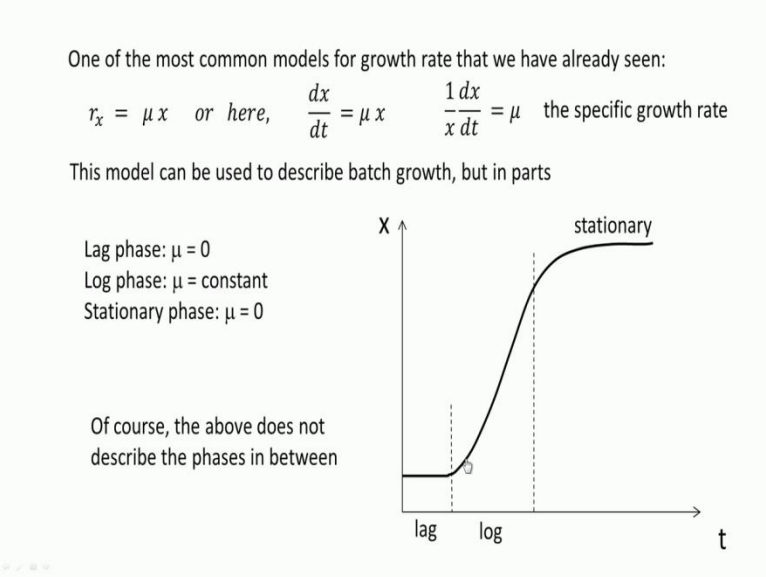


And 1/ x dx/dt = μ, it is easy to see why it is called the specific growth rate. This we have already seen earlier, where even in a more generic representation of $r_x$. This model can be used to describe batch growth, but only in parts. The lag phase, the log phase, the stationary phase is what we had seen in a typical batch growth. In the lag phase, there is no increase in cell concentration over time, therefore the specific growth rate is zero. In the log phase, this you will see immediately after the specific growth rate happens to be a constant. And, in the stationary phase, again, the specific growth rate is zero, because there is no increase in cell concentration with time. Right?

Among the various stages of growth, this phase, the specific growth rate and this phase happens to be the maximum, and therefore this is also called the maximum, the, the growth rate here, the specific growth rate here is also called the maximum specific growth rate in a batch, okay, that is another term that is used, and usually it is a constant here. But this model does not describe what is going on here when there is a variation, does not describe what is going on here, and so on and so forth. Therefore it does not describe the phases in between, okay? This we need to keep in mind.

But probably we do not need that, okay, all this is dependent on the need. Therefore we will realize the limitations of its representation, and use it appropriately. In other words, we cannot use it to represent things changes here, and changes here. Let us begin, or let us consider the log

phase where μ is a constant. And we can write dx/dt = μx. If we solve this simple first order differential equation, with the initial condition that at time t = 0, the beginning of the log phase, the cell concentration is $x_0$, okay?

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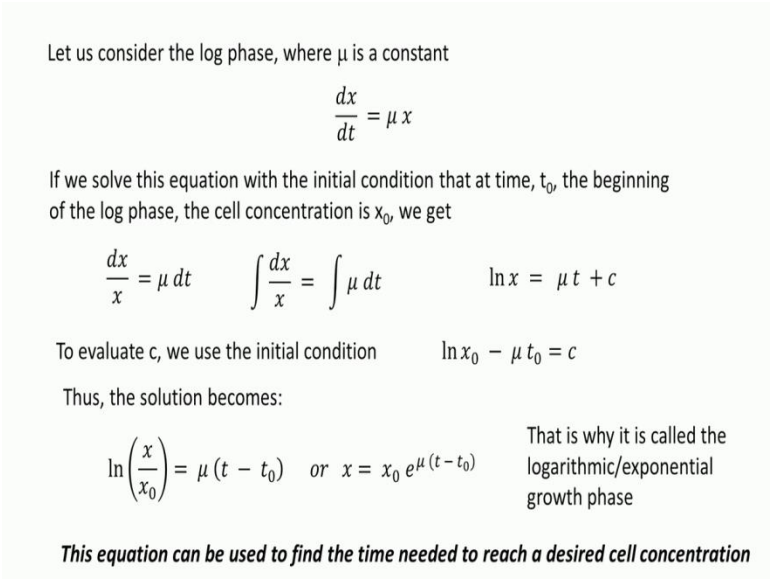


It is not the beginning of the batch, it is a beginning of the log phase, time t0, and the cell concentration is $x_0$ at that time. If we do that, and we will solve this dx/ x = μdt, integrating both sides, we get ln x = μt + c. This constant can be evaluated using our initial condition, that at t = $t_0$, x = 0, we substitute that $\ln x_0$ - $\mu t_0$ = c, and therefore the solution becomes $\ln(x/x_0) = \mu (t - t_0)$, or $x = x_0\, e^{\mu(t - to)}$.

And that is the reason why it is called logarithmic growth phase or the exponential growth phase. Logarithmic in this form, exponential in this form, and that is the reason why it is called so. This equation can directly be used to find the time that is needed to reach a desired cell concentration, starting from a certain initial cell concentration. And that is a very important design aspect. We would like to know what is the maximum cell concentration that needs to be reached, how long do you need to operate the bioreactor to reach that and so on and so forth apriori. Okay?

And if we know the specific growth rate apriori, this is a straight forward simple calculation to find out the time t that is needed to reach a given maximum cell a given cell concentration, okay? It is always important.

And of course, we are assuming that the cell concentration is less than the maximum cell concentration that is possible to achieve under those set of conditions. I think that is where we will sign off here, , we have seen culture growth or population growth, we have seen the need for quantification, insistence and the importance of sticking to time rates in trying to, when, when we quantify systems, dynamic systems, including biological systems, that is a cell, and then we looked at batch growth and quantification of batch growth through specific growth rate and in application of that, to find out the time that is required to reach a certain desired cell concentration starting from a certain cell concentration.

Batch growth happens to be the work horse of many industries, many biological industries, now even nowadays. See you later in another module. Bye.

**Biology for Engineers and other Non-Biologists**
**Professor G.K.Suraishkumar**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Lecture Number 16**
**Mendelian Genetics: Genetic Disorders**

Welcome to the set of lectures on 'Mendelian Genetics'. Mendelian refers to Mendel, Gregor Mendel. We will refer to him during the course of this lecture and it will also become clear to you why we are looking at something that is so old. What is the relevance nowadays?

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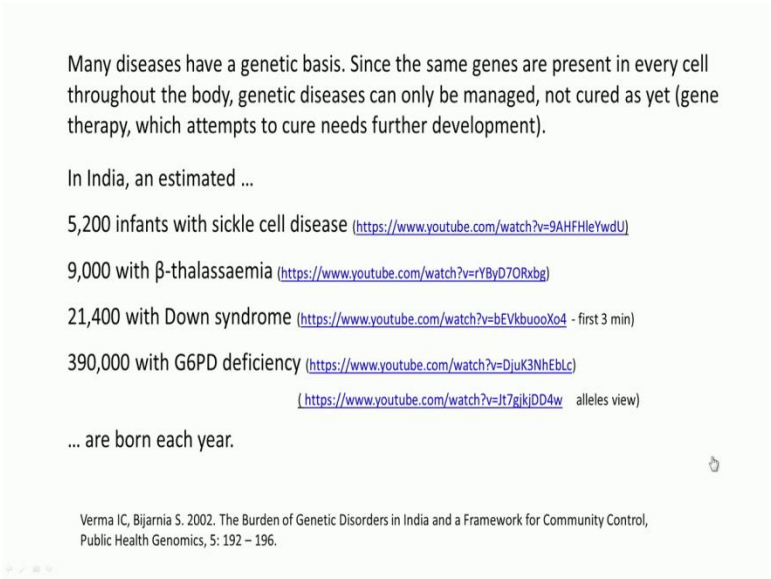


Many diseases have a genetic basis. You know that each cell in the body has the same set of genes; each somatic cell in the body has the same set of genes, and it does not matter whether it is the brain cell or the liver cell or a skin cell and so on so forth, they all have the same set of genes that we inherited from our parents.

They could be expressed differently depending on where they reside and so on and so forth, we will not get into that. But, the basic material is the same. And since the same genes are present in every single cell throughout the body, genetic diseases will manifest across and there is nothing much can be done, it can only be managed. It is rather difficult to cure them as yet. Genetic, or gene therapy has some promise, it is , it has been studied for quite a long time; it has had a few

successes, but I think it needs quite a bit of development before it gets to a good acceptance stage.

Let us start with some data. In India, an estimated five thousand two hundred infants with sickle cell disease, nine thousand with something called (beta) beta thalassaemia, twenty-one thousand four hundred with Down syndrome and (thirty-nine), three hundred and ninety-thousand with G6PD deficiency are born each year, okay? These terms, some of which we have seen earlier; sickle cell disease you know, sickle cell anemia, the same thing, it is called sickle cell disease. If you want, you could look at this video, which is also given in the pdf file, which can be clicked from the pdf itself. This is an optional video to know about sickle cell disease, nice video, but it is an optional video.

Similarly, you could look at this video for knowing what thalassemia is, especially beta thalassemia; there is alpha thalassemia and beta thalassemia, you could click on this, again an optional video. Down syndrome, I would suggest that you watch the first three minutes of this video. It has a very nice overview of the genetic basis itself, and it gives an introduction to the Down syndrome, and then it gets into a lot of research aspects, that might be a little beyond this course, the research aspects, the first three minutes are very nice, I would recommend that you watch this, the first three minutes of this video.

And then there is something called G6PD deficiency which seems to affect a large number of infants born every year, right? This is a social aspect that is given, an optional video, and this is an allele view that is given of G6PD deficiency, which you may want to look at, okay? These are optional. Except the first three minutes, I would say is highly recommended. This is actually from a source because these are numbers and these are estimates; this is actually from a source, a paper published in two thousand two by Verma and Bijarnia; 'The Burden of Genetic Disorders in India and a Framework for Community Control'. It was published in 'Public Health Genomics'. This was in two thousand two, the numbers, of course would be different now, but atleast we have something that we can hold on to, in terms of numbers. That is why this is chosen. Gives you an idea of the prevalence of such diseases in India.

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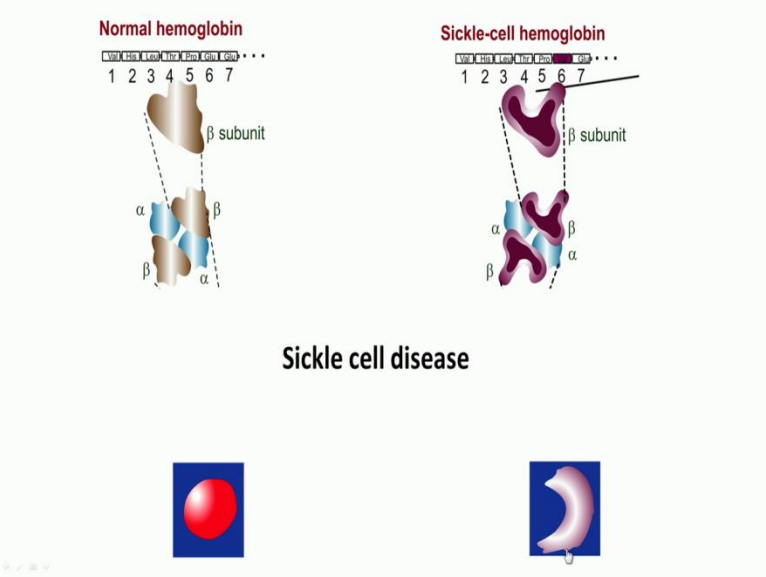


We have already seen the sickle cell disease; let us review this. We know that normal haemoglobin is made from the haemoglobin, haemoglobin is a protein, therefore it is made from the genes that correspond to the various sub-units, that code for the various sub-units. In a normal haemoglobin gene, there is a glutamic acid in the sixth position. If that glutamic acid becomes a valine, right? If, if that is changed, then just that one change can bring about a different conformation, different three dimensional folding in the case of the various protein sub-units, and we get a haemoglobin molecule that can easily crystallize out, it will not have an ability to carry oxygen as a normal haemoglobin molecule does.

And therefore, we get sickle cell anaemia, right? This is, it is as simple as that. The, if the haemoglobin is normal, the red blood cell would look nicely disc shaped, and if it happens to have, if it happens to be diseased with sickle cell anaemia, then the red blood cell takes on a sickle shaped, sickle shape. And this causes major difficulties as you have already seen in an earlier video, in an earlier recommended video in this course.

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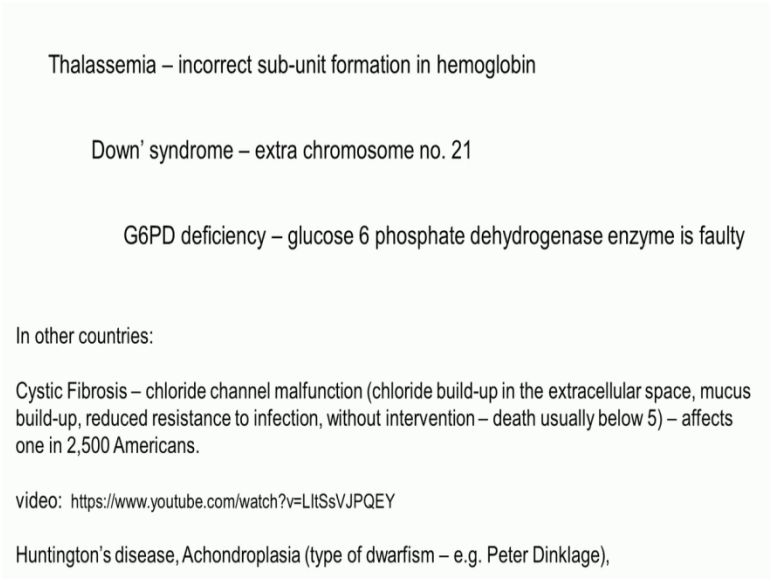


Similarly, thalassaemia is incorrect sub-unit formation in haemoglobin; either the alpha sub-unit or the beta sub-unit are incorrectly formed, it is different from sickle cell anemia. Down syndrome is caused by an extra chromosome number twenty one. Typically people have two chromosomes, right? The chromosome twenty one has three; there are three number twenty one chromosomes. If that happens, this happens in each cell, if that happens, it results in Down syndrome, and it manifests in different ways that you can pick up from the video that was recommended, that was suggested, it is an optional video.

And then, this G6PD deficiency, which stands for 'Glucose 6 Phosphate Dehydrogenase' deficiency, this enzyme is faulty; and because this enzyme is faulty, it leads to a lot of difficulties, major difficulties, right? Again recall that 'Glucose 6 Phosphate Dehydrogenase'; It is an enzyme, which means it is a protein, it is coded by a gene, right? And therefore, it is a genetic disorder. This is in India. In other countries, cystic fibrosis; it is a very very common genetic disorder in the US; it arises because the channel for chloride in the membranes malfunctions, okay, because the protein which is actually the chloride channel protein, that is not properly formed and therefore, that malfunctions, the chloride cannot move in and out of the cell.

If that happens, there is a chloride build up in the extra cellular space, and that results in mucus build up, reduced resistance to infection, and without proper intervention, death usually occurs below five years of age, okay? With intervention, people live much longer, and it affects one in

two thousand five hundred Americans, it's a very prevalent. You can look at this video which gives you some idea of cystic fibrosis, it is a let us say an optional video. And similarly, Huntington's disease, which actually manifests above forty five, is a genetic disorder. Achondroplasia, which is a kind of dwarfism, okay. You might recall Peter Dinklage, from 'Game of Thrones', right? Tyrion Lannister, is it? , that person, has Achondroplasia; it is a type of dwarfism. That is an inherited disorder; a special type of inherited disorder as we will see later.

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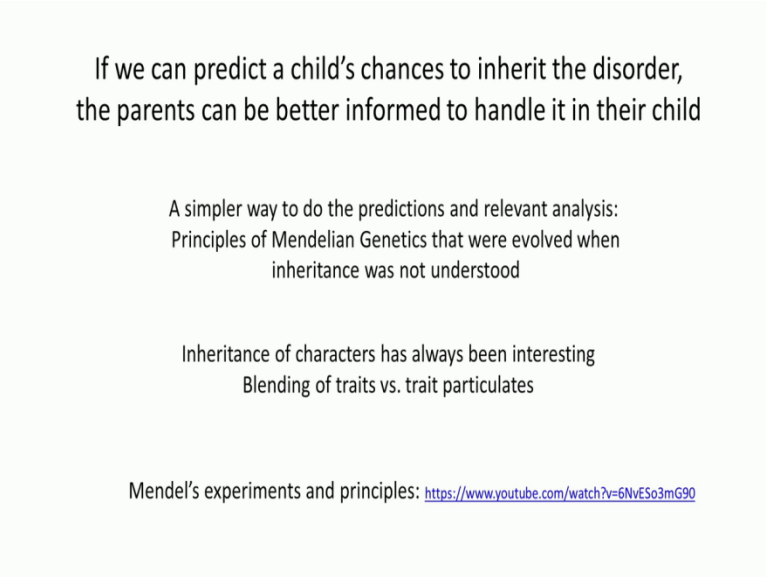


Now, we said all this for this reason: if we can predict a child's chances to inherit a disorder, okay, something as major as cystic fibrosis, the parents can be informed to handle it in their child, okay? They will be atleast expecting this difficulty in their child, they would be mentally prepared to handle it and so on and so forth, in their own ways, and that would be a big thing, that is already being done, and the basis for that is what we are going to see in this particular lecture.

A simpler way to do the predictions and the element analysis, we know a lot today and there are various ways to do this analysis; but a very simple way in which this analysis can be effectively done is by using the principles of 'Mendelian Genetics', and the principles of Mendelian Genetics evolved, may be a century and a few decades ago, when inheritance was not even understood, when people did not understand how, what is the basis of the son or the daughter

looking like their father or the mother, and sometimes looking like their grandmother or the grandfather and so on and so forth. People just did not understand.

Right? It was during that time, late eighteen hundreds, that these principles were developed; we will very briefly look at it by Gregor Mendel, seminal piece of work, and those principles are very effective today, although they are, you know basal principles and there are huge variations known to them, those principles are still very useful to do this, to be able to predict a child's chances to inherit a disorder; and that is the reason we are studying or we are looking at Mendelian Genetics even today.

In any case, historically it is nice to know what Mendel did and the development of that if you are interested in genetics; but there is a very useful angle to it. When inheritance was not understood, may be a century ago, the inheritance of characters, of course has always had a good appeal with people. People were always curious to know why people inherit characters, why a son behaves like the father or the mother, or the daughter behaves like the father or the mother and so on, or sometimes even like the grandfather or grandmother.

Many felt that there was always a blending of traits; there was some trait from the mother, some trait of the father, the son or the daughter has, or the offspring has the traits that are a blend of these two traits, that was what was believed for a very long time. Although, there were many different situations where this clearly was not valid, okay. That has always puzzled people but they were skeptic about it and so on and so forth, till Mendel came and told us that the traits are actually passed on by trait particulates. There are aspects that determine traits in an appropriate fashion and if you understand this, you can predict to a certain extent what will happen.

So that was a big contribution by Gregor Mendel, and I would like you to watch this very nice video. I would I would say it is a required video; it is a very nice video, slightly long, about twenty, twenty five minutes; so long, old, but it is a very nice video, okay, I would like you to watch this video to know some parts of Mendel's life, which is very interesting and Mendel's experiments and some of the principles that he brought forward, okay? In this lecture, or these set of lectures, we will see what is relevant for us.

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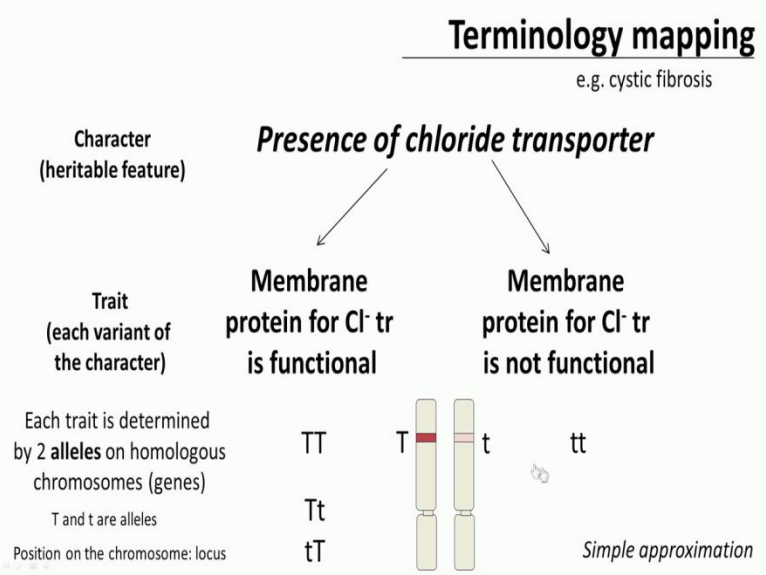


To do that, we will have to map our current terminology to the terminology that existed during the development by Mendel, okay, or, soon after that for many years. To do that, let us consider cystic fibrosis. The presence of the chloride transporter is important, because if that is absent, cystic fibrosis arises. There are two possibilities; the membrane protein for the chloride transporter, is either functional or the membrane protein for the chloride transporter is not functional, right? These are the two possibilities, normal or cystic fibrosis.

The presence of the chloride transporter is a character or a heritable feature, okay? Heritable feature is called the character. Sometimes it is not as, not in molecular terms as this, it could be the height of a person, or it could be the colour of the eye, and so on and so forth. Those are typical observable traits, or in other words, the characters usually an observable thing, I am just using a terminology mapping here, so this aspect, if it can be observed, it is called a character, and it is a heritable feature.

The variants of each character, each one of them is called a trait. Okay? For example, the functionality is a trait, the non functionality or the absence is a trait. Tall is a trait or short is a trait, okay? Blue colored eyes is a trait, brown colored eyes is another trait, and so on. So, if the character happens to be eye colour, blue eye colour and brown eye colour are the two traits, or many, two of the many traits of that particular character. If height of something is a character, then tall is a trait and short is another trait, and so on. You get the idea.

So we are going to deal in terms of characters, traits and so on. Each trait is determined by two alleles on homologous chromosomes, and this is what is equivalent to the genes that we know of, right? On homologous chromosomes means, you know that they exist in pairs and therefore, we just talked of pair number twenty one, if there is an extra one, that's Down syndrome and so on, but those two twenty ones or those two twenties or those two, let us say one two three, they are all homologous chromosomes; and two alleles on homologous chromosomes determine each trait is the equivalence here.

So if these two are homologous chromosomes, capital T is an allele, and small t is another allele. So you could have various combinations, both could be capital; they could be alternate, one could be, this one could be T, this one could be small t, or both could be small ts. And these different combinations result in different traits of that character; different yeah different traits of that character. The position on the chromosome of that allele is actually called a locus, this is just for the terminology, okay?

Remember that this is a simple approximation, there are variations possible, we will point them out when we come to them. I think at this point in time, we will take a break and cover the other things in the next lecture. It will be very nice if you can see the recommended video or the required video on Mendel and his work, we will see that, okay. And then, let us meet in the next lecture to take things forward. See you then.

**Biology for Engineers and other Non-Biologists**
**Professor G.K.Suraishkumar**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Lecture Number 17**
**Mendelian Genetics: Mendelian Inheritance Principles**

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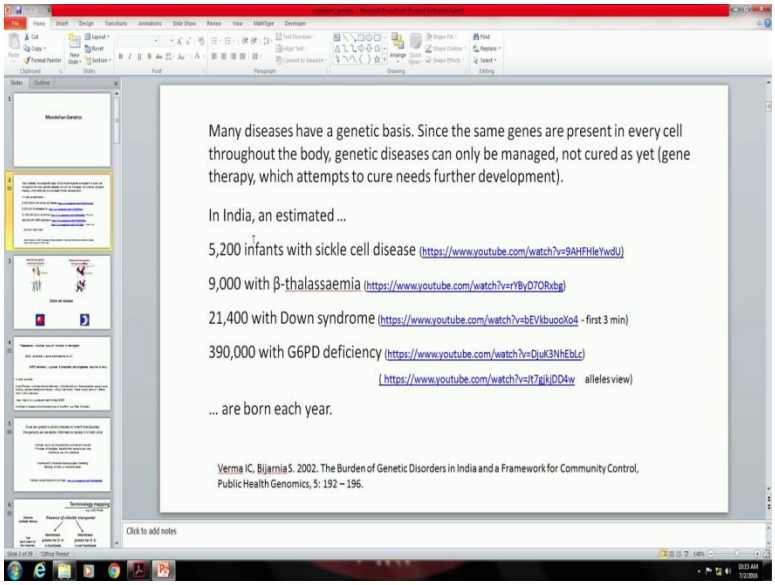


Welcome to the set of lectures on Mendelian Genetics. We saw that many diseases have a genetic basis; for example in our country, sickle cell anaemia or sickle cell disease, beta thalassaemia, Down syndrome, glucose 6 phosphate dehydrogenase deficiency are all occurring genetic diseases and in other countries such as the US, cystic fibrosis is a widely occurring genetic disorder. So are Huntington's disease and Achondroplasia and so on and so forth; these are just examples.

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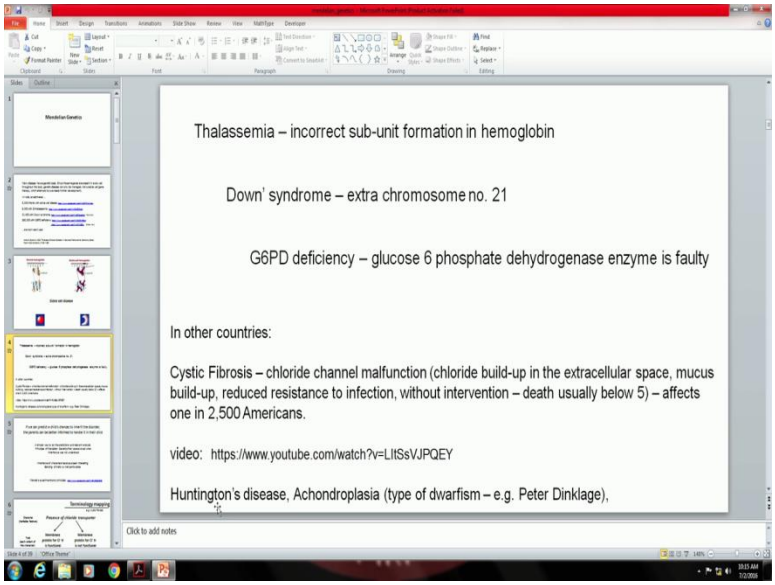


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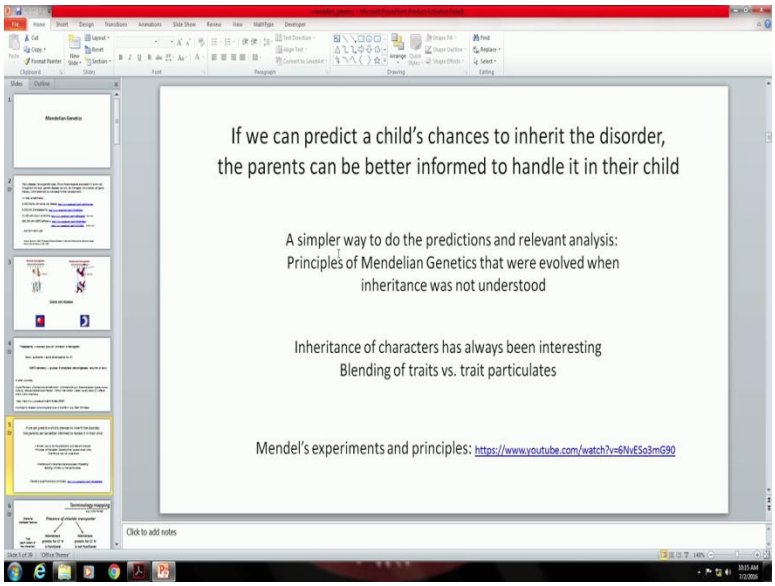


Our overall aim was that if we can predict a child's chances to inherit the disorder, the parents can be better informed to handle it in their child, we know quite a bit about the way the information is inherited or the characteristics are inherited and so on, whereas for this kind of an aim, the principles of Mendelian Genetics which were developed about a century and a half ago, or more than a century ago, are very convenient to use; and that is the reason why we are looking at Mendelian Genetics here. I had pointed out this video to you, please take a look at this video,

if you have not already done so; slightly longish, but a very nice video on Mendel's experiments and principles.

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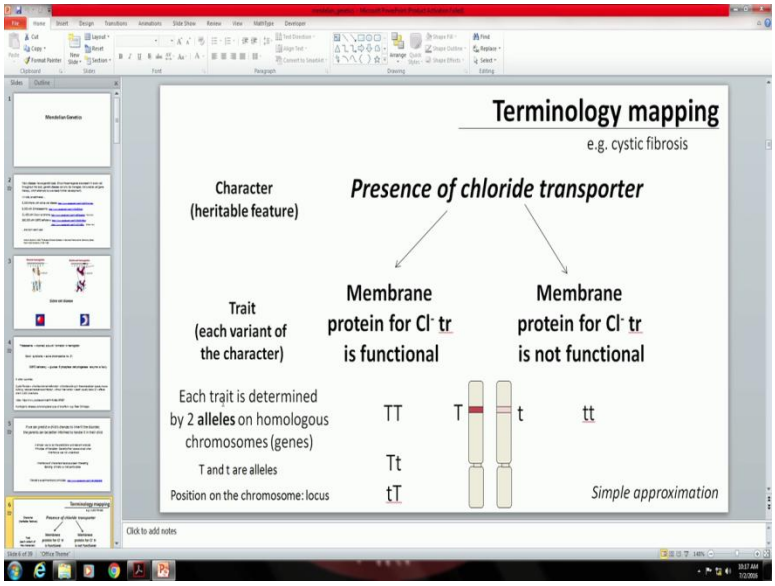


Before we went into Mendel's experiments, we needed to map whatever terminology that we use nowadays in the context of genetics to the equivalent terminology that goes with a Mendelian analysis of inheritance; for example, we had taken cystic fibrosis and we said presence of a chloride transporter, this is rather molecular in nature, it could be somebody being tall and short and so on so forth, that could also be and we said that this was a character which is an (inheritable) which was a heritable feature, and the presence of the chloride transporter, it could be of two kinds, if the membrane protein for chloride transporter is functional then it is fine; if the membrane transporter for chloride transporter is not functional, then we get the disease.

Therefore there are two traits, the functionality and non functionality and these two traits are variance of this character and that is the terminology here. In other words, in terms of classic terminology; tall and short, these are the traits of the character height. Then we said that each trait is determined by two alleles on homologous chromosomes which are the equivalent of genes; for example, this could be determined by TT, Tt, tT or tt, depending on what is present on these two loci, locus here, these two loci of the homologous chromosomes.

If both capital Ts are present, then the membrane protein for chloride transporter is functional; if at least one of them is capital T, then it is functional; if both are small, then it is non functional.

This is the mapping between a molecular aspect that we know of nowadays to the olden times, the Mendelian times, when nothing of this was known, and it is very insightful to come up with this kind of a model to explain inheritance. And as you can see, this is a simple approximation, there are various different diseases that do not follow this approximation, we will first look at this approximation which is very useful and then I will extend that to show you the differences.

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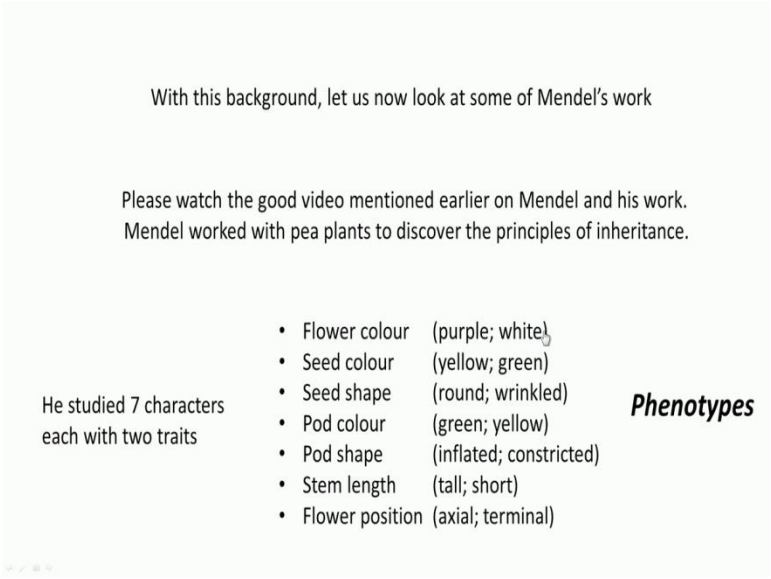


I think this is where we stopped last time, so let us go further with the lecture material of this lecture. We are going to review, we are going to see some of Mendel's work. Again let me emphasize the watching of this video. Mendel worked with pea plants to discover the principles of inheritance, you know, the very first lecture, the introductory lecture, I had mentioned about studying something for the sake of it, studying something because we want to understand something that exists around us. This study was one such kind and look at where it has led to.

Coming back to Mendel's work, Mendel studied seven characters, each of which had two traits. The characters that he studied were flower colour, seed colour, seed shape, pod colour, pod shape, stem length and the flower position. Let us go further to see, to explain this and these characters, flower colour, seed colour, seed shape, pod colour, pod shape, stem length and flower positions are called 'phenotypes'; for example, the flower colour could be either purple or white; both are phenotypes, flower colour is a phenotype and so on.

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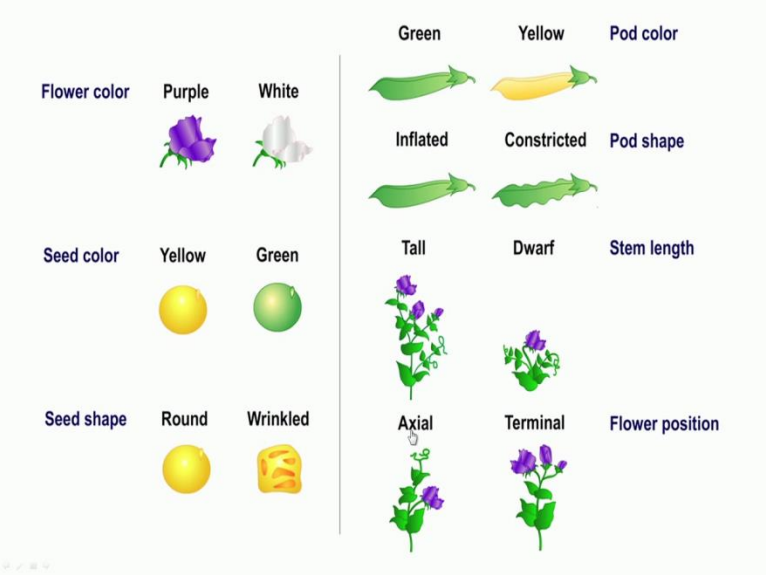


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Here, we see it in a pictorial form in the particular case of pea plants; the flower colour would either be purple or white; the seed colour would either be yellow or green; the seed shape would be round or wrinkled, by round it is actually smooth, round or wrinkled; the pod colour could either be green or yellow; the pod shape could be either inflated or constricted; the stem length could be either tall or dwarf; and the flower position could be either axial or at the end, terminal, okay?

So these were the characters that Mendel studied, most of these characters were rather simple, it just happened by chance that they were simple and therefore the basic principles of inheritance

became amenable to study by Mendel; he could come up with the basic laws of inheritance, most of which is, was applicable for simple inheritance. Now let us look at one character, okay, which is flower colour. Mendel started his experiments with true breeding plants. What does true breeding mean?

It means, in the context of flower colour, the same (color) of flower results in all the offspring in all the generations, okay; which means it is true breeding amongst itself and therefore if it is purple; if the flower colour is purple, it is purple throughout in all the generations forward; if it is white, it is white in all the generations forward. Such plants are called true breeding plants and Mendel was careful to spend the time to make sure that they were indeed true breeding plants before he started out his experiments.

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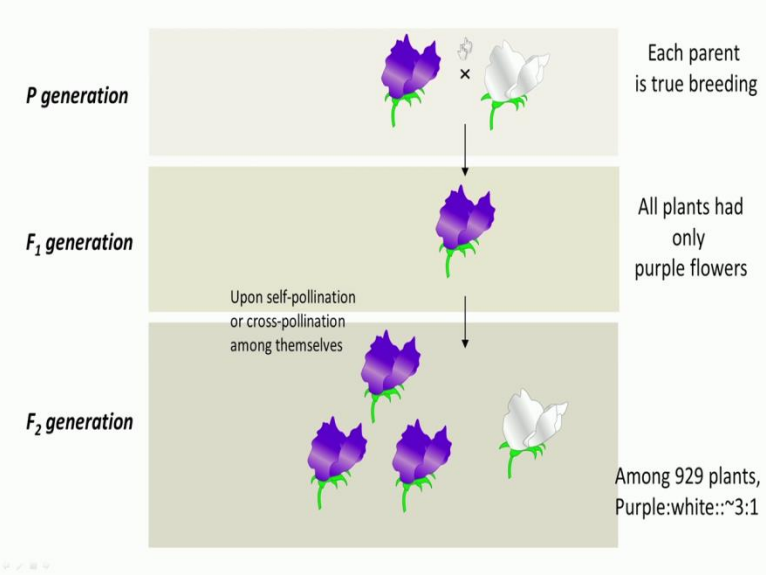


So when he started out with true breeding plants, the ones that yielded purple colour flowers and the ones that yielded white colour flowers, and he crossed them with each other. The pollen from and the female part of the plant were made to interact in different ways and this cross resulted in a purple flower in all the plants of the first generation; this is the parent generation; the first generation had only purple flowers, there was no sign of the white flower at all, and there was nothing in between; it was either purple, rather in this case, it was purple or white, and in the F1 generation, it was all purple. This was the observation, and he had observed this over a large number of plants and he did a statistical analysis to find that, a statistical analysis of course does

not mean much here; he did statistical analysis over the entire set of experiments to come up with these various findings.

Here it does not make a difference; here everything had only purple flowers. And then what he did was, he either self-pollinated or cross pollinated the purple flowers amongst themselves, okay, this cross with another purple flower of the F1 generation itself. So when that happened, in the F2 generation, something strange happened. Three fourths of the flowers, he had studied - among nine twenty nine plants, about three fourths were purple, and one fourth was white approximately, in a very large number of plants. Okay. So the white colour which was missed in the first generation made an appearance in the second generation; that is what he found.

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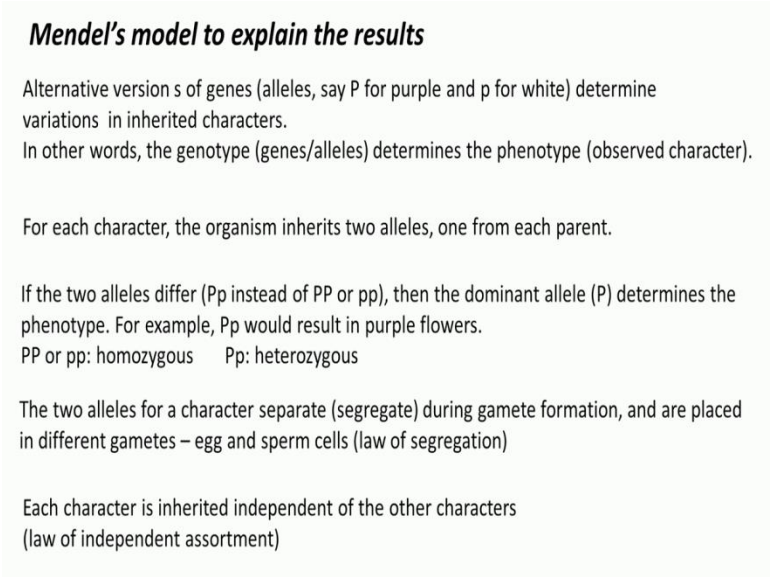


So after a lot of experiments with a lot of single characteristics, dual characteristics and so on so forth, Mendel came up with a model to explain the results which can be given as follows: alternative versions of genes or alleles, say capital P for purple and small p for white determine the variations in inherited characters, this is the first aspect of the model; in other words the genotype, genes or alleles determine the phenotype which is the observed character. The P determines the purple and the small p determines whether it is white.

For each character, the organism inherits two alleles, one from each parent. If the two alleles differ, you know, capital P small p, instead of both being capital P or both being small p, the dominant allele P determines the phenotype, okay? For example, if there is a capital P and a

small p, the trait of capital P is what would be seen. In other words, this would result in purple flowers. In terms of terminology, if both are the same, and either both capitals or both smalls, then they are considered homo - same, zygous. And whereas if they are different, they are called hetero, different, zygous, okay?

Homozygous and heterozygous, these are commonly used terms, it is nice to know. If both are the same, homo, so homozygous, if both are different, hetero, so heterozygous. The two alleles for a character separate out or segregate during gamete formation, right, you would have gone through meiosis, you would have studied meiosis, so you know what a gamete is; this is what results during, in the cell division, that is gone through by the germ cells. Here we are talking about the separation of the two alleles, and what Mendel said was that they segregate during gamete formation and are placed in different gametes, the egg and the sperm cells. Okay? And this is called the law of segregation.

The two alleles separate, they are placed in separate gametes; and each character, for example flower colour, seed colour, seed shape, these are all different characters. Each character is inherited independent of the other characters. Right now we are looking at only one character, but all characters are being inherited simultaneously. That we all know. And what Mendel said through his studies was that each character is inherited independent of the other characters, and this is called the law of independent assortment. These two are supposed to be major contributions; the ‘law of segregation’ and the ‘law of independent assortment’. Why this is so will become clear very soon. It blends itself to very easy analysis.

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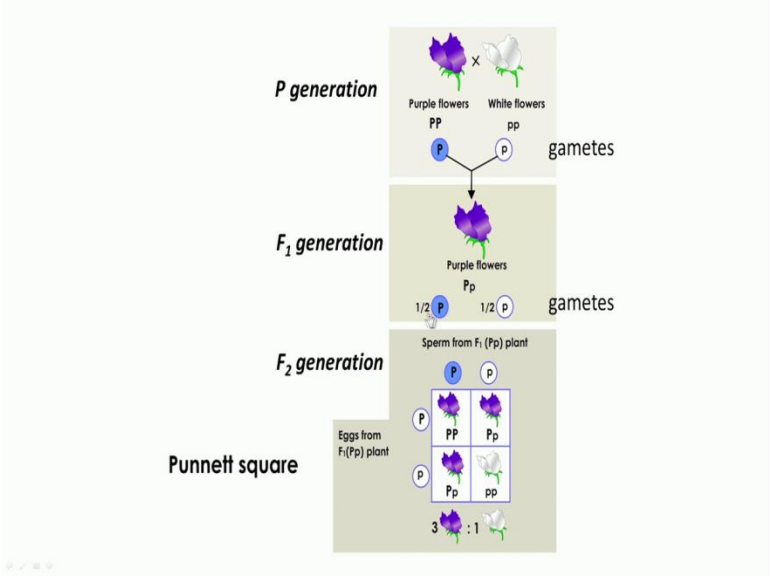


Therefore, if you look at the earlier experiment again, the P generation had purple flowers, white flowers. The purple flower, these were the true breeding varieties and therefore the purple flowers means both are capital P, and the white flowers means both are small p, okay? In other words, there is no heterozygosity here. So the gametes would have both capital P, in this case, and both small ps in this case; and upon fertilization, the genotype is capital P small p, which results in a purple flower because capital P is dominant, right?

And the gametes from this, half of them would have capital P, and half of them would have small p, and therefore in the F2 generation, if you look at all possibilities, this is p and p from the sperm of the plant, and P and p from the eggs of the plant; and the combinations would PP , Pp, pP and pp.

If atleast one is a capital P, then it will result in a purple flower because it is dominant, and therefore you have three out of four being purple and one out of four being white, okay? This kind of an analysis where you look at all possibilities by using this kind of a square with the gametes from the sperm and the gamete from the eggs is called a ‘Punnett Square’, okay? This is heavily used in this kind of analysis. It's rather nice if you are not dealing with very complex situations; one or two traits, one or two characters it is quite good to do, it gives you a visual feel for what is happening, okay? Whereas, if you have, if you are comfortable with probabilities, that is a much easier way to do, okay? We will, we will look at that in a little while.

If you look at what is happening during meiosis, this is what happens.

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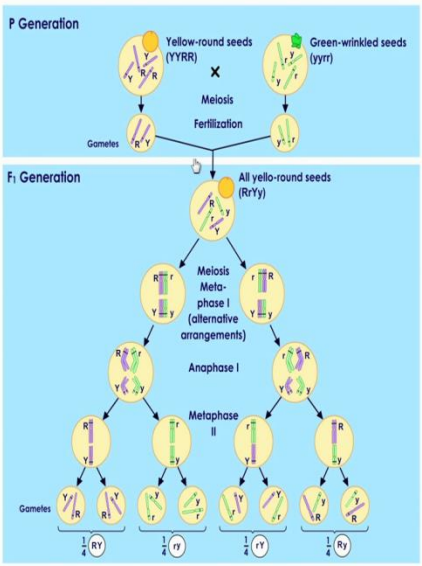


The P-generation here, I think slightly different example is considered; yellow round seeds, these are two traits being considered together, that is fine and these are green wrinkled seeds. Yellow round seeds are true breeding, therefore YYRR, and green wrinkled seeds would be yyrr.

During meiosis they form gametes. These gametes would only be YR; and yr and upon fertilization, these are the two possibilities, let us look at them one by one. They get mixed up and by the way they arrange themselves, it would be Rr, Yy, please go and take a look at what happens during meiosis, especially during metaphase one and anaphase. In the anaphase these start separating out and metaphase two, you have these gametes being formed, RY and ry and therefore the gametes here would all result in either YR, or yr, okay?

Alternatively, if the arrangement is like this, r capital Y capital R small y, then during anaphase, they segregate out resulting in small r capital Y, capital R small y and small r capital Y, small r capital Y here, capital R small y capital R small y here. Therefore, one-fourth, you know, two out of eight is capital R capital Y, one-fourth is small r small y, one-fourth is small r capital Y, and one-fourth is capital R small y. This is essentially what has resulted from this kind of a segregation. This will become clearer when we look at two things together, but this gives you the molecular basis for the Punnett Square itself, in this case two characters, but you could also look at it in terms of one character.

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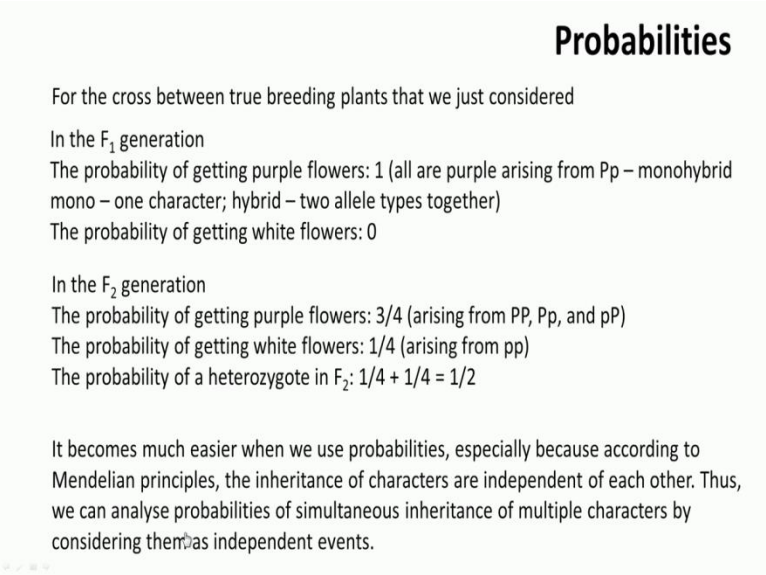


Now let us look at probabilities. It is, as I said, if you look at it in terms of probabilities, if you are comfortable that way, then it becomes much easier to do the analysis. Let us apply this or let us look at whatever we did just now, the cross that we did just now, in terms of probabilities. For the cross between true breeding plants that we just considered in the F1generation, the probability of getting purple flowers was one; all were purple, either resulting from, rather as a rising from capital P small p, okay? So monohybrid, mono is one character, hybrid means it is a mixture of two allele types. And the probability of getting white flowers is zero.

Whereas in the F2 generation, the probability of getting purple flowers is three-fourth arising from either homozygous condition or heterozygous condition; capital P capital P homozygous, capital P small p in both the, both these kinds is heterozygous. And the probability of getting white flowers was one-fourth, arising from only small p small p of all the possible combinations. And therefore, the probability of getting a heterozygote in F2, right, is ¼ + ¼ which is half and so on. You could do various different calculations of this kind in terms of probabilities; you can find the probability of various different things.

So you don't have to go and draw squares which is somewhat elaborate and so on, if one is comfortable with using probabilities. It becomes much easier when we use probabilities, especially because, according to Mendelian principles, the inheritance of characters are

independent of each other, okay, law of independence. Thus, we can analyze probabilities of simultaneous inheritance of multiple characters by considering them as independent events, okay? It will become clearer here when we consider two characters. This is what I had shown the molecular basis for.

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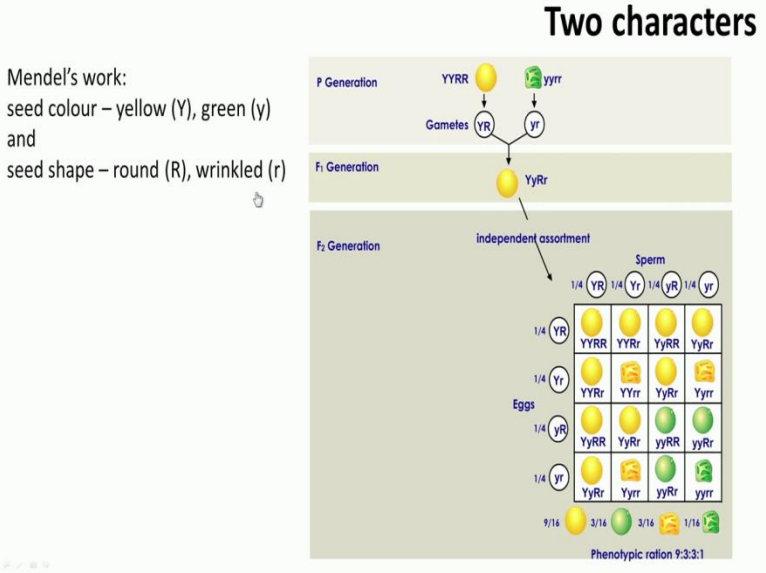


Now let us see the way Mendel saw it. For that, let us look at seed colour, yellow (Y) or green (y), seed shape round (R) and wrinkled (r), being inherited together, or being considered together. Same kind of a cross, YYRR with yyrr. The gametes in this case would be YR and yr; upon fertilization it is going to be completely heterozygous, YyRr and then they are going to be independently inherited or independently assorted.

Therefore the seed colour would be independent of seed shape, the inheritance of seed colour would be independent of the inheritance of the seed shape, that is what it means. If we draw a Punnett Square here of all the possibilities, you can see if you count, that nine out of the sixteen possibilities would be yellow and round, both dominant characters; three out of sixteen would be round and green; three out of sixteen would be yellow and wrinkled and one out of sixteen would be green and wrinkled. This is how it happens with two characters which we can get an idea by using the Punnett Squares here.

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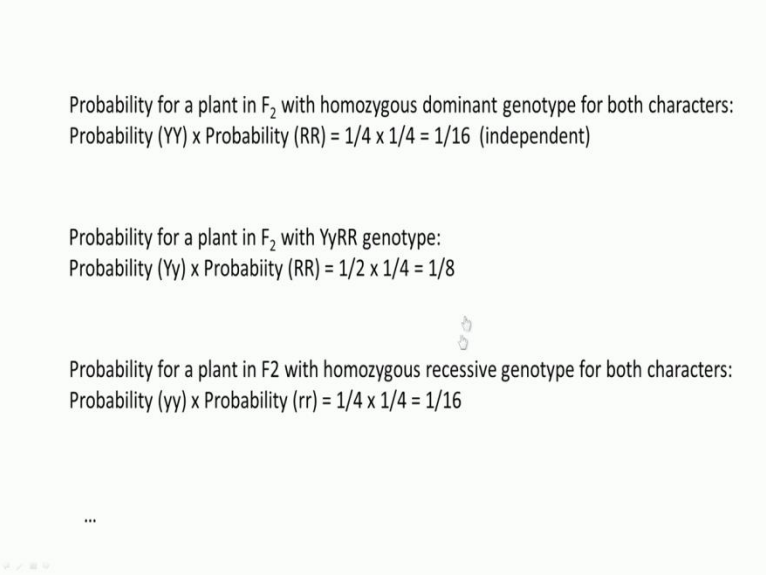


In terms of probabilities; probability for a plant in F2 with homozygous dominant genotype for both characters. What does it mean? Homozygous dominant genotype. 'Homozygous' means both should be the same; 'dominant' means, in our terminology, both should be capital, right? So in other words, probability of YY and RR. We know that the probability of YY= 1/4 and the probability of RR= 1/4, and therefore, since these are independently inherited, independent assortment, you can multiply these, ¼ x ¼, that is 1/16 . So for independent events, you can multiply the probabilities.

Now the probability of a plant in F2 with YyRR genotype, these two are independent. Therefore it can be determined as the probability of Yy into the probability of RR which is ½ x ¼ and that is 1/8. One more thing, just to illustrate how you could use this, it becomes very easy if you are comfortable with probabilities. The probability of a plant in F2 with homozygous recessive genotype for both characters, 'homozygous' both the same, 'recessive' both small letters in our terminology. In other words, probability of yyrr would be ¼ x ¼ which is 1/16 and so on and so forth. You could do a lot of possibility probability calculations with this approach. You could also bring in three characters and so on and so forth; four characters and so on, then you can work this out.

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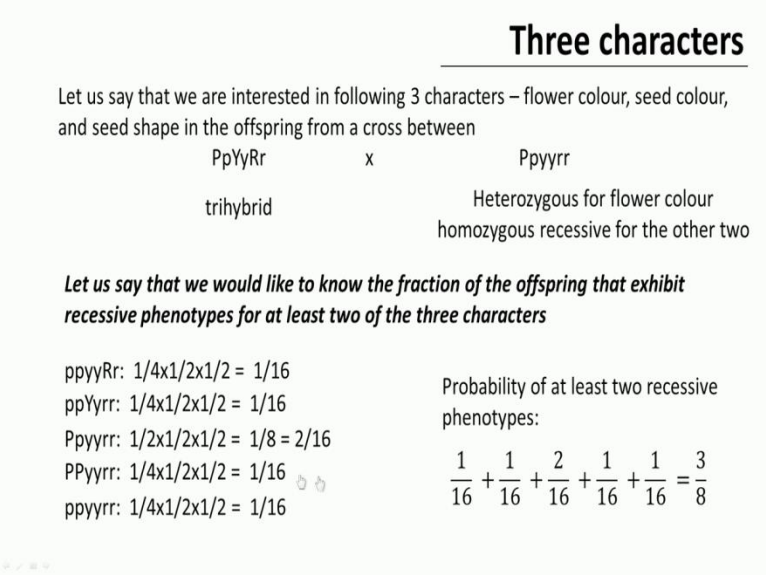


There are three characters here. Let us say that we are interested in the following three characters: flower colour, seed colour and seed shape in the offspring from a cross between PpYyRr and Ppyyrr (this is heterozygous in P, whereas homozygous in Y and R).

And let us say that we would like to know the fraction of the offspring that exhibit recessive phenotypes for atleast two of the three characters. This is what we are interested in. Okay. If you go through the Punnett Square, it is some amount of work, whereas if you know probability, you can quickly write down this, okay? So atleast two of the characters exhibit recessive phenotypes? So taking two at a time. So the various combinations are

ppyyRr = ¼ x ½ x ½ = 1/16

or

ppYyrr = ¼ x ½ x ½ =1/16

or

Ppyyrr = ½ x ½ x ½ =1/8 = 2/16

Or

PPyyrr = ¼ x ½ x ½ = 1/16

Or we could also consider all three being recessive since the questions requires ‘atleast ‘ two characters to be recessive

Thus another combination is

Ppyyrr = ¼ x ½ x ½ = 1/16

So we just have to add all these things together, total probability; so probability of atleast two recessive phenotypes, it is just addition of all these probabilities that turns out to be 6/16 or 3/8. So a lot of probabilities this way are found and they turn out to be useful as we will see in some ways later on when we discuss diseases.

I think this is right time to close this lecture. Here we saw Mendel’s experiments with pea plants, their results and analysis of those results using simple probabilities and the law of independent assortment that Mendel found. When we move forward, we will take things further towards diseases and see the use of ‘Mendelian genetics’ to predict diseases. See you in the next lecture.

**Biology for Engineers and Other Non-Biologists**
**Prof. G. K. Suraishkumar**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Week- 04**
**Lecture - 18**
**Mendelian Genetics: Pedigree Analysis**

Welcome to the next lecture on Mendelian genetics. We have been talking about the use of Mendelian principles to analyse inheritance especially toward prediction of the occurrence of of some inherited disease in an offspring, so that the parents would be able to handle it better.

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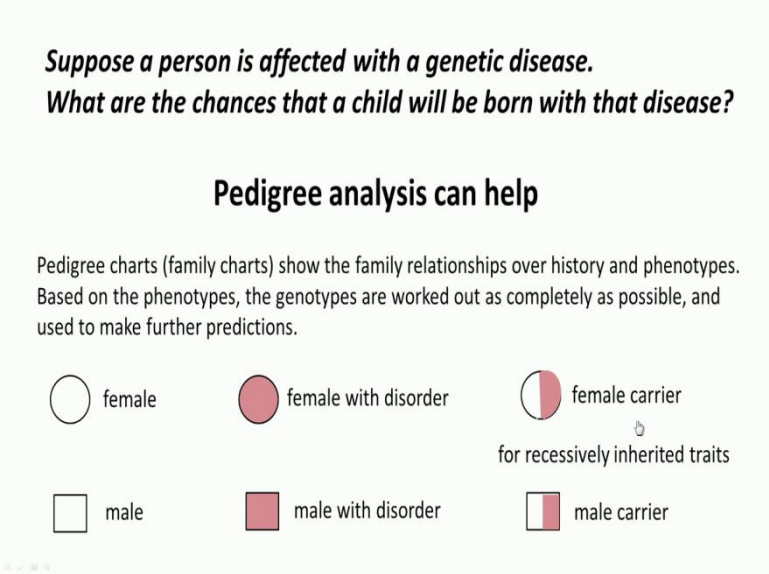


Let us look at a person affected with a genetic disease. Suppose a person is affected with a genetic disease. What are the chances that a child will be born with that disease? That is a question that we are going to ask now, okay? In this lecture this is what we are going to see the various possibilities of that happen. To do this, something called a pedigree analysis can help. Pedigree charts or family charts show the family relationships over history and phenotypes characters, characteristics.

So based on the phenotypes the characters the observable characters, the genotypes are worked out as completely as possible and used to make further predictions, okay? We cannot go and do experiments with humans and therefore we need to use all the information that is available in terms of observable characters and draw our conclusions. Of course if somebody's genotype can be tested, just by taking some part of the saliva or some blood and so on and so forth, that can be done. That is that is acceptable to most and that can be used to help in the analysis.

However in many of these cases it may not be even be possible because maybe the grandparents and the previous generations have passed on and therefore the analysis of the phenotypes and the guess of the genotypes are used to get as complete an information on the genotype as possible and thereby make further predictions. We will see a couple of examples of those.

For that you have a certain terminology, if we have a circle it means a female, if you have a square it means a male. These are standard terminologies for Pedigree analysis. If you have a filled square it means that the female has the disorder, the disorder is apparent; if it is a filled square it is a male with the disorder. If it is a half-filled circle then something called a female carrier, it will become clearer, if the person does not show the disease with (the) person carries a part one allele which can cause the disease.

And this is true for recessively inherited traits, you know, if both need to be small, let us say pp for a disease to manifest, then if it is Pp, then this becomes a carrier, right? The small p is still there, that can be passed on to the next generation although because of the capital P this disease may not manifest in this particular person in the carrier. That is what the carrier means. The disease is not manifest but the person has a potential to pass on the allele to cause the disease in the offspring, or in the offspring's offspring.

And this is a male carrier. So circle is a female, square is a male, if it is filled than the person is showing the disorder, if it is partly filled then the person is a carrier, okay? These 2 can be you know, filled in or these 2 can be decided just by observations and records, whereas this requires a genetic analysis or at least genetic possibilities which we can deduce with surety.

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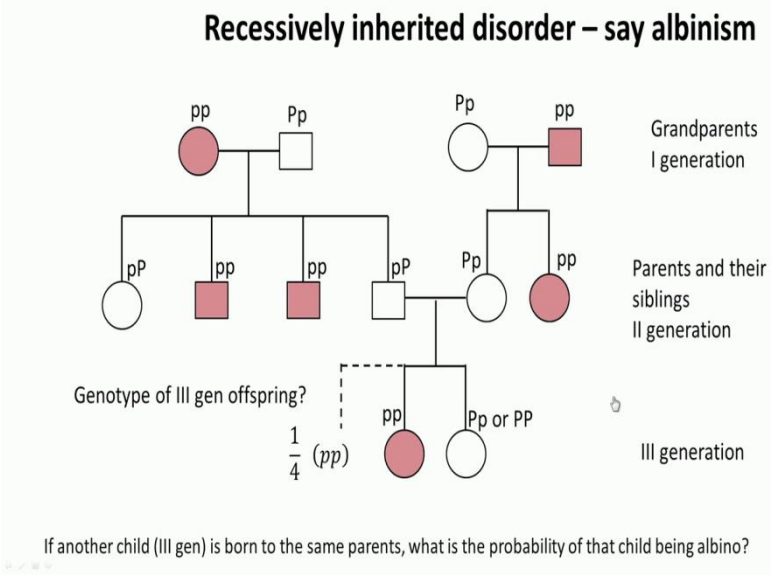


Let us take the case of a recessively inherited disorder, okay, which is what would be natural to think if it is both small then the person has the disease, that is not always the case but let us take that for the time being, that is what one would expect given the background that we have been through.

Albinism, you know the person who has light coloured skin patches or sometimes even the entire body. That is a recessively inherited disorder. Let us say that this is a family that we are considering. A female who has albinism marries a male without albinism and they have children. A female without albinism, a male with albinism, and these 2 marry and they produce 2 children, a female without albinism and a female with albinism, okay?

Then these 2 marry, these are 2 different families, their children, the 2 among their children, each one, a child from each family marries the other and they have children. So far the observations are: one has albinism, is a female with albinism and another is a female without albinism and this is the grandparents generation, the first generation terminology, this is the parents and their siblings, okay, the second generation and this is the third generation that we are currently concerned with. This is the family tree on which we are going to do pedigree analysis.

If we are interested in finding the following, if another child in the third generation, in this generation is born to the same parents, what is the probability of that child being an albino, okay? Here itself by now it is not very surprising, but to see here this person is not affected with albinism, this person is not affected with albinism, whereas the offspring of that those

parents is affected with albinism. That is because it comes from the grandparents, okay? Now that we know Mendelian inheritance, we know how this can happen, the recessive possibility- it is skipped here whereas it is manifested in the third generation.

Let us go to the analysis now. This is the way the various linkages in the family are shown and the various phenotypes are shown according to our code, the generally accepted code. If we look at obvious genotypes, this is a male with albinism therefore it has to be pp, or anyone with albinism has to be pp because it is a recessively inherited disorder.

So let us start here small p small p, small p small p. This one should also be small p small p, that we can fill in for everything. Whereas here it's a female who does not have albinism, okay, whereas this parent has both small p alleles and since one allele comes from each parent, definitely one of the alleles has to be p. And since this person is not showing albinism, other allele has to be capital P. If the other allele had been a small p then this person would have also been affected.

Similarly, this person is from this parent therefore must have inherited a small p, and this, the other allele must have been a capital P from the other parent, okay? With this, and taking a look at this, this father or the grandfather should have had at least one capital P and one small p. Only then these kind of possibilities would exist, right? Otherwise if both had been capital P, then these 2 people would not have had albinism, both the other allele would have been capital P and as long as one allele was capital, then the disease would not have been manifested here. And since it has manifested here and also it has not manifest here, this person must have had capital P, small p, okay? This is the analysis.

Similarly we can do an analysis here. This is small p small p definitely, and this is small p small p. These both are affected and this must have been capital P because this person does not have the disease and therefore it is quite easy to see why this must have also been capital P small p because the person does not have the disease. Now this is heterozygous, this is also heterozygous, it is a cross between heterozygous parents and what is their probability that this person, whether male or female would have the disease.

It would be one fourth the probability you know these are heterozygous cross, therefore PP, Pp, pP and pp are the possibilities, therefore one fourth is the probability that it will be pp, result in the child being albino. We could also ask what would be the genotype of the third generation offspring which is these 2 and quite easy to see it has to be either Pp or PP, both

can arise. We cannot rule that out from just this observation that the person is not an albino. The person could be a carrier, okay? Whereas here it is very clear that it has to be pp, okay?

So this is lot of information that we can gather just by looking at what the various relationships between people and their observed characters. And we have deduced the genotypes, given that albinism is a recessively inherited disorder, okay? Now, some of you might have been wondering why did I keep harping on recessively inherited, okay. You could think how else could the disorder be inherited. It so happens that a disorder could be a dominantly inherited disorder too.

And Huntington's disease which is so prevalent in the US is actually a dominantly inherited disorder which means if one allele is capital then the person has the disorder which also means that most of the population is homozygous recessive, okay? Both are small small, right, only then will the person not have the Huntington's disease. If one of them is capital then the person definitely has the disease, that can also happen. At the same time the dominant allele is not the one that is found the majority of people, okay? This could also happen, we will not get into how it happens and so on so forth. If you are interested you can go and read later chapters of your textbook, it does talk about that.

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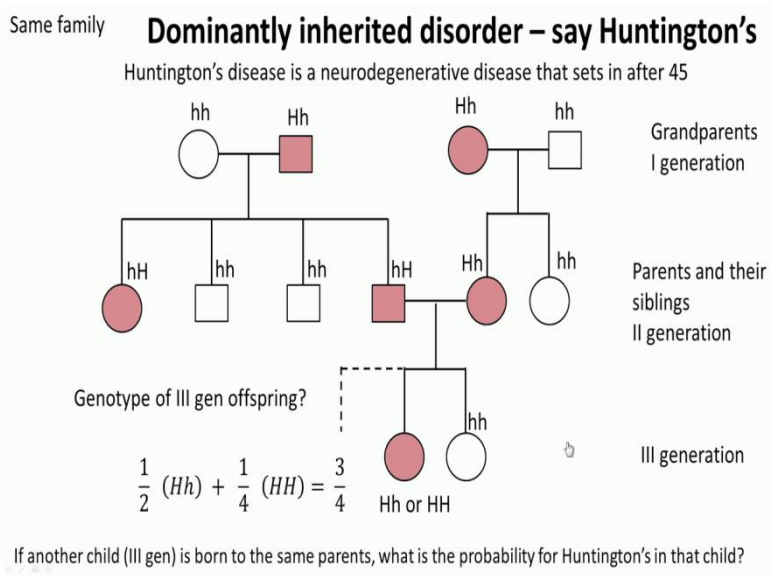


But for now, basic course let us look at what happens if you have a dominantly inherited disorder and let us do a pedigree analysis for the very same family, okay? It is the same family so this is what it was, right? And these things refer to the Huntington's disorder. By the way Huntington's disease or a disorder is a neurodegenerative disease that sets in after 45, okay? So it is sometimes a little difficult for the people and they could actually have their genotype tested to see whether they have Huntington's disease.

If they have Huntington's disease then it is a badly degenerative disease that sets in after 45 and its bad life after that. So it has a lot of ramifications - social and cultural and so on, social essentially and of course health wise and the fact that you could be sitting on a ticking time bomb. So it is one such disorder which is predominantly found in the US, not much in India. Here this shows the family members who have Huntington's disease by a shaded square or a circle, okay, a shaded square is an affected male and the shaded circle is affected female, okay?

Now let us do the analysis here, the same way that we did analysis for a recessively inherited disorder which was albinism, it was an example of that. Same question if another child is born to the same parents what is the probability for Huntington's in that child, okay? This is more serious than albinism, albinism is merely looks and maybe it makes the person prone to skin cancer but it is not as dangerous as this, okay, although it does have a social angle to it.

Very clearly this has to be both smalls for the person not to show the disease, both small here and both small here that can be very easily deduced. Since this person is showing the disease

and this is both recessive, it has to be at least one dominant for the person to show the disease and therefore this has to be hH. This has also got to be hH, same parents, and because this is hH, the only way this can result is if this is Hh. This cannot be HH, otherwise these 2 people would have also had the disease.

Similarly this would be hh for no disease. This one would also hh for no disease. This has to be hH with the disease because this has resulted in one small h coming from here therefore at least one is a small, the other one has to be capital for it to show the disease, therefore it is Hh, and by the same argument this is Hh.

And what is the probability that this person will have Huntington's, you can work at the probabilities here. If it is heterozygous dominant or homozygous dominant the person will show the disease, so it is a very high three fourth or a 75% probability that the child, born to the same parents will have Huntington's disease, Huntington's gene which will develop into a disease at around 45 or so.

And genotype of the third-generation offspring is? If you work out the details here we will not be sure. It would either be this and this or this and this or this and this. Therefore it is either Hh or HH. At least one has to be capital because the person is showing the disease. Okay, these kind of questions can be answered and the parents can be alerted to these possibilities, the people can themselves be alerted to these possibilities.

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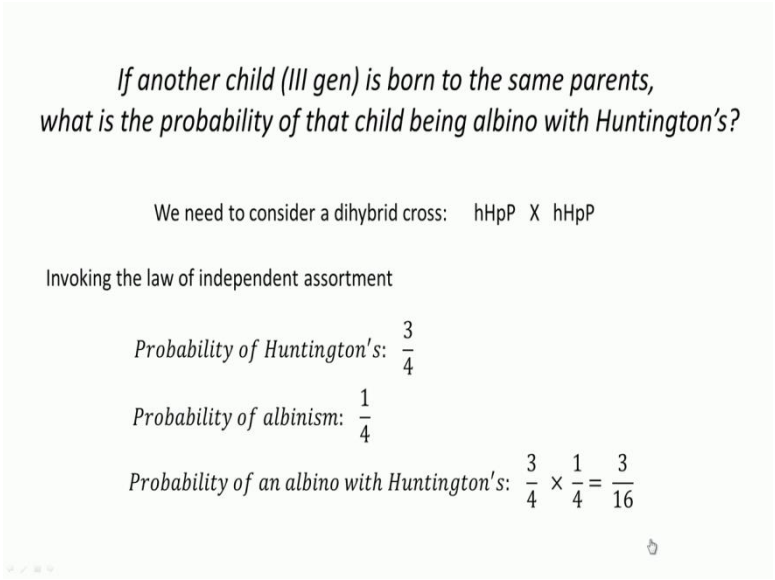


Now let us look at both of them together, okay, and use probabilities here. If another child of the third-generation is born to the same parents, what is the probability of that child being an albino with Huntington's? Okay? Albino is 1 characteristic, Huntington is another character, they are independently inherited according to Mendelian principles and to come up with the probability we need to consider a dihybrid cross in which albino character and Huntington's character are considered. If we do that, invoking law of independent assortment, the probability of Huntington's is three fourth; the probability of albinism is one fourth according to the various combinations here.

Therefore, the probability of an albino with Huntington's, both are independently inherited independent assortment, ¾ x ¼ , multiplication rule, gives 3/16, so this is the probability. So you can work out various different things with these probabilities and the law of independent assortment.

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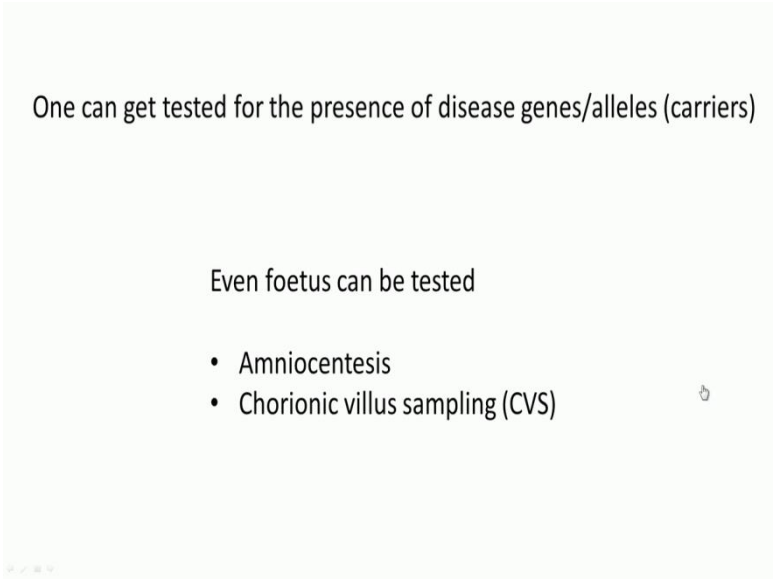


One can get tested for the presence of disease genes and alleles whether you are a carrier or not. If you have the disease you know that your genotype could be a certain way, if you do not have the disease you would like to know whether you are a carrier, whether you are going to pass it on to the next generation, one can do that. Even foetuses can be tested through procedures called amniocentesis or chorionic villus sampling called CVS. These 2 are done, especially for disease kind of a situation and so on, okay?

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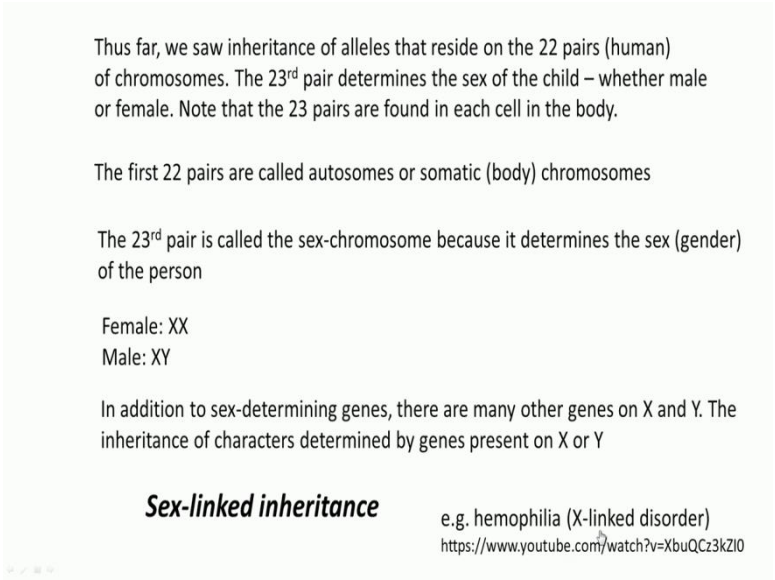


Whatever we have seen so far is valid for a certain kind of inheritance. It is for the inheritance of alleles that reside on the 22 pairs of human chromosomes. The 23rd pair, XY if you recall, determines the sex of the child, okay, whether it is a male or a female. And the 23

pairs are found in each cell in each body, that that you already know. Whatever we have seen so far is valid if the allele sits on the first 22 chromosomes, not the 23rd. If it is, if it sits on the sex chromosomes, then things are going to be different and let us see how the things become different, if the allele sits on the sex chromosome.

The first 22 pairs are actually called autosomes or somatic chromosomes, somatic for body, somatic chromosomes. And the 23rd pair is called the sex chromosome because it determines sex of the person. Now, gender I think in this lecture we would use interchangeably with sex but in actual terms gender has a social connotation and sex has a biological connotation but we could use this, or I could use this interchangeably in this lecture. The female has an XX sex chromosome occurrence and the male has a XY sex chromosome occurrence.

In addition to sex determining genes, there are many other genes that are present on X and Y, the inheritance of characters determined by the genes present in X and Y is what is called sex linked inheritance. If it is sex linked inheritance the probabilities would be different from what we have seen if they had been autosomal inheritance.

An example of sex linked inheritance is haemophilia, haemophilia is inability of the blood to clot and if that happens then the person has a wound then the person continues to bleed. If it is outside, there is a way of handling it but if it happens inside then it can be dangerous, the person can bleed to death for no fault. And that condition is actually called haemophilia.

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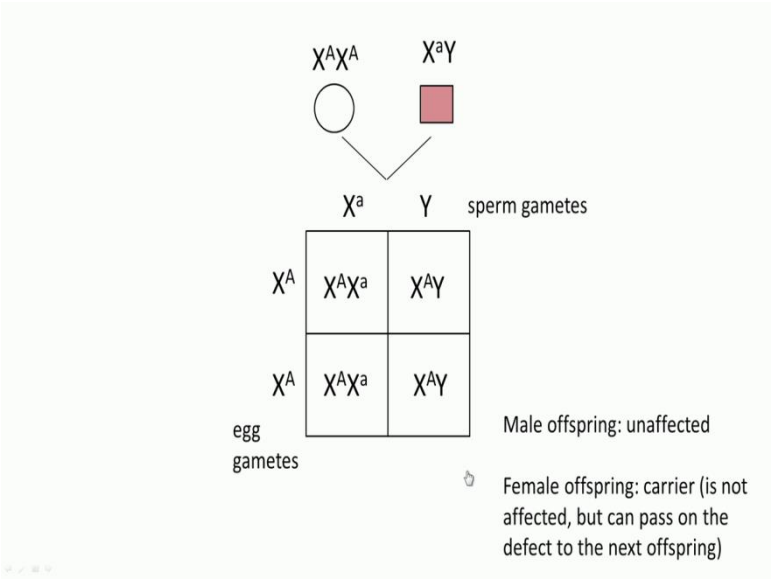


You can take a look at this youtube video, if it is a female it is XX and let us say that the person has A and A genotype there, we are using a superscript to determine the genotype,

that is associated with the X chromosome. Since it is a male, it has to be X and Y, since the male is affected it is a small a. The gametes from a cross between these 2 would result in: the gametes are $X^AX^A$ from the eggs and the sperm gametes are $X^a$ Y. And a Punnett square from this kind of a cross would result in capital A small a, capital A, capital A small a and capital A.

And as you can see the, if there is a Y, the offspring is going to be a male, both these are males and both these have the capital A on their X chromosome so therefore they are unaffected. The female offspring, these 2 they are again unaffected because they have at least one capital A, but they are carriers, they have the other small a, right? Therefore female offspring is a carrier, is not affected but can pass on the defect to the next offspring, this we have already seen, okay, this is the way it happens. The probabilities would be different if you calculate, it is dependent on the sex of the person, sex of the offspring.

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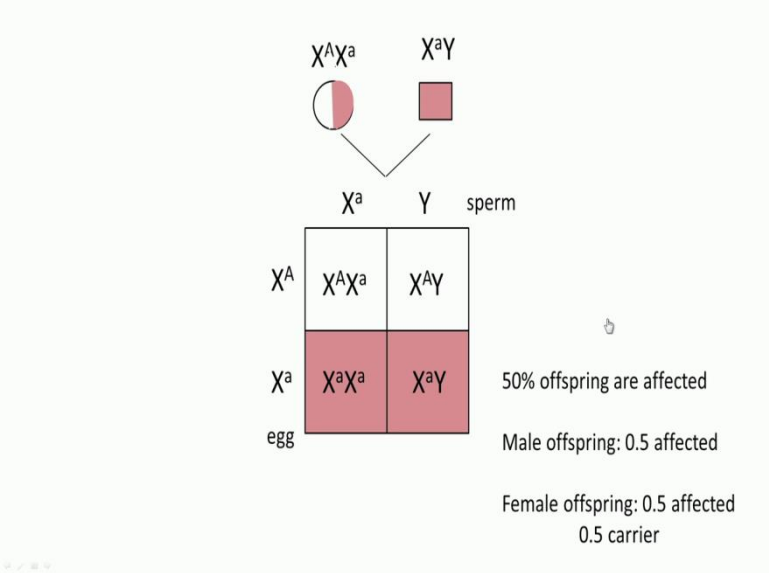


Now let us say, it is a cross between a carrier and an affected male, carrier female and an affected male. If we work out the Punnett square, it is going to be something like this. The one female and one male are going to be affected, okay? Therefore 50% of the offspring are affected this shaded ones are the affected ones, 50% of the offspring are affected. The male offspring this and this are males, male offspring - again 50% affected and female offspring, 50% affected and 50% are carriers.

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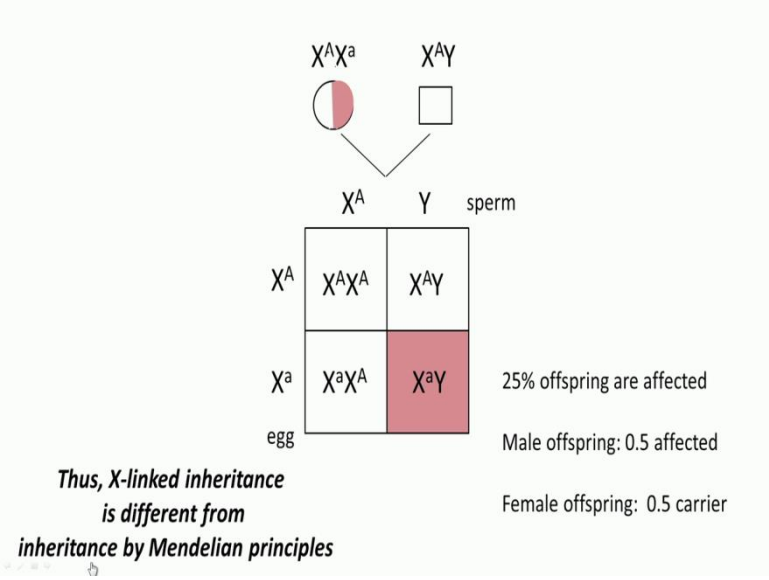


Another example between a carrier female and an affected male; in this case only 25% of the offspring are affected, it happens to be a male because the small a comes here, the Y does not have anything, if you consider the male offspring alone, 50% are affected. If you consider the female offspring alone, no female offspring is affected but 50% are carriers. So if the allele lies on the X chromosome, then the probabilities are going to be different from that we found with autosomal disorders, or the alleles that reside on autosomal chromosomes.

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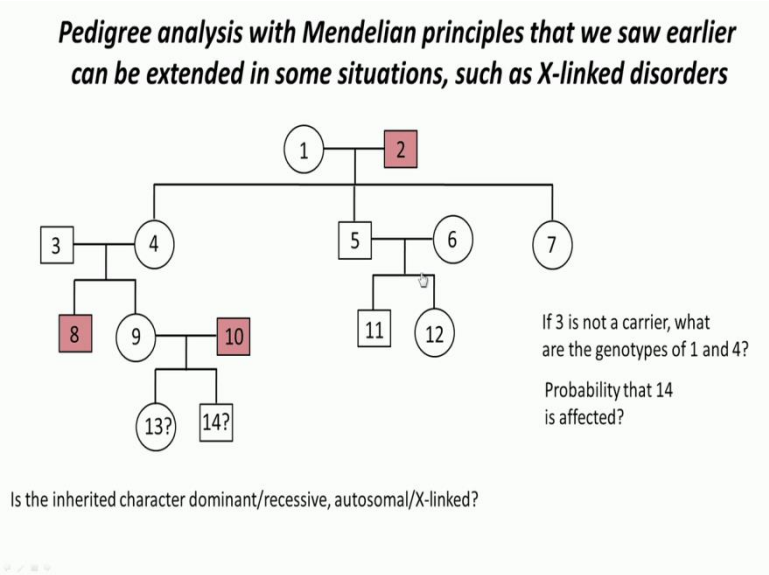


Thus X linked inheritance is different from inheritance by Mendelian principles. Pedigree analysis with Mendelian principles that we saw earlier can be extended in some situations, such as X linked disorders, to a certain extent. This is one such possibility that has been shown here, why don't you work this out with whatever we have learnt in this particular class

and when we begin the next class I will begin by solving this, okay, there are these question marks here, this is what we need to find whether these are affected or not, that is the question. These are various numbers that are given for our reference. The other representations are the normal representations and these 3 are affected.

You would like to find out what kind of a disorder it is. These are the questions; is the inherited character dominant or recessive, autosomal or X linked; that is the first question. If it is given that 3 is not a carrier, what are the genotypes of 1 and 4? That is what you are asked to find. The probability that 14 is affected is what you are asked to find, okay? Why don't you work this out and then when we meet you can check the solution with the solution that I will provide in the next lecture. See you then.

**Biology for Engineers and Other Non-Biologists**
**Prof. G. K. Suraishkumar**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Week- 04**
**Lecture - 19**
**Mendelian Genetics: Non-Mendelian inheritance**

Welcome to the next lecture on Mendelian genetics. This will most likely be the last lecture on Mendelian genetics. We said that many diseases are genetically linked and to usefully analyse such disease inheritance, we could use Mendelian principles. We showed some examples, we looked at some examples and then came to the situation of X linked disorders, the disorders that arise due to alleles that are inherited from X linked chromosomes. We saw how the inheritance patterns could be different from those predicted by Mendelian principles and said that you could work out this example and we would start this lecture by solving this example.

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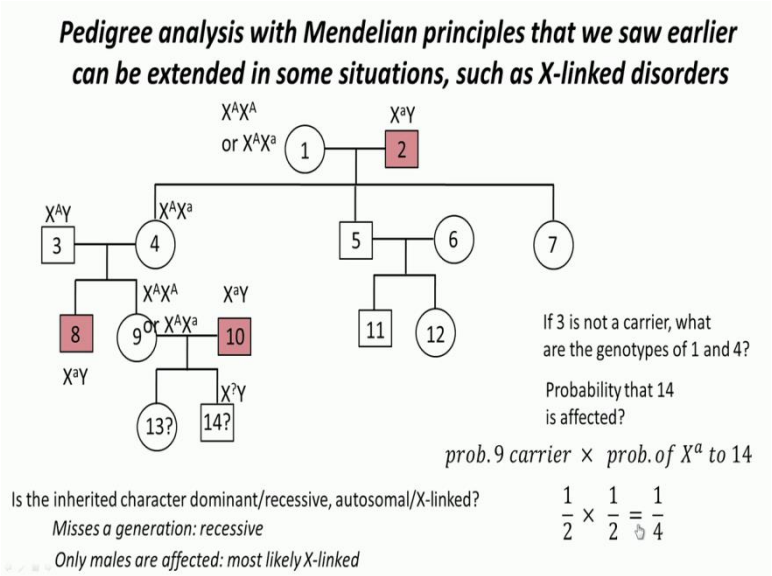


This is a family tree - parents and then the 3 offspring here, they marry and they have children and there is one more generation that is occurring here and after mating here, there are children here and we are trying to answer some questions related to these. The first question was; is the inherited character dominant or recessive, is it autosomal or X linked; that is the first question. The second question was; if 3 is not a carrier, probably the person has genotypic analysis results and we know that the 3 is not a carrier of the disease. If that is a case, what are the genotypes of 1 and 4; that is the second question. The last question was what is the probability that 14 is affected, okay?

So let us look at how to solve this. As you can see this disease misses a generation completely; 4, 5 and 7 who are offspring of 1 and 2, is completely missed here, okay? If it is dominant, then this kind of a missing will not arise because, as long as one of the genes is dominant the disorder will manifest. Therefore it is most likely recessive, right, since it has missed a generation that will happen only if it is a recessively inherited disorder and if you see here only males are affected, 2, 8 and 10 are affected, all are males and that will happen most likely if it is X linked, okay? Therefore it is a recessive disorder and it is most likely X linked.

Now let us try to answer this question; if 3 is not a carrier what are the genotypes of 1 and 4. We know that it is a recessively inherited disorder and most likely X linked so let us work it out. This person has to be $X^AX^A$ or a heterozygous person, $X^AX^a$, the person is not showing, it is recessive therefore even this kind of a genotype will not result in the disorder being manifest. And this is manifested here and it is X linked therefore this has to have a recessive allele on the X for it to be manifest in this male here, okay? So it is $X^aY$ here.

We are interested in 1 or 4. So far on this, 1 could either be this or this. Let us look at 8 here. This is an affected person therefore it has to be X affected male therefore there has to be a Y chromosome and it is X linked therefore the disease allele needs to be carried by X, $X^aY$.

We know that this person is not a carrier therefore $X^AY$, male and this person is a female and not affected since it is a female it has to be XX. Since person is not affected and the father is affected this would be an $X^aX^A$ from the mother, okay, so that is the genotype of 4. So 1, we cannot say anything more than $X^AX^A$ or $X^AX^a$, it could be one of the 2 types whereas 4 is $X^AX^a$. So that is the answer to that question.

Now what is the probability that 14 is affected? 14 is here, therefore we need to look at the parents here, this is a female, the mother is a female therefore its XX and the mother is not affected therefore either both capitals homozygous or capital small heterozygous in both cases the person will not be affected. The father is affected and therefore it has to be $X^aY$ and therefore the probability that this male will be affected is? The Y is coming from the father. And what is the probability that this would be a $X^a$, that is what we are asking.

The probability that this will be an $X^a$ depends on the probability that the mother is a carrier. Therefore it is something like this; probability that the mother is a carrier times the probability that $X^a$ goes to 14 or this has become $X^a$, the person as has inherited the $X^a$ from

the mother, okay? The probability that 9 is a carrier, see for example, if the mother is not a carrier then the mother is $X^AX^A$, then 14 will not be affected at all, because Y comes from the father. The father's Xa does not matter. So the Y will be affected only if the affected allele comes from the mother, this $X^a$. So it is the probability that 9 is a carrier and Xa goes to 14.

Probability that 9 is a carrier is half, okay these are the 2 possibilities and one of which is we are considering and the probability of $X^a$ going to 14 when $X^A$ is also there, is another half and therefore the probability that 14 will be affected, 14 will have genotype $X^aY$ is half into half, that is one fourth. So that is the answer to the question. I am sure many of you have the right answer, you can check this.

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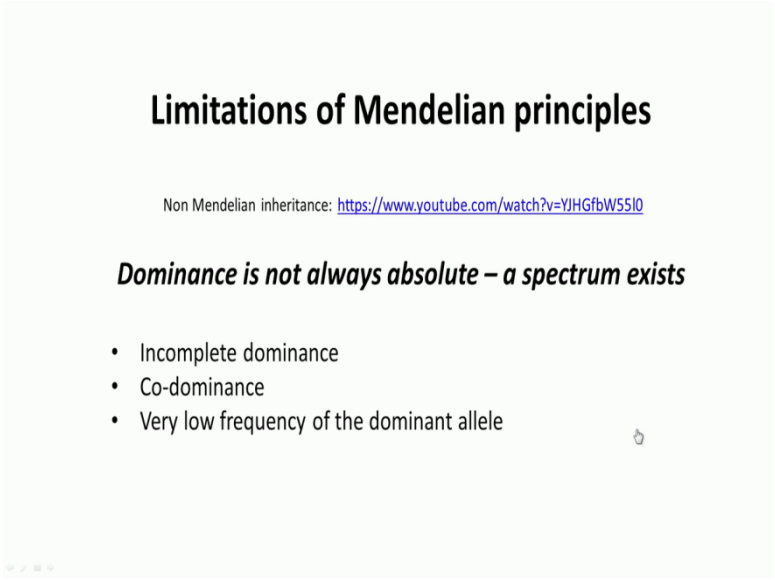


Let us finish up with what we said earlier. The Mendelian principles as we know have limitations. We cannot apply it blindly to everything but we can apply it to large number of diseases that show Mendelian inheritance, okay? That was the use of learning Mendelian principles. There are a lot of non-Mendelian inheritances, please check out the video that is given here. The first non-Mendelian principle that we are going to look at is that dominance is not always, absolute, a spectrum exists. It is not either purple flowers or white flowers, okay? There is something in between also that could arise. It does not happen in the pea plant but it happens in many other things.

That is called incomplete dominance or co-dominance is a variant of that and there could be very low frequency of the dominant allele that we have already seen. This is slightly different

from what we expect. That is the reason why I have put it down as something that is different from expected. It does not go with the other 2. We have already seen this in the case of Huntington's disease, okay? The very low frequency of the dominant allele as long as you have the dominant allele you are going to get the disease, but the presence of the dominant allele itself has a very low frequency in the population, okay? So that is what this means. So this is not always absolute, a spectrum such as this exists from incomplete dominance or co-dominance to complete dominance.

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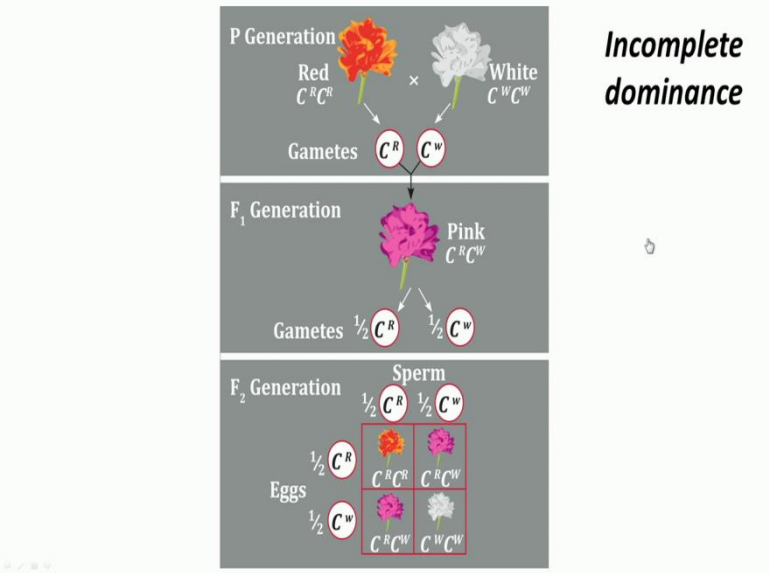


An example of incomplete dominance is something like this in the case of a different plant. The, if you take true breeding red and white flowers and cross them together, the gametes will be capital R and capital W. In the F1 generation, all the flowers would be pink, somewhere in between red and white, okay? So it is neither red nor white, $C^R$, $C^W$ has resulted in a pink flower, no longer a red flower. And this is an example of incomplete dominance. You take this further, you have the gametes from the F1 generation as $C^R$, $C^W$, half and half and then if you do a Punnett square analysis, one fourth would be red, half would be pink and one fourth would be white in the F2 generation. This is an example of incomplete dominance which is different from that predicted from Mendelian principles.

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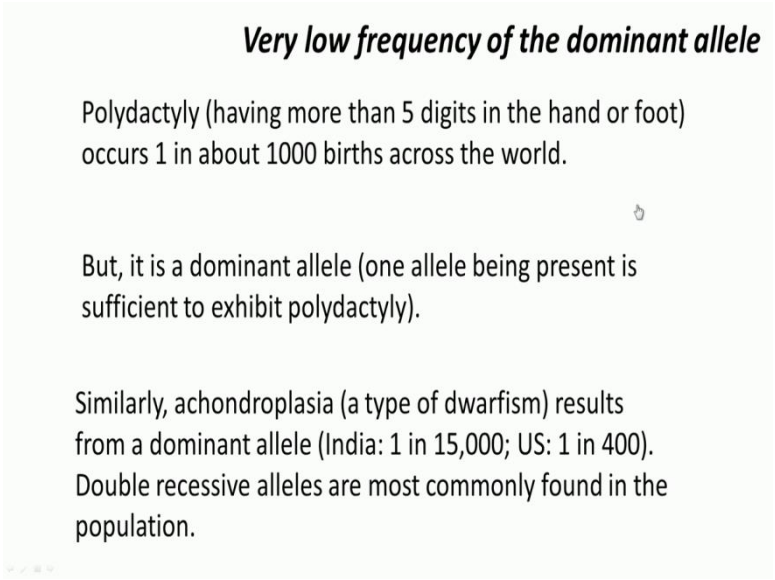


Very low frequency of the dominant allele, we have already seen the example, one more example is polydactyly, more than 5 digits in hand or a foot, okay? It occurs in about 1 in 1000 births across the world. It is the dominant allele, one allele being present is sufficient to exhibit polydactyly, but we know that a majority are not polydactyl, right? So dominance does not always mean a majority. So that is something that we need to keep in mind.

Similarly achondroplasia, a type of dwarfism, results from a dominant allele again. In India, about 1 in 15,000 have this achondroplasia, in the US about 1 in 400 have achondroplasia. And double recessive alleles are most commonly found in the population because if one allele had been dominant, the achondroplasia would have settled whereas we know that only 1 in 15,000, have the disease which means most in the population have recessive alleles, they do not have even one of the dominant alleles. This is the very low frequency of the dominant allele. We have already seen an example of Huntington's disease earlier.

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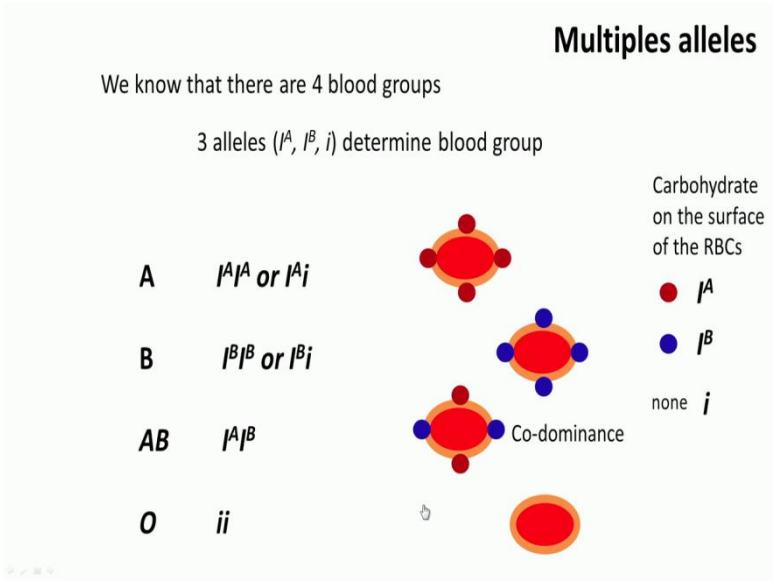


The next non-Mendelian aspect is that, multiple alleles can determine a trait. Okay, not just 2, multiple alleles. For example, it is a very good example that of blood groups, we all know. We know that there are 4 major blood groups, A, B, AB and O, right? And of course Rh positive, Rh negative, we will leave that aside for the time being. We know that there are A, B, AB and O and this is determined by 3 alleles, okay, 3 alleles, capital A, capital B and nothing at all and written i, with a small i. $I^A$, $I^B$ and small i, that we name it that way. These determine the blood group for us.

The A type results, if it is either $I^A$ $I^A$ or $I^A$i, okay? If it is a B group, it is $I^B$ $I^B$ or $I^B$i, and if it is AB, it is $I^A$ $I^B$ both capitals and if it is O, when neither A nor B are present or in other

words, ii. These are the alleles. In terms of what actually happens, this A and B refer to carbohydrates on the surface of RBCs, A and B are different carbohydrates.

And $I^A$ or an A carbohydrate is represented by a maroon circle, a B carbohydrate by a blue circle and if there are no carbohydrates neither A nor B and it is small i. So if this is the red blood cell disc shaped red blood cell, if it has all A carbohydrates being expressed then it is blood group A. If it is all B carbohydrates being expressed, it is blood group B.

If it is both A and B carbohydrates being expressed, it is AB and if none are present it is group O, okay? And this is also an example of what is called co-dominance where both A and B are expressed are shown are showing up simultaneously, okay? That is what is called co-dominance which is again a difference from the Mendelian inheritance.

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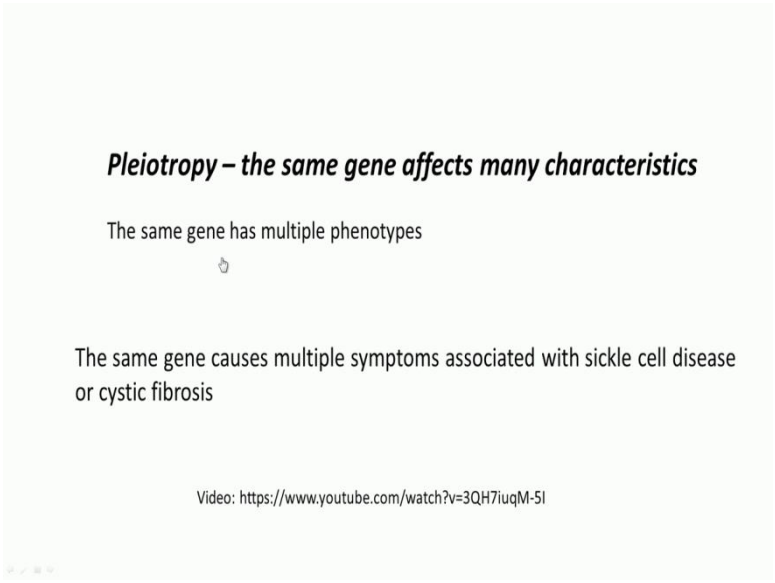


Pleiotropy is again Non-Mendelian. This means that the same gene affects many characters, not just one, it affects multiple phenotypes. An example is from this the same gene causes symptoms associated with sickle cell disease as well as cystic fibrosis, okay? You can look at the video that is given here, the last video that is shown that will give you some more details about Pleiotropy.

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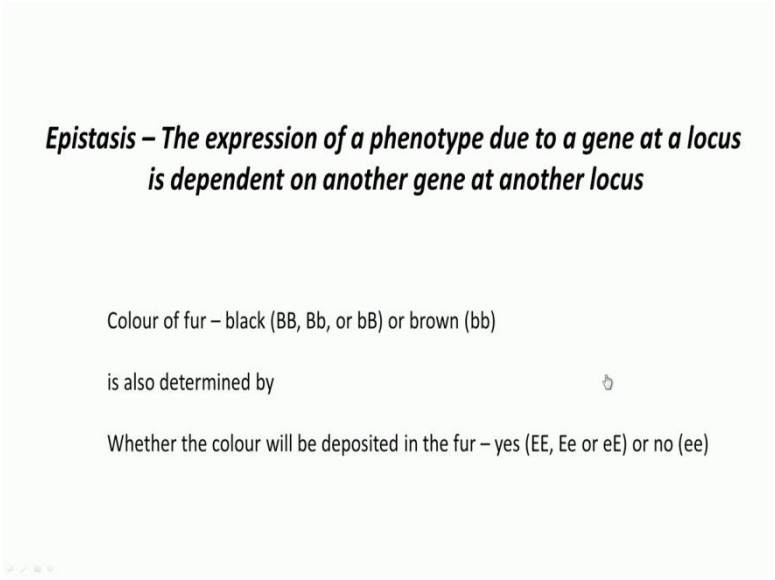


Epistasis arises when the expression of a phenotype due to a gene at a locus is dependent on another gene at another locus, okay? Expression of one is dependent on the other. If that happens then it is called epistasis and again this is clearly non-Mendelian, this happens.

Example is the colour of fur - it could either be black which arises from a dominant B, capital B or brown from a double recessive small b. But, the colour of fur is determined whether the colour itself is expressed or not, okay, whether the colour will be deposited in the fur or not. If it is deposited then again it is dominant, if it is not deposited it would be recessive, double recessive, homozygous recessive. So not only what colour it is, whether the colour will be deposited in the fur, both determine whether the animal turns out to be black, the animal fur turns out to be black or brown or no colour at all, white, maybe.

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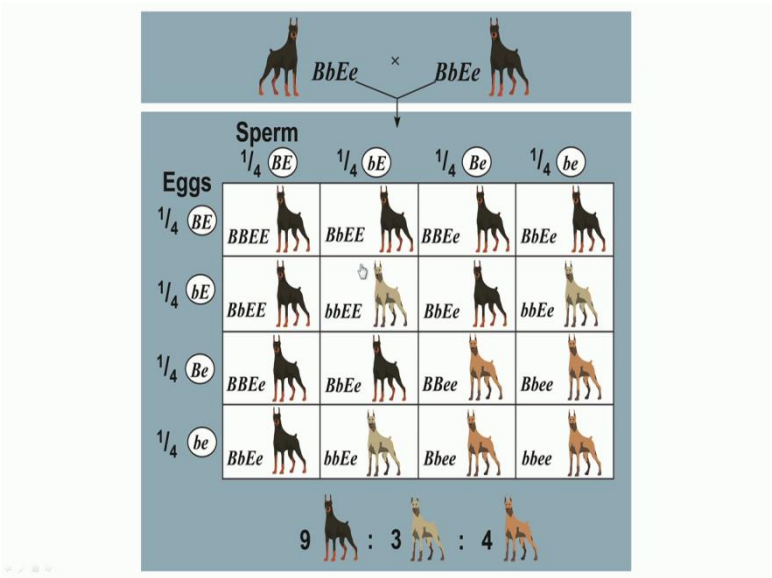


This is an example of such a cross, okay, in other words you could analyse this using a dihybrid kind of cross situation. If you do a dihybrid between colour and expression of that colour, okay, whether the colour is deposited is shown by this E, then this kind of a Punnett square results from these 2 characters for these 2 aspects, or these 2 characters and both those characters together determine whether the colour is seen ultimately in the dog or not.

For example in this case, 9 would have the colour, 3 will not have the colour, 9 would be black, and 3 would not have the colour at all whereas 4 would be brown; the dominant, recessive and no colour at all, right? So this is different from the classic Mendelian Punnett square, for 2 characteristics.

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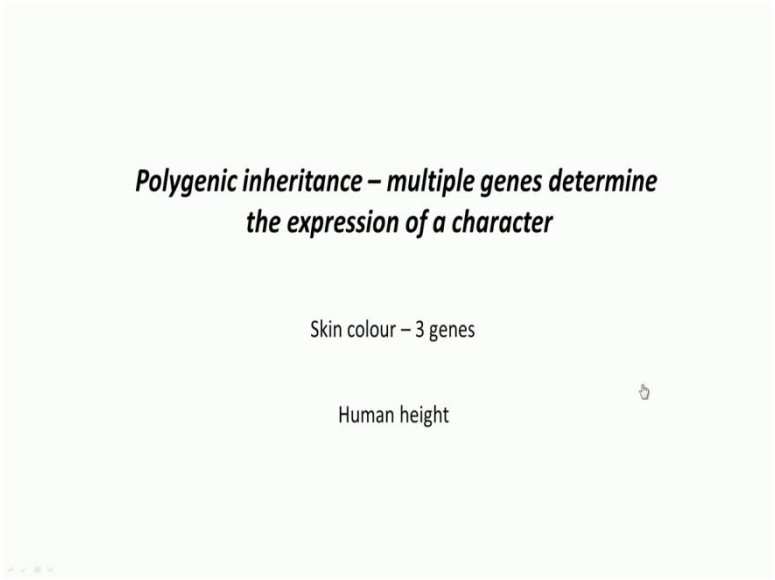


Polygenic inheritance is another variant again from Mendelian inheritance where multiple genes determine the expression of a character. The skin colour is determined by 3 genes. The human height is again a good example of polygenic inheritance where multiple genes determine the height of person. And therefore there is a smooth variation, there is a continuous variation in heights, in skin colour and so on so forth, because multiple genes are determining this, in the case of skin colour it is 3 genes.

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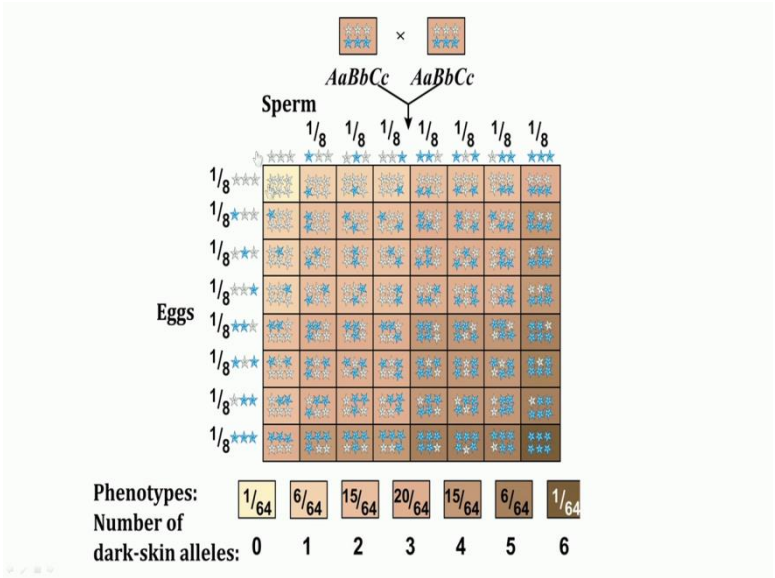


This is a Punnett square it shows a tri-hybrid cross. As you could see at least 7 different possibilities exist, no colour at all when everything is recessive here and then 6/64 of this colour, 15/ 64 of a slightly darker shade, 20/ 64 of a darker shade, 15/64 of a darker shade

and this can be obtained if you go through the various numbers of the blue, bluely shaded stars that are given here, that would directly show the intensity of the colour here and as you go along the intensity increases, 15/ 64 for higher intensity, 6/ 64 even higher and 1/ 64 the darkest, the darkest possible here that arises of all these 6 are of a certain kind, okay? So these are the various phenotypes, the number of dark skin alleles and so on and so forth where 3 different genes determine the skin colour.

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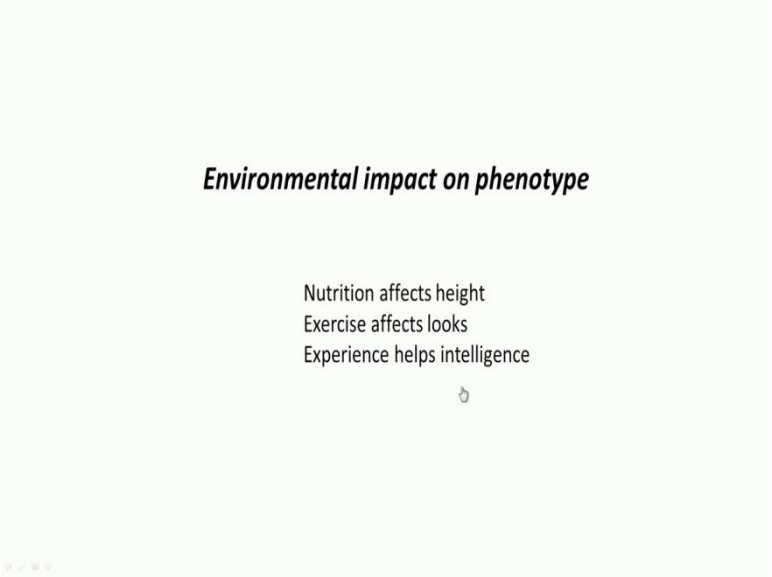


The last thing that we are going to see is that even the environment could have an impact on the phenotype, okay? Not just the gene, the environment in which the gene is expressed could have an impact. For example nutrition affects height. The height could be determined by a number of genes but whether the person grows up to the height potential, depends on the nutrition, right? So the environment is determining the height. The exercise affects the looks, maybe you are born with the looks but you need to exercise to reach the potential in terms of the looks. Experience helps intelligence and so on.

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The pictorial example that I have here is the colour of a hydrangea flower. It could either be this colour or this colour depending on the acidity level of the soil, okay? Nothing else, it is a same gene, same expression and so on and so forth but which colour is expressed is determined by its environment and condition, in this case the soil acidity, okay?

I think that is good enough basal information in terms of whatever we looked at after an introduction, we looked at biomolecules, in terms of stories and so on, just to make sure, it is just not a set of information. We saw that there were 4 major biomolecules, carbohydrates, proteins or amino acids, whichever you want to call it. Then nucleic acids, lipids and each one has their own structure and the structure determines its function and so on and so forth; that was the major take-home lesson from that particular set of lectures on biomolecules.

And we also looked at cell growth; why is it important to quantify cell growth; by cell growth we mean the population growth, the number of cells or the mass of cells increasing, mass of cells per unit volume, number of cells per unit volume increasing with time, okay? How do you go about quantifying that you can do that using the first order relationship and how you could use that to find out the time for operation of a bioreactor, that also we saw?

And then some principles of Mendelian genetics. The Mendelian genetics that we had developed a long time ago, it was given as a part of a basic course because although they were developed a long time ago, they are simple enough to be easily used to predict the occurrence of certain genetic disorders. After that we saw some variations such as X linked

inheritance of disorders and the non-Mendelian inheritance characteristics, very many different kinds of those, okay?

You could look at this in association with what you pick up from Dr Madhulika Dixit's lectures, and that would give you some basis on the molecular aspects of biology which you could apply to your own requirements later, to address your own challenges later. At least you have been exposed to this, you know where to go and pick up more information more specific information as and when the need arises, okay?

One last thing, I would like to leave you with this thought to tell you the level of complexity that we are dealing with even in understanding the cell completely. Once we understand the cell completely then possibly the manipulations aspects can get more rigourous and we have come to a stage where you could manipulate life. But, to give you an idea of the complexity that is involved let me just show you this.

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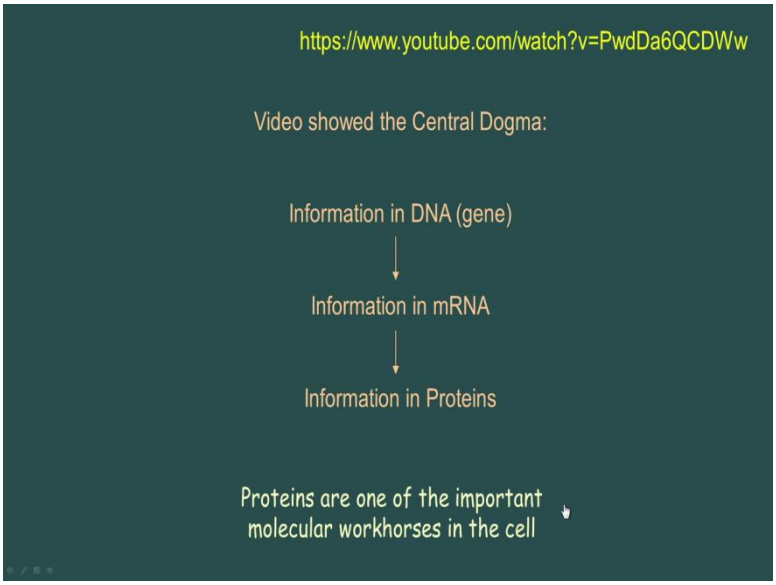


I am sure you watched the video from nature publishing which was on the human genome project, which showed that the information in the DNA is transcribed to the information in the mRNA which gets translated to the information in the proteins, okay? And that is actually called the Central Dogma, as you already know by now. And we are so interested in proteins because they are important molecular workhorses in the cell, they do lot of things in the cell, each biomolecule is very important and there could be more of a focus on proteins because it does so many things that are very apparent, that the basis of things that are very apparent, okay?

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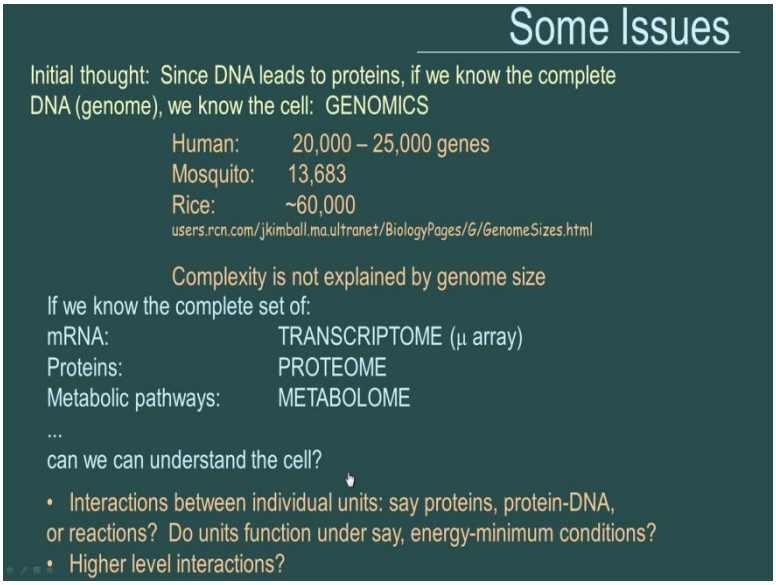


Let us look at some issues. When the human genome project was initiated, the initial thought was, since DNA leads to proteins, if we know the complete DNA or the genome as it is called, we know the cell, okay? The source is Genomics view of the cell. If we know the blueprint we know the cell, okay, that was thinking and that was the motivation behind knowing the entire genome structure of humans which we came to know about 15 years ago.

However we were in for a lot of surprises. Humans had only 20 to 25 thousand genes, okay, that is from this site 20 to 25 thousand genes whereas even a pesky mosquito has about 13,683 genes, okay, not, not far apart from humans as we thought. And not just that rice has 60,000 genes. And there goes the concept of us being superior, right? This has 3 times the number of, approximately 3 times the number of genes that we do, okay, 2 and a half to 3 times, okay? So we can say that, if we assume that we are the most complex I, I am not very sure about that, but if we assume that we are the most complex we can say that the complexity is not explained by genome size, right?

Then people started thinking, if we know the complete set of m RNA, because DNA information goes to mRNA information through transcription, so you know the complete mRNA information which is called the transcriptome which can be done through micro-array analysis and so on, then we know the cell, and if you know the complete set of proteins, the proteome, we know the cell.

If you know the complete set of metabolic reactions that the proteins catalyse through and so on so forth, we know the cell because there are so many interactions that are taking place and

so on. And this can go on and on, okay, the level of interactions between these various kinds, are so many. So when can we understand the cell really? That is the question, you do not really know.

Also, interactions between individual units, say proteins, amongst proteins itself is 1; protein-DNA, okay, or protein-something else or maybe carbohydrate-something else and so on or reactions that occur in the cell. And do these units function under energy minimum conditions - unlikely, right? And are there higher level interactions, ya definitely there are and how do you go about understanding this.

And as we speak, people are working on all these things. Hopefully sometime in the future we will have a better understanding of the cell and therefore a better understanding of life itself. Hope that you enjoyed this course (and) there will be a few more lectures that throw light on some of these aspects such as DNA, the processes related to DNA and so on. Wish you had fun. Bye.

**Biology for Engineers and Other Non-Biologists**
**Prof. Madhulika Dixit**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Week- 04**
**Lecture - 20**
**DNA Replication**

Hello and welcome back to these series of videos on biology for engineers and other non-biologists. Today we are going to talk about a very important process, the process of DNA replication. Now, before I get into the nitty-gritties of exactly how DNA copies itself, it is important to know and refresh our memory about the DNA structure. Now we all know that DNA is a double standard molecule and it is made up of smaller units which are called as the nucleotides. Now each nucleotide itself is made up of a phosphate sugar backbone. So it has a phosphate, a pentose sugar and the nitrogenous base.

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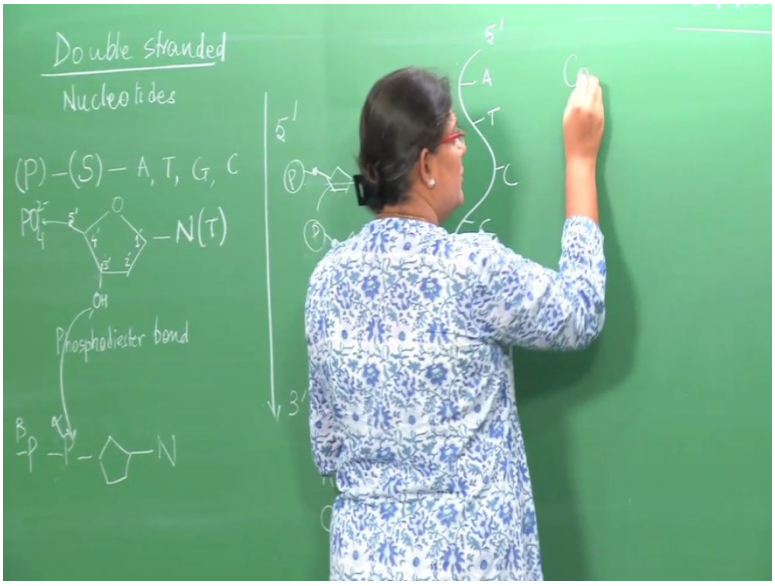


Now this nitrogenous base could be either an adenine or a thymine or a guanine or a cytosine. So each nucleotide basically has a nitrogenous base and this nitrogenous base is attached to a pentose sugar. And the fifth carbon of the pentose sugar in turn is attached to the phosphate group. Now we know that each nucleotide, so let us say this is nucleotide 1; now this nucleotide 1 in its pentose sugar has the first, the second, the third, the fourth and the fifth carbon.

Now we know that every time this nucleotide has to interact with the next incoming nucleotide, it is the hydroxyl group present in the third carbon of the pentose sugar which is

deoxyribose in this case, has to interact; this hydroxyl group at the third, 3 prime carbon, interacts and forms of phosphodiester bond, with the next incoming nucleotide.

So if you have let us say a next, let us say this nucleotide was anything; let us say it was a T. And then it has to form phosphodiester bond with the next incoming nucleotide; this 3 prime hydroxyl in the pentose sugar will form the phosphodiester bond with the next incoming nucleotide which again will have its own pentose sugar, will have its own nitrogenous base and will have alpha, beta and gamma phosphates. So this 3 prime hydroxyl is then going to form the phosphodiester bond, will have a nucleophilic attack on this, on the first alpha phosphate and hence you get the first phosphodiester bond formed.

So what do you get? You get the first nucleotide, with its phosphate, with its sugar and with its nitrogenous base let us say N1, and this forms, this third carbon forms again a bond with the next incoming nucleotide, let us say N2. Now what you notice is that it is always the 5 prime, the phosphate which is attached to the 5 prime carbon, so you will have another carbon here and then this.

Similarly in this case this will be the fifth carbon attached to the pentose ring. So you always find at, this is the third carbon of the sugar which is forming the phosphodiester bond with the phosphate which is attached to the fifth carbon. That is why you find that the Strand or the polymerisation, building up of more and more nucleotides for DNA happens from a 5 prime to a 3 prime direction.

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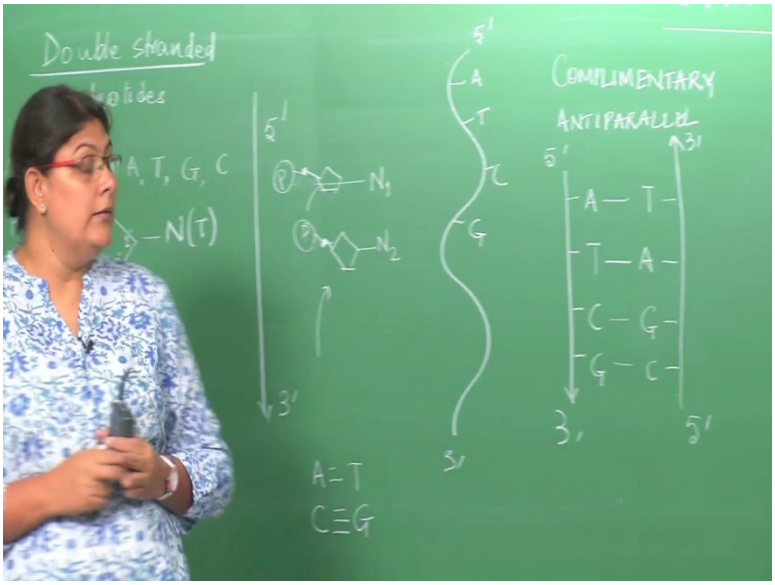


So before I get into DNA replication, there are few things which is very important to know. The first one, as I told you that DNA is a double stranded structure, it is a polymer of nucleotides, each incoming nucleotide attaches to the previous nucleotide through a phosphodiester bond and this incoming always happens at the 3 prime end of the given Strand. So this is one thing that I want you to remember and I will come back to this when we are talking about the mechanism of DNA replication.

The other thing which you have to remember is that when you look at a DNA double helix; let us say this is one strand which is going 5 prime to 3 prime, the other strand is going to be complementary to it. Now what do you mean by complimentary, we have already studied that always the adenine base will form a hydrogen bond with the thymine group while the cytosines will always form hydrogen bonds with the guanine. So every time in this strand, 5 prime to 3 prime, you let's say have an adenine, a thymine, a cytosine and a guanine, what will happen is the second strand will be complementary to it.

So the DNA structure is also, the 2 strands are complimentary. So if this is the first strand with a certain sequence going 5 prime to 3 prime, the second strand will be complimentary to it but will also be antiparallel. So let us, for simplicity sake, let us say this is one strand which is going 5 prime to 3 prime with this sequence, A, T, C and G, then the complementary strand at this position will have a C, at this position will have a G, at this position will have an A and here it will have a T.

And the sugar phosphate bone which holds it together will be going in the opposite direction, 5 prime to 3 prime. So this is, these are 2 major features I want you to keep in mind, one that the 2 strands of DNA are complimentary, so in a sense if you know the sequence of one strand, that one strand knows and has information as to what all would be the sequence of nucleotides in the second strand.

So that information is kind of coded already in the DNA, thanks to the ability of base pairing. The second thing is that the 2 strands of DNA are antiparallel. And how do you decide the directionality as I just mentioned through the formation of phosphodiester bonds that it is in the direction of 5 prime to 3 prime because every incoming nucleotide will form a phosphodiester bond with the 3 prime hydroxyl of the previously sitting nucleotide. So the polymerisation, adding in of successive nucleotides is happening in a 5 prime to 3 prime direction.

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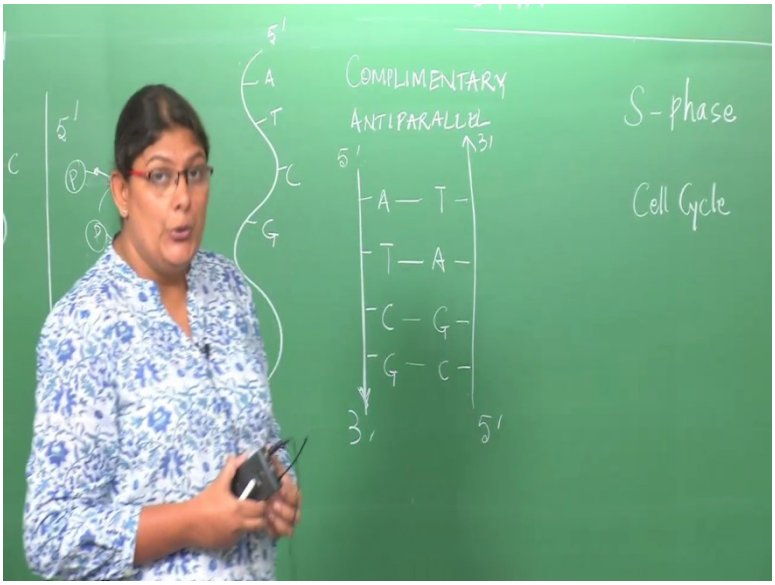


Now this is some of the important basics about DNA structure and the relevance of this will become more apparent when we start looking at exactly how DNA replication happens. Now, you may ask me what is DNA replication. Well, DNA replication is nothing but a process in which a DNA will copy itself and as we had seen in cell cycle this process wherein a DNA copies itself happens in case of eukaryotic cells during the stage of S-phase in cell cycle.

Remember I told you that in cell cycle a cell prepares itself to pass on the information to the daughter cells and the way it does that is that it first duplicates its DNA before actually dividing and undergoing the process of cell division. So that is what we are talking today, we

are talking today exactly how a DNA is able to copy itself and pass on the same information to the daughter DNA molecule.

So let us look at what happens during DNA replication. Now, I will, want you to remember again I am reiterating 2 things, antiparallel strands, complementarity and formation of phosphodiester bonds from 5 prime to 3 prime, or the incoming of the newer nucleotide and formation of a phosphodiester bonds and hence are directionality in 5 prime to 3 prime.

Now, let us look at the DNA replication itself. Now DNA as I had mentioned earlier also, does not exist as a linear molecule in our cells, it kind of remains in a condensed form because of its interaction with histones. And around the time that the cell is getting ready to replicate, it loosens up and it exists as a chromatin. Now it is important to note that DNA is a helix. It is an alpha helix, it is a twisted helical structure and; so let me draw DNA molecule.

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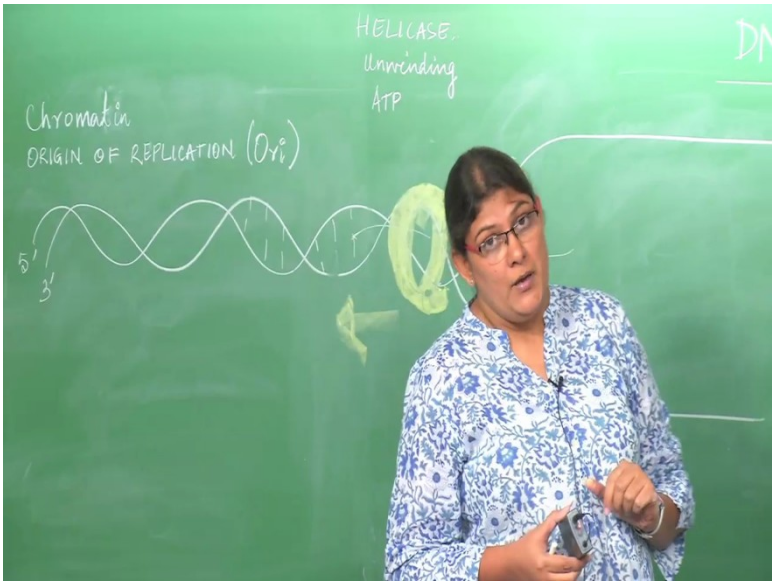


Let us say this is a DNA molecule, this is the first strand going 5 prime to let us say 3 prime. And then you have a second strand which is complementary to it, where this becomes the 5 prime end and this becomes the 3 prime end. Now it is important if the DNA has to replicate, it has to first unwind. And this unwinding has its consequences and imagine it like this, you have a nylon rope and you are trying to pull apart the 2 strands of a nylon rope. Now what will happen is, every time you are trying to pull apart the 2 strands of the nylon rope, they will try to recoil back. Now that is a tendency.

Now the same thing, and same problem one encounters when there is going to be the separation of the 2 strands and how is it that the cell makes sure that once the strands have

segregated and separated, they are prevented from recoiling back till the process of DNA replication is over. Now that is one thing to ponder.

So let us look at how DNA replication happens. Now, the human DNA as I told you is really big. Now what I am going to talk today about DNA replication is going to hold true, what I am going to cover is just the basic features of DNA replication and just give you the common features. They are slightly different in prokaryotes than in eukaryotes, we will talk about that later, but right now we are just looking at exactly how DNA copies itself.

Now DNA has these signature sequences on its arrangement of nucleotides, which act as recognition for a point where the replication has to start. Now these regions where the DNA replication starts is called as origin of replication, okay? In bacteria it is called as the Ori sequence. So there are specific regions on the DNA double strand which will direct the entire machinery of the DNA replication to that point telling them, that this is where the DNA replication should start. So that region where the replication actually starts is called as the origin of replication.

Now let us come back to this DNA strand. So we have these 2 strands and the unwinding has to happen. So this strand has to segregate. So it is like you imagine that this is your double helix DNA and then it has to open up. So it is like you have a zip and then you have to unzip this entire molecule of DNA. And then this is the next strand. Now this opening of the 2 helices, somewhere here will be the origin of replication and this is where the replication has to start, the first thing that has to happen is the 2 strands have to unwind. And this unwinding happens with the help of a particular enzyme, So it is like a clamp like enzyme.

And this enzyme is called as Helicase. So what this enzyme really does is nothing but, since the 2 strands are held together the complementary strands are held together through hydrogen bonds, it basically breaks these hydrogen bonds, so it starts moving inwards, so it would have first bound here, starts moving inwards and it starts breaking the hydrogen bonds and as it moves in this direction it keeps unwinding, keeps unzipping. So it is like the clamp on the zipper which is just unzipping the 2 strands which are held together through hydrogen bonds.

So the helicase is the first enzyme which comes in and it causes the unwinding of the 2 DNA strands. Obviously this process is not going to happen spontaneously, it does require some sort of energy and this energy comes from ATP. I mentioned to you during cell cycle that before a cell commits itself to cell division and cell replication it ensures that sufficient

energy is there and now you can appreciate why because this process of unwinding is a pretty energy intensive process and it does require expenditure of ATP, so helicase is the first enzyme which will come and open up the 2 strands of DNA.

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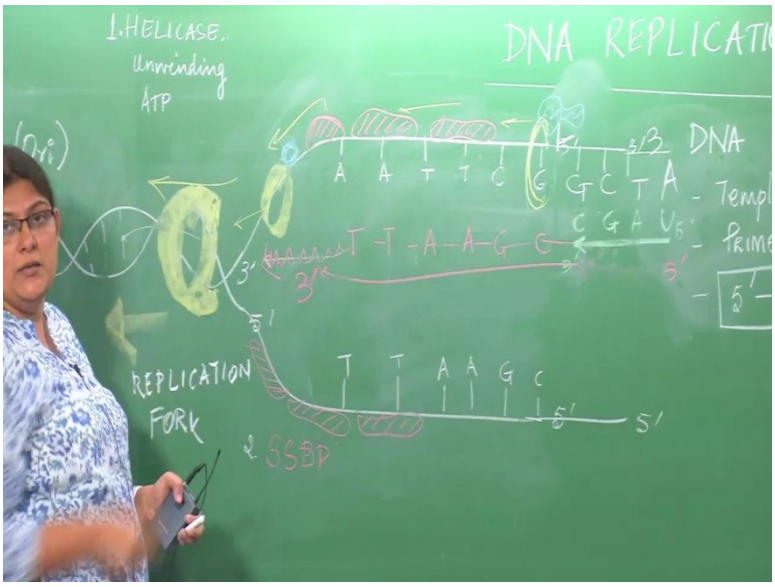


Now let us look at what will happen. Now as I mentioned the strands will try to come back together and that should not happen because the DNA has still not finished its job of replicating it and the way DNA prevents this is by a clause of another proteins which as soon as the unwinding has happened bind to these strands. These proteins will bind on either of the 2 strands, both the 2 strands actually, and it will prevent it not only from binding to each other; if the DNA the separated strand tries to recoil with itself, it will prevent that also.

Now these proteins which prevent that are called as single strand binding proteins. So this SSBPs will now keep the separated strands separated. That is fine. But how does a new DNA come into existence? Now that happens because, so let me before I get there let me again tell you the there is origin of replication that the process of replication will start; you have the first enzyme which comes in and which helps you in unwinding the DNA which is the helicase. Once the DNA has been unwound the 2 separated strands remain separated thanks to the second class of proteins which are called as single strand binding proteins.

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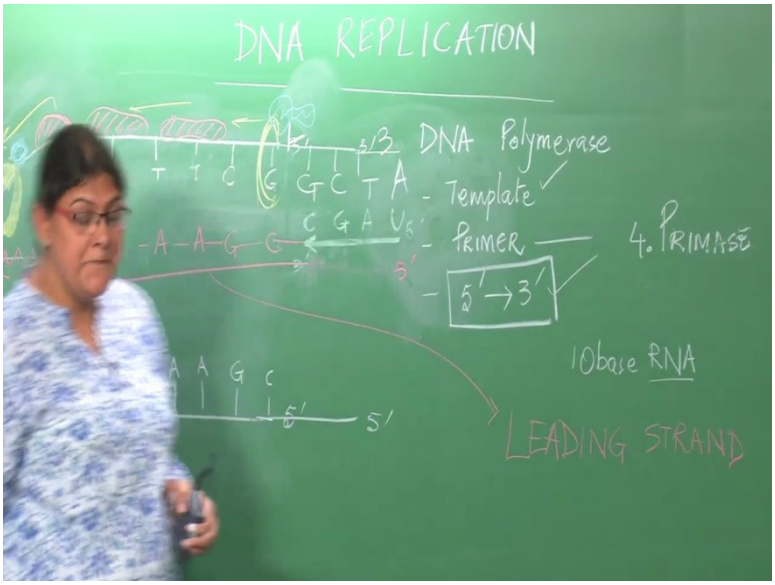


Now once that has happened the there has to be a enzyme which will then come and, you know, meticulously will start bringing in 1 nucleotide at a time and make a second strand. So let us take this separated strand and let us let us give it some sequence. Let us say it has a sequence of an A, another A, a T, another T, say a C and a G. Now since this strand was complementary to this strand, it should exactly have the complementary information, which is it should have had a C here, a G here, right, and A here, another A here, a T here and a T here.

So, now this separated strand has to give a new copy, it cannot go back and reanneal with its partner which was there earlier, it has to give rise to new strand. Now this polymerisation, this bringing in of new nucleotides as per the sequences just dictated by this strand is done by an enzyme called as DNA polymerase. Now there are different types of DNA polymerases both in prokaryotes and eukaryotes, I am not getting into details of those, I want to keep this class very simple.

So these DNA polymerases will bring in 1 nucleotide and attach it to the next nucleotide in the 3 prime end. So what happens is this DNA polymerase needs 2 things, first and the foremost it needs something to guide it, something to tell it that after this nucleotide this nucleotide has to come. So it needs what we call as a template. So this separated strand acts like a template for DNA polymerase, the first requirement is a template.

The second requirement is the more bigger of a deal because it is this stringency of DNA polymerase which will create some problems for us and we will see how. The DNA

polymerase de novo cannot start making, so if the new strand which has to come here has to get synthesised in 5 prime to 3 prime direction. Remember there is a formation of phosphodiester bond and this happens because the incoming nucleotide, which is, has a phosphate at its fifth carbon of the sugar has to form a phosphodiester bond with an existing nucleotide having a 3 prime hydroxyl.

So it has to make a chain in this 5 prime to 3 prime direction. The problem is, polymerase on its own cannot start making a new strand. It needs, one it needs a template to tell how to put in and in what sequence to put in the nucleotides, the second is it requires a primer. So it needs something to go and tell it that from there you need to start adding the nucleotides. So it has to have some sort of an indicator which will tell us that from here you need to start adding nucleotides in a certain direction.

And DNA polymerase always adds nucleotides in a 5 prime to 3 a prime direction. So basically this is a requirement of a DNA polymerase. So when we have separated the 2 strands, the separated strand acts as a template, so this requirement has been taken care of, it will polymerize only in the direction of 5 prime to 3 prime; that also we know it happens. But how will it decide where to start adding? Now this requirement of where to start adding these nucleotides one at a time, is brought about by another enzyme which is called as the primase.

Now primase is a very interesting enzyme. It kinds of tags along every time with a helicase, and as the unwinding happens, this the, let us say, earlier the helicase was sitting here and then started moving in this direction, reached here, now it has continued to move like that. Now, as it started unwinding the helicase, the primase kind of piggybacks itself along with the helicase, and as and when there is unwinding happening, it can immediately form a primer.

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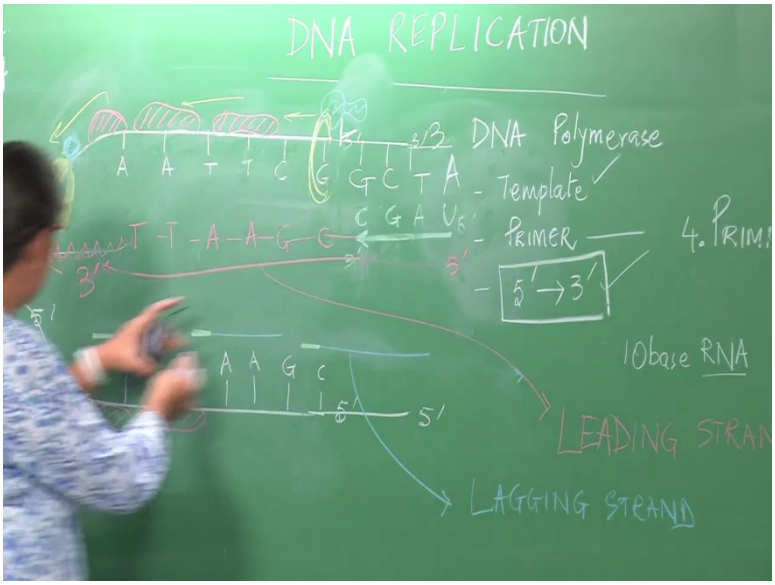


Now primer is nothing but about 10 bases long RNA sequence, so this is like an RNA polymerase. So what it does is that if you have this as a sequence, it will form, let me continue this, this is 3 prime, this continues as 5 prime. There are let us say another set, set of bases here, G, C, T, so on. So what it does is, reads this, immediately synthesises a small RNA molecule. T will always pair with an A, C will pair with a G, G will pair with a C and if it is an RNA molecule and let us say there was an A here; now RNA will not give thymine it will give a uracil. So it synthesises this small stretch of RNA as a starting point.

So this primer now acts as the base on which the DNA polymerase can start adding the nucleotides. Now this is formed by the enzyme primase and once the RNA primer is there DNA polymerase, so this is 5 prime, so the 3 prime hydroxyl here is free, right, of the pentose sugar. So this will now form of phosphodiester bond with a new nucleotide which is coming in and what will decide which nucleotide will come in; that is decided by the sequences on the template.

So this is how the polymerase will start, so let us give another colour, so let us say it starts acting so it will put a C here, we will put a G here, it is an A here and A again. Now this is DNA, this is not RNA. So this will put a thymine here and a thymine here. And this process will keep on continuing. So what happens here is all that DNA polymerase needs is somewhere to start. And it is the primer which gives the direction that start adding nucleotides from here and that is exactly what happens. Once the primer is there in vicinity the DNA polymerase hops along and moves, and it started to polymerise in the direction of 5 prime to 3 prime.

Now, what you notice here is 2 things, one; as soon as the 2 strands have opened and they have got separated you have a formation of a structure which has taken place and this structure thanks to the uncoiling by the helicase is called as the replication fork. So this is a replication fork. Now in that replication fork for one of the strands, the 2 strands remain separated thanks to the single strand binding proteins, once they remain separated for one of the strands, what happens is you start having a quick polymerisation. As soon as the primer has been put in, thanks to the primase you find that the DNA polymerase continuously starts adding the nucleotides because DNA polymerase works only in the direction of a 5 prime to 3 prime direction.

Now this newly synthesised strand which is pink in colour, this one, right, till here it is a RNA and then it continues as a pink strand; it keeps on continuously moving, so as the helicase keeps on unwinding this particular strand is continuously getting synthesised because DNA polymerase works in the 5 prime to 3 prime direction, and one primer is enough to keep the extension going. This strand which is continuously synthesised is called as the leading strand.

Everything is fine so far so good. But then the problem comes for this one. Now here the 5 prime to 3 prime direction is going this way. This strand has a 5 prime end here and a 3 prime end here. And if the DNA polymerase has to work, the DNA polymerase will work in the complimentary fashion 5 prime to 3 prime. But there is another problem it encounters because the helicase is moving faster, keeps on unwinding and as it keeps on unwinding the piggy backed primase keeps on adding primers. So in this strand what has happened is for this particular complementary strand the DNA polymerase cannot, at a stretch, synthesize the new nucleotides; it has to keep on adding these things in stretches.

So let us say, the DNA polymerase started polymerising this thing, started adding the bases in the 5 prime to 3 prime direction, by the time it finished it, this region further uncoiled and when a new primer was formed, so the DNA polymer has to come back and jump and then synthesise again. Then this uncoiled further, again there was a primer formed here and then it again had to extend it in a 5 prime to 3 prime direction. So what you find is that when you have this short stretches synthesised, the second strand is not getting synthesised in a continuous manner. Though it is getting synthesised in a 5 prime to 3 prime direction it is not happening in a continuous manner. And this, what happens for this one is that you end up having small small stretches of new strands synthesised.

Every time you have a RNA primer,RNA primer and a RNA primer. So this strand is a little is slower than the first strand and it is called as the lagging strand. And this process keeps on continuing. And then once this has been formed, what we'll now have is let us say, this is how I am just redrawing this with much simpler form, so you have this DNA molecule, and then you had this one as the 3 prime end, this point give you the 5 prime and then this was a 3 prime and then this was the 5 prime. So what is happening now is that you are getting a new molecule synthesised, with the leading strand which is moving towards the replication fork and a lagging strand which is getting synthesised in bits and pieces.

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Now each of these tiny pieces of the lagging strand are called as the Okazaki fragments. Now, so what has happened, this one molecule is now giving rise to 2 molecules and these Okazaki fragments have to be later rejoined; not only are they supposed to be rejoined, whatever RNA which was sitting, there was primer which was sitting here, there was one primer which were sitting here, you have primers here, primers here, primer here. All these primers have to be removed and have to be replaced with a DNA sequence, because in the end it is about copying the DNA and not DNA-RNA molecule.

So what happens is the primers get removed after the Okazaki fragments have been formed by another type of DNA polymerase. That is why I told you there are different kinds of DNA polymerases; I am not going into all of them, this is a different DNA polymerase. This DNA polymerase will re-synthesise the missing pieces which were originally occupied by the RNA and then for the lagging strand once these have been synthesised, these pieces have to be

stitched together, because it has to be a continuous stretch and that stitching happens by the one of the another crucial enzymes called as DNA ligase.

So what do you notice here is, you started with a double helix, the helix got uncoiled, thanks to the helicase and the 2 separated strands were maintained in a separate position because of the single strand binding proteins. Not only that, as soon as the uncoiling happened you had the primase come in; it started putting in small stretches of RNA which you call as the primer. You need this primer because DNA polymerase has to then build upon it, also RNA primer kind of acts as a foundation for this DNA polymerase to then keep on adding the nucleotides in a 5 prime to 3 prime direction.

Of the 2 separated strands, one strand just grows continuously so that is called as the leading strand but since here the uncoiling is happening at this end and the DNA polymerase only works in 5 prime to 3 prime, for the other strand the DNA polymerase adds these stretches in small pieces, which is what you call as the Okazaki fragments. The Okazaki fragments are finally stitched together as a single strand by the DNA ligase while the RNA sequences are removed by another DNA polymerase. So this is how the DNA replication happens and then finally once it has copied itself the DNA polymerase will reach a point the way you have origin of replication, you will have another signature sequence sitting on the DNA which is called as the terminator, a terminator sequence in case of prokaryotes, in case of eukaryotes you have regions which are called as telomere.

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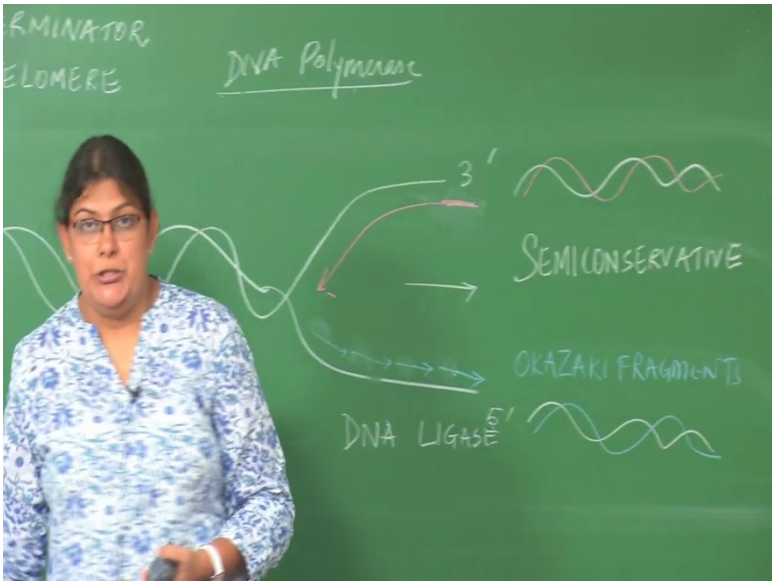


So this is how DNA replication takes place. Now you notice at the end of this entire process, once this DNA replication has happened what you end up getting are 2 daughter DNA molecules, this one formed because of a leading strand, right, and this one formed because of the lagging strand. But what you notice is in each new daughter DNA molecule one strand is the parental strand which came from the original DNA while one strand is the newly synthesised strand. The same thing happens in this molecule, one strand is from the parental DNA, the other strand is the newly synthesised strand.

So such mode of DNA replication, where it still has the original parental strand, one of it and one of this is newly synthesised because of complementarity is called as semi-conservative mode of replication. So with that we have covered how DNA replicates itself. Before I wind up, I just want to highlight few points.

The 2 strands of DNA are antiparallel, the base pairing provides the complementarity between the 2 strands and then you have a whole array, array of enzymes which are required for uncoiling of the DNA, for polymerisation of DNA. The DNA polymerisation always happens in the direction of 5 prime to 3 prime. And that is simply because of the chemistry by which the sugar phosphate bond in DNA is actually formed.

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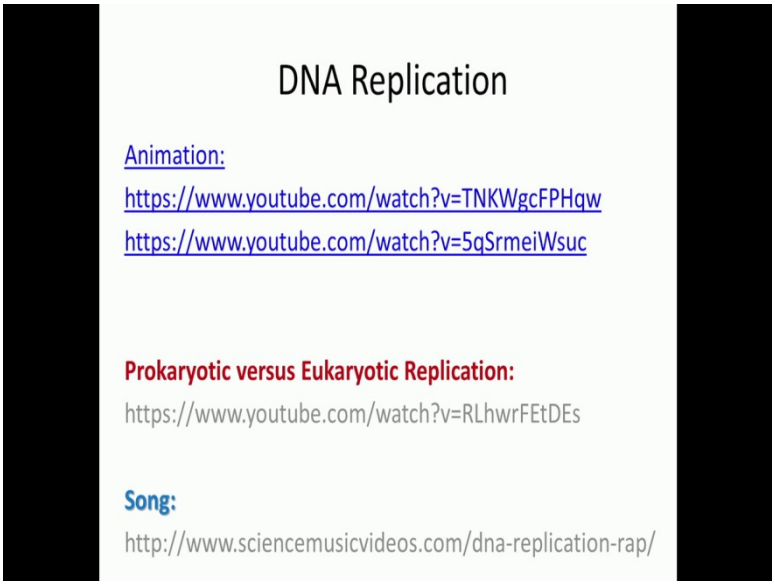


Now, I would like you to, if you have time, to go through some of these fun videos and the first one is, there are 2 videos which very briefly in animation actually show to you exactly how this process of replication takes place, what has taken me about 30 minutes to explain you can see it in about 5 to 10 minutes video. And this is a very interesting video.

There is another video which also will tell you if you are interested to know, what is the difference between DNA replication in prokaryotes versus eukaryotes. And the last is a fun rap song again by Glenn Wolkenfeld which will just help you kind of quickly grasp the various quick players of DNA replication. Thank you and I will see you later. Bye-bye.

**Biology for Engineers and Other Non-Biologists**
**Prof. Madhulika Dixit**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Week- 04**
**Lecture - 21**
**Transcription:**
**"The Decoding Mechanism"**

So, hi and welcome again to these series of videos. Today we are going to talk about one of the most interesting problems in biology and that is, how is it that in our day-to-day lives and in each and every cell of our body whatever information that is stored in the DNA is read and interpreted. Now this decoding mechanism is what I am going to cover today and in my next video. And for the sake of conceptual understanding of the topic I am going to skip a lot of details.

I do not want to bother the audience with too much of the mechanistic nitty-gritty and mechanistic details. So we are trying to understand it at a very superficial level but it is important to understand the concept as to how the DNA information is read, decoded and interpreted and then what does it lead to. It obviously leads to formation of the working horses of our cell which nothing but the proteins. Now protein is one of them, there are quite a few others but we will stick to proteins in this particular video.

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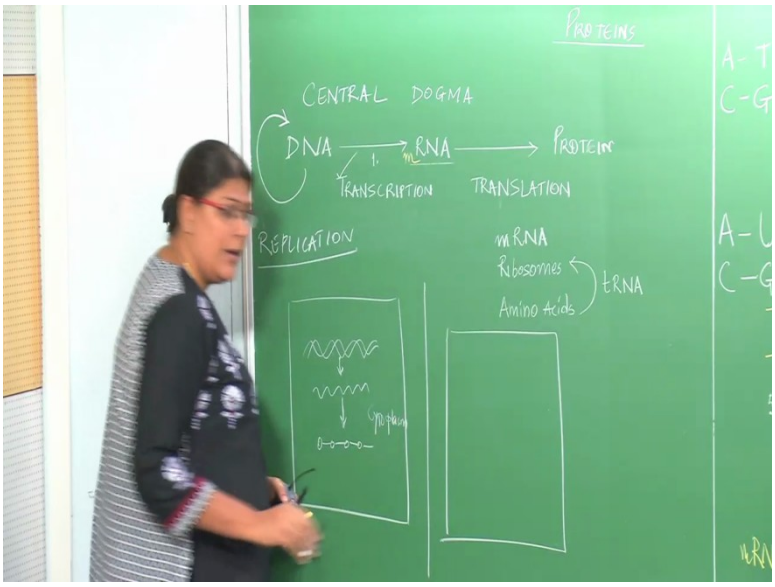


So I want you to start imagining that DNA which is packaged in our nucleus is like our huge instruction manual, and it is in this instruction manual that all the information is kind of catalogued and kept. And then there has to be a mechanism which needs to, as and when the

need is, to pick up the relevant instructions, read it and then convert it into a product. So we will start with what is called as the Central dogma of biology. Now Central dogma, what it says is that most of our information is stored in DNA, our genetic material. And then, this is like what I said is your instruction manual.

Now these instructions which are stored in DNA are then copied into RNA. You know, imagine like this, you have a instruction manual which has got a series of recipes and you need to pick out one specific recipe to prepare, let us say, a protein. Now this process of copying the recipe from the instruction manual, let us say into cue cards. So this RNA is like your cue card in which you are going to note down the recipe for the synthesis of a particular protein. So that is DNA to RNA.

And then the RNA has all the information it carries this information from the nucleus to the cytoplasm which happens in case of eukaryotes. We will also see how it happens in prokaryotes in a bit. So this cue card is then read by the chef and then it will bring in all the necessary ingredients which are needed to make this protein. Now this protein is the final product. Now, this process, the step 1 in which the instructions are read and are communicated from the nucleus into the cytoplasm by one specific kind of RNA which we call as the mRNA.

There are other species of RNA as well, but this conversion from DNA to RNA is process one which is called as transcription. And then, all the information which is present in the RNA is then decoded, is understood and accordingly the ingredients are brought in for the formation of a complete protein. That process of decoding and the actual formation of proteins is what you call as translation. Now in today's video we are going to talk about transcription, I will come to details of translation in the next video.

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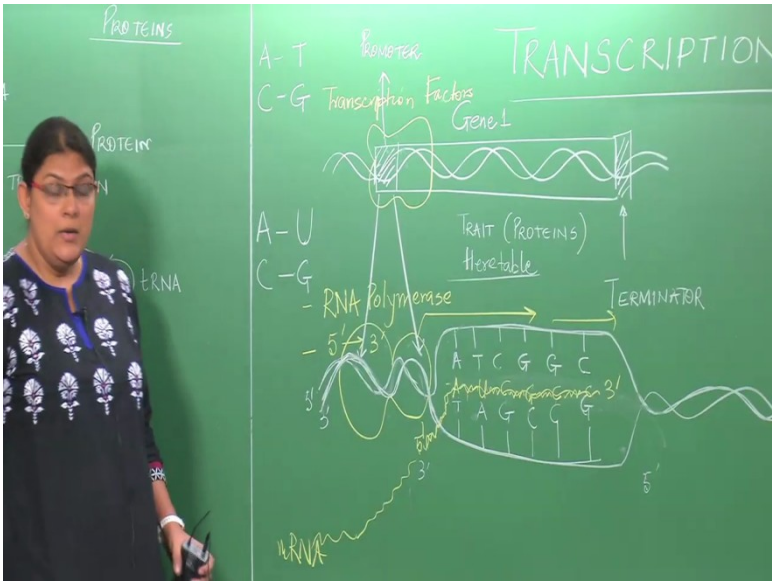


So what are the ingredients for a protein formation to happen? Well you definitely need a messenger RNA. You need the chef is going to put in all these ingredients together and the chef is nothing but the ribosomes which are present in the cytoplasm. There are also ribosomes which are present on the endoplasmic reticulum in eukaryotic cells that is also a site for synthesis of proteins.

So you need mRNAs, you need ribosomes which are the chefs, and these chefs will read the information in the mRNA and bring in the other ingredients which are the amino acids.The amino acids are the building blocks of the proteins, as you would have read in your biomolecules class. And these amino acids are brought to the chef; they are ferried to the chef by another class of RNA which is called as the tRNA. Now we look at all this in translational video. For this video we will stick to transcription.

So in transcription, what is happening is that the information which is present in the DNA is read and copied onto an RNA molecule, and then the RNA molecule moves out of the nucleus into the cytoplasm. So how is it that, where is it in a DNA that the information is stored. Now, let us assume that this is your long, linear, uncoiled DNA molecule. Now the DNA molecule in a stretch will have specific segments which will carry this information or in simple terms these are like specific files or folders in which the information is kept.

Each unit which codes for a particular trait, let us say this entire set from here to here has a stretch of nucleotides which are coding information, let us say, for synthesis of a protein, protein 1. So that information is coded in a stretch of DNA, so each stretch of DNA which

codes for a particular trait; in this case we are talking about proteins, you can also have traits like, which are coding for the tRNA itself. So these sections of DNA which are coding for specific traits and the traits have to be heritable. They have to be passed on they have to passed on from one generation to other.

This basic unit which carries certain information is what you call as a “gene”. And each gene has a section which determines from where you need to start reading the code, from where you need to start copying the code. So if I give you the instruction manual and I tell you, start copying from the start page, so you need to know from where to start copying it so that portion from where it gives an the instruction that the gene can be copied from here on is called as a “promoter”.

Similarly the gene will end, the code message for a particular recipe will end at a certain point and that end point where it ends is called as a terminator. So at the time of transcription the DNA is loose and it is loose chromatin, their promoter is present and let us say this gene has to pass on and it has to be copied into an RNA, read by the RNA, so the script which is written here is going to be rewritten into an RNA molecule.

Now remember, unlike DNA, RNA is single-stranded but just like DNA, RNA shows complementarity with DNA. So let us see, if you were to have this DNA, okay, this DNA has to first uncoil, this segment of the gene has to uncoil, the hydrogen bonds have to be broken to set apart and this is where the gene has to read.

Now this uncoiling has to happen, the reading has to take place and all this; remember in DNA replication the uncoiling was happening by helicases and then the nucleotides were being added by an enzyme called as DNA polymerase based on complementarity. Now what is that complementarity?

As I mentioned, in DNA, an adenine will always base pair with a thymine and a cytosine will always form of base pair with a guanine. Now in case of RNA, wherever there is an adenine, there is no thymine so instead of thymine in case of RNA you will see a uracil. And then again as seen in DNA for cytosine you will always have a guanine.

So this is the rule which has to be followed, but rest of it is the same, it is still complementarity. The hydrogen bonds have to be broken to kind of separate the 2 strands and then whatever is the information, let us say there is a set of sequence here, it has a A, it has a T, a C, a G, a G again and a C. Now the other sister strand on the DNA will obviously be

having a T, a A, a G here, here it will have a C because G pairs with a C, here again G pairs with a C and here you have a G.

And let us look at, let us give it some directionality, let us say this strand is 5 prime, this one which is going all the way from here, this strand is a 5 prime to 3 prime. Then obviously the second strand is going to be antiparallel, so this will be 5 prime and it will go 3 prime. Now whatever is the enzyme which is going to read it; so enzyme is like the pen which is going to copy it and write it down, now that enzyme in case of transcription is similar but not exactly same, similar, it is called as RNA polymerase.

Now just like DNA polymerase, it needs a template to read, the first thing, second just like DNA polymerase it will keep on adding the ribonucleotides; now these are not deoxyribonucleotides, here you are bringing in the ribonucleotides; it will read the ribonucleotides, or it will polymerise the chain of RNA in the 5 prime to 3 prime direction. And I had explained why it happens in 5 prime to 3 prime because of the phosphodiester bond formation.

And the only difference, or rather one of the major differences the RNA polymerase has from a DNA polymerase is that DNA polymerase had to have a primer, it had to have a foundation on which it had to then go on adding the deoxynucleotides. That is not required in case of RNA polymerases, in that sense RNA polymerase is a smarter enzyme. So now its the RNA polymerase which will do this conversion from DNA to mRNA.

Now, where does a RNA polymerase bind? RNA polymerase actually goes and binds to this region which is called as the promoter and there are a whole bunch of proteins which allow these RNA polymerase to actually go and bind to the promoter, they are called as transcription factors. Now to avoid confusion I am not getting into details of different kinds of transcription factors, they are far more complex in eukaryotes than in prokaryotes, but just for the time being remember that it is these transcription factors which assist the RNA polymerase to go and hop on a to the promoter and then sit on the promoter.

Once it sits on the promoter, so let us say this was the promoter region, right, the RNA polymerase sits on it and then it starts moving in that direction, and as it moves along it starts breaking these intermediary hydrogen bonds, causes the separation of these 2 strands and then this strand becomes the template strand. Why? because this strand is running from, I

would say this particular strand, which is going from here to here and is then opening, is running at the 3 prime to 5 prime end.

But the RNA polymerase, the strand has to be antiparallel, and it has to be complementary. So the RNA polymerase will then start reading this. So this strand will start reading. So this has separated and wherever it encounters a T, here we will put a A, ribonucleotide, here there is an A, but it does not have a thymine so it will form a uracil, then it will form cytosine, a guanine, a cytosine and here since there was a guanine it will form a cytosine again.

And then this synthesis is happening in the 5 prime to the 3 prime direction. And this RNA is then coming out and it pulling out and as RNA polymerase is moving forward it further unwinds it, reads this strand, keeps on adding the ribonucleotides in its 5 prime to 3 prime direction and the mRNA molecule is coming out at the other end.

So what has happened by the end of transcription, so you had this DNA, and then you had the promoter, to which the RNA polymerase is bound started moving in that direction and then it starts copying and starts giving you an mRNA molecule which is the exact complimentary to the template strand. And that happens from 5 prime end to 3 prime end.

So now you have the mRNA molecule which is ready. And in case of eukaryotes the mRNA molecule then undergoes further processing because remember in case of eukaryotes, the mRNA has to travel a long journey, it has to come out of the nucleus and then dock on to a ribosome and then approach the chef. So, it has to travel. So that protection is provided by some additional modifications in case of eukaryotes wherein again the mRNA at its 5 prime end will have some additional nucleotides added, and this, and a few phosphates, and this is called as a 5 prime cap.

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Similarly the 3 prime end of the mRNA in case of eukaryotes will have a stretch of a few adenines, this is like an adenine tail and this is also called as a poly-A tail. So what has happened is unlike prokaryotes; in case of prokaryotes the transcription stops here, but in case of eukaryotes the RNA is further processed because it has to travel outside the nucleus and reach to either the endoplasmic reticulum or the ribosomes in the cytoplasm.

So it has a 5 prime cap, and it ends up having a poly-A tail. Now this process; so you still do not call this as an mRNA in case of eukaryotes; this process is called as RNA processing. Some of them will also call this as post, post means after, post-transcriptional modifications.

So that also happens in case of eukaryotes. So now you have; whatever was written, the instructions which are written in the instruction book which is your DNA; has been, the script has been copied into an mRNA molecule, the molecule has been appropriately processed to further take it out to the ribosome where the actual process of translation will happen, and I will cover that process of translation separately in a separate video.

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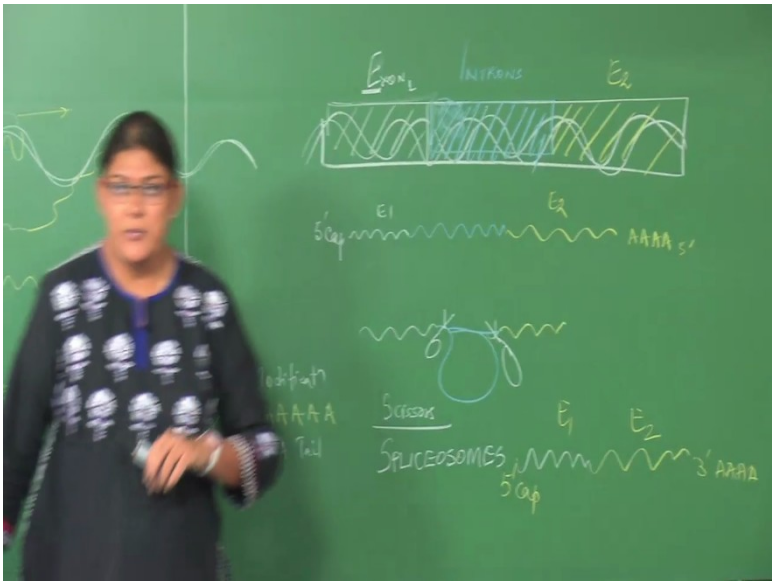


But, I want to cover 2 other important things. In case of eukaryotes what we observed is that the gene which is coding for a particular character, is not in continuity. What happens is the gene will have certain segments; so let us say this whole thing is one gene. Now this gene will have regions which actually code for a protein so they are called the “exons”; E for expressing parts, the parts which get expressed into proteins; so it will have exons, let us say this is exon 1 and then, in between it has a region which does not code for anything. It is, so these regions which do not code for anything are called as the “introns”.

And then you will again have the next exon coming in, let us say this is exon 2. So when the RNA which is being copied is formed, you will have an RNA which will have a 5 prime cap. It will have the exon 1 and then it will have the intron in between. And then it will have the next exon coming in, which is exon 2 and then you end up having a poly-A tail at the 3 prime end.

Now this cannot be read, I mean completely, if this is read, the recipe will get messed up. The recipe cannot afford to have this intron, the chef cannot have this intron. So there is a process wherein the editing takes place. It is almost like you make a video film and then you kind of cut it wherever the regions you are not happy you do a crisp editing so that the final video looks absolutely crisp and continuous.

Similarly this RNA, it is still a pre-RNA, right, what happens is you have a machinery, which are like scissors. Now this scissors are called as “spliceosomes”. Now do not get into the details of it, what they do is they bring in these intros, the exons together and the kind of loop out and they cut it. So you will have a scissor cutting it here, you will have a scissor which will cut it here. You cut it and you remove the intron out, and then you get a continuous mRNA, with exon 1, exon 2, 5 prime cap and a 3 prime poly-A tail. So this happens in case of eukaryotes.

So this was a little bit of a detailing which had to be told because I told you, remember, that the DNA is a little more complex in case of eukaryotes than prokaryotes and part of it, one of the examples is this process which is necessary for the processing of the information. Now again I want to go back to Central dogma. So, Central dogma is the main thematic. There are exceptions to it but by and large for simplicity's sake, the information flows from DNA to RNA which is what you call as transcription; then from RNA into protein which is what you call as translation.

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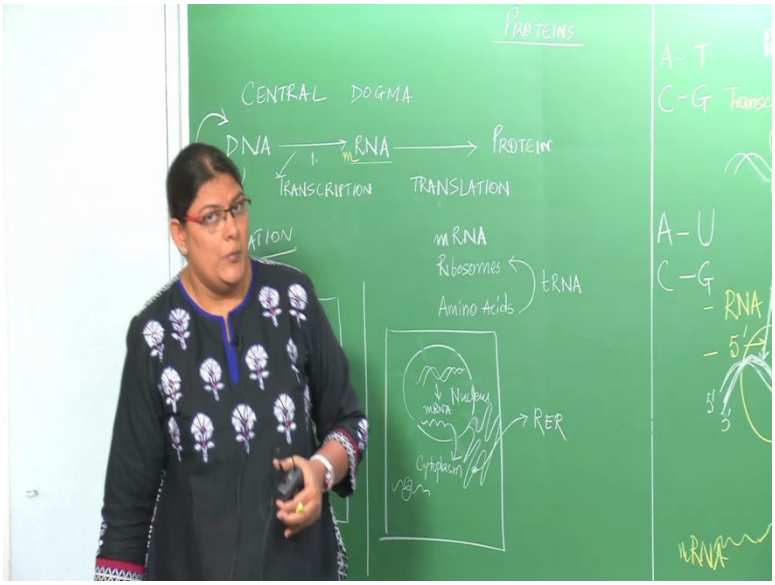


When a DNA is re-copying itself this is what you call as replication. Now if one were to compare the differences in these processes, let us say in a prokaryotic cell; so this is your prokaryotic cell and this is a eukaryotic cell. So in a prokaryotic cell, there is nothing called as nucleus, the DNA is loosely sitting in the cytoplasm; the DNA gets copied into an mRNA. So the transcription happens in the cytoplasm itself and then the mRNA is read and is used for formation of proteins. The translation is also happening in the cytoplasm. So this is like in the cytoplasm.

But in case of eukaryotes you have a nucleus, and then it has these openings which you call as the nuclear pore. The DNA is sitting in the nucleus. The process of transcription, formation of mRNA processing of mRNA, 5 prime capping, 3 prime poly-A tail, post-transcriptional modifications, all that is happening in the nucleus. After that once the mRNA is ready, it comes out of the nucleus into the cytoplasm and then in the cytoplasm there will be either free ribosomes to which the mRNA will go and the process of translation will happen or it will go and sit on those ribosomes which are sitting on what you call as the rough endoplasmic reticulum. So you can have protein synthesis in either of these 2 areas, either in the cytoplasm or in the rough endoplasmic reticulum.

So in today's video what we have learnt is that through the process of transcription which starts at the promoter, where the RNA polymerase binds, and this RNA polymerase separates the strands of the DNA, uses one of them as a template and then from a 5 prime to 3 prime direction it starts polymerising the ribonucleotides leading to formation of an RNA molecule. So in transcription, whatever instruction which was stored in the DNA has been copied into a single-stranded mRNA molecule.

And then in case of eukaryotes this mRNA is further processed, because it needs to go out of the nucleus through the process of post-transcriptional modification. Hopefully this has helped you understand the first step in Central dogma which is transcription. In the next video we will talk about translation. Thank you.

**Biology for Engineers and Other Non-Biologists**
**Prof. Madhulika Dixit**
**Department of Biotechnology**
**Indian Institute of Technology, Madras**
**Week- 04**
**Lecture - 22**
**Translation:**
**"The Decoding Mechanism"**

So, welcome back to the video on translation. Now in the last video when I was talking about transcription we saw how the information which was written in DNA was copied onto an RNA molecule. So RNA molecule was your cue card which then moved, can move from the nucleus into the cytoplasm. So today we are going to start, starting point of the video is going to be that the mRNA has been already synthesised. Now how is it that this mRNA is read, And having read how is it that the protein is synthesised?

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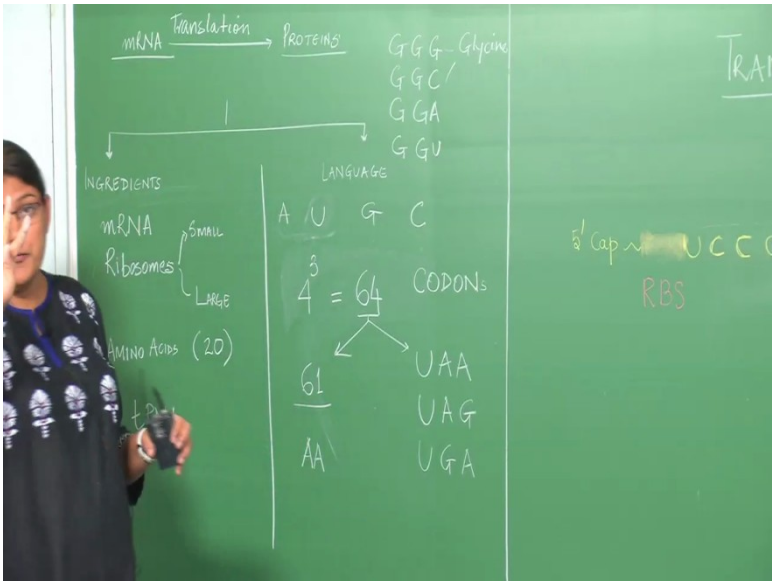


So to understand translation it is important to understand that translation is the process by which the information which is written in mRNA is finally converted to the product which are the proteins. So that is the process of translation. Now our starting material is this stretch of mRNA molecule which has, you know, meticulously copied all the information which was written in the DNA, so this is now the script which the ribosome needs to read. So we need to understand 2 things in this process of translation, 1. What are the ingredients which are required to make the proteins and 2. How do you read that language? So let us come to the ingredients first.

The first ingredient is the script itself which is in the mRNA molecule, then the chef where this entire synthesis happens which are the ribosomes. The ribosomes have 2 units; there is a small subunit and a large subunit. We will come to this little later again but remember it is a structure made up of 2 units, a small and large unit. So you need the chef, you also need the ingredients with which the proteins are made and proteins are made up of amino acids. So you need the amino acids.

There are 20 different amino acids and these 20 amino acids arranged in different permutations and combinations because of which you get different proteins. So you have amino acids and then you have a molecule called as the tRNA. It is also called as transfer RNA because this molecule will actually bring in the amino acids to the ribosome, the site of protein synthesis. And this tRNA is; so you will have different kinds of tRNAs which can then handpick the amino acids and bring them to the ribosomes.

Now that is what are the ingredients, you need the messenger RNA which is the script, you need the site of protein synthesis which is the ribosome, you need the basic building blocks of proteins which are the amino acids and these amino acids are ferried to the ribosomes by a molecule called as transfer RNA or tRNA.

Now let us come to the language. Now we know we basically have 4 bases, so you have 4 bases; in case of DNA it is A, T, G and C. But the script is written in the RNA molecule so instead of a T in case of RNA we are going to have a U. So there are 4 letters or I mean in simpler words the language of DNA has 4 alphabets, A, U, G and C. Now these alphabets have to come together to form words and each word should mean a particular amino acid.

So if you have each alphabet coding for one amino acid you have only 4 possibilities. Let us say if 2 of them at a time are coding for one amino acid, A can code with U or A can combine with G, or A can combine with C, then you will have 4 to the power 2, about 16 different possibilities in which these combinations can happen. So you essentially, will have 16 different words. But that is still falling short because you have about 20 amino acids.

So what we know now is that actually there are 3 of them who come together at a time and then they code for a word. So if you have 4 bases which you are combining in groups of 3, then you have about 4 to power 3, about 64 different possibilities. You have 64 different words, each word meaning something. Now each, so in biology the alphabets are 4, but these alphabets arrange in groups of 3 to form a code which is called as the “codon”. And there are

64 codons in our system out of which, 61 of them code for amino acids, while 3 of them do not code for any amino acid.

For example you will have UAA, UAG and then UGA. Now these 3 codons, they do not code for any amino acid but you have 61 of them which are coding for amino acid. Now that is, again brings one interesting point; you have 61 codons, but you have only 20 amino acids. Now there is seen that the more than 1 codon can code for an amino acid. For example you will have say GGG, can code for an amino acid. The same, let us say this codes for glycine, for example.

Now the same amino acid can also be coded by another codon, like GGC. This code also codes for a glycine. So you can have multiple codons for the same amino acid but a single codon cannot code for 2 different amino acids. Let me make this clear again. So let us say the amino acid is a glycine, the glycine can be coded by GGG or GGC. So the glycine can be coded by more than 1 codon. In fact glycine is coded by 4 different codons.

So there are 4 different codons which code for the same amino acid which is glycine, but it is not possible that the GGG will code for glycine will also code for alanine, that is not allowed. So the genetic languages, the 3 nucleotides come together to form one word which is the codon and each word codes for a specific amino acid. You can have more than one word for a particular amino acid. But a meaning of the word is unambiguous. It means if it is GGG it will always code for a glycine, it cannot code for any other amino acid.

So that is the language, and now that language is there in that language is sitting on the mRNA molecule. But that language has to be read and interpreted. Now that interpretation of the language is done in part, by the tRNA molecule. Now tRNA is a very interesting RNA molecule. If one were to look at its two-dimensional structure, it has a clover leaf structure, I mean; it looks like the cloves. And what you find is this tRNA molecule which is usually about 80 nucleotides long, so there are different tRNAs; each tRNA has a region which is called as the “anticodon”.

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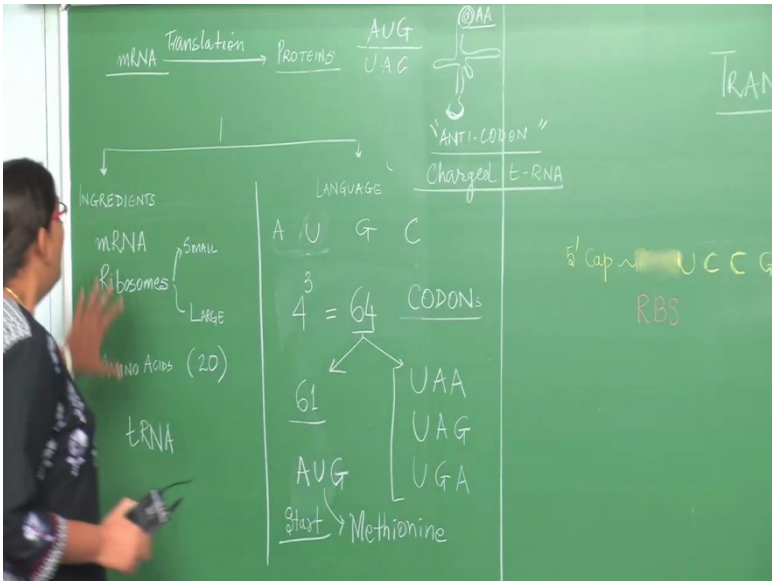


So if you have, let us say a codon AUG; now this codon has to be read by the anticodon. So the anticodon will be complimentary to the codon, which will, in this case will be UAC. because there is no thymine so you will have a uracil; this is again an RNA it is not a DNA. The uracil will always pair with an adenine and guanine will always pair with a cytosine; that is the complementarity.

So every codon, likewise all 61 codons will have the complementary anticodons. So each tRNA molecule in itself will have a complimentary anticodon and at the other end will also have an amino acid attached to it. Now such a tRNA molecule which for a given codon carries that specific amino acid is also called as a charged tRNA.

Now I am not going to get into details of how all this charging happens because then it becomes too mind-boggling and I do not want to bother you with all those details. Just remember that the codes are the codons, the 3 letter codes and each code codes for a specific amino acid, and then the code is decoded by the tRNA because of the anticodon which is complementary to the codon. And each tRNA will then bring in the respective amino acid.

Now among the codons as I told you, 3 of them are stop codons, so for UAA, UAG and UGA there is no tRNA molecule available. And for the AUG you have, this is always the start codon. It is also the first codon so it is like you are writing a script, if you are writing on a letter and you start with a capital T for “the”, right, so the first word is always a AUG which codes for methionine. So out of the 64 codons 4 codons are special; the start codon which is

AUG and the terminator codons which are UAA, UAG and UGA. So I hope till here you have understood what are the ingredients and how the language is read in translation.

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Now let us look at the actual process of translation. So what I have done here is I have drawn a mRNA molecule. So this mRNA molecule has a 5 prime cap. It has a 3 prime poly-A tail. And now this mRNA is sitting in the cytoplasm. And then it has this whole sequences, we need to make sense of this sequence. So now each RNA molecule also has a specific signature which is called as the ribosome binding site. Ribosome has a small subunit and a large subunit.

So the ribosome binding site is kind of scanned by this small subunit and wherever the small subunit will see this, the ribosomes will start assembling. So the ribosome will recognise this and the small subunit of ribosome will then start assembling, this is the small subunit of ribosome.

Now, in this sequence if you see carefully, what is the start codon? The start codon is AUG. So if you look at the sequence this is UCC. Then you have GAU, but then if you read a little carefully this becomes AUG. Now this is your start codon. So there is another important aspect in the reading of language that you have to understand is about punctuations. I am really simplifying this. So AUG becomes the first code, the next code is always read without any break in sets of 3. So this becomes your second codon. So this becomes the second codon, this becomes the first codon which is always AUG; first codon, second codon.

Then again you read the next triplet without the break, so this becomes your third codon and then you have the fourth codon and then you come to the fifth codon. If you notice the fifth codon is a UAA which is nothing but the stop codon. So here the process will stop. So this is the RNA molecule and by just looking and scanning through it you can understand that it has AUG followed by GGG then the third codon then the fourth codon and then you bump into a stop codon. So let us look at translation.

Now translation has 3 stages; the first stage is initiation, the second stage is elongation and the third stage is termination. So let us look at the first stage which is initiation. What is happening in initiation is the ribosome, the small subunit of ribosome scans this mRNA and attaches itself based on ribosome binding site and then here is a start codon. Wherever there is a start codon the first tRNA which will have the complementary anticodon. So let us say this is the anticodon site, will come and bind, so I am just drawing this like a clamp like structure, just a hook like structure. So this is the amino acid it is bringing which is nothing but methionine.

So the first tRNA has come and it has docked itself onto the start codon, the small subunit of ribosome has come in sitting, and now when this happens, this is the first step during the process of initiation. Once these are juxtaposed and are put together only then the large subunit of ribosome comes and sits. So this is the large subunit of the ribosome which is sitting. So, it is like this; you have the RNA molecule, let us say this represents the RNA molecule, the small subunit comes and binds and then the big subunit will come and sit on top.

Now if I were to take a cross-section, this is I am, this is a 2-D diagram unfortunately, it will become clearer in some animation videos which I will show you; I cannot show you but I will definitely give you the links for those. So when you cut it across and see a cross-section, when this entire large subunit and the small subunit along with mRNA in between; so there is a groove here in, through which the RNA is passing through, you, the whole assembly is ready for translation.

Now once the large subunit arranges the largest subunit has 3 sites, it has 3 sections; it has a section which is called as the E-site or the “empty site”, the P-site and the A-site. Now, the system is ready to bring in the next amino acids. The first amino acid is methionine. Now the next tRNA comes, again it binds with the help of its anticodon sequence, and it will bring in

the next amino acid, let us say amino acid 2; I think GGG we discussed codes for glycine so this comes in as the second. Now the incoming tRNA is coming at the A-site.

Once this has happened, now the 2 tRNAs are next to each other you will have the process of formation of peptide bonds; remember to amino acids are joined by the process of peptide bonds. So this methionine will be passed on to the second amino acid through the formation of peptide bond. And once this happens, so what will happen then is that this entire ribosome will shift by one codon to the right. So this now will shift by one codon to the right.

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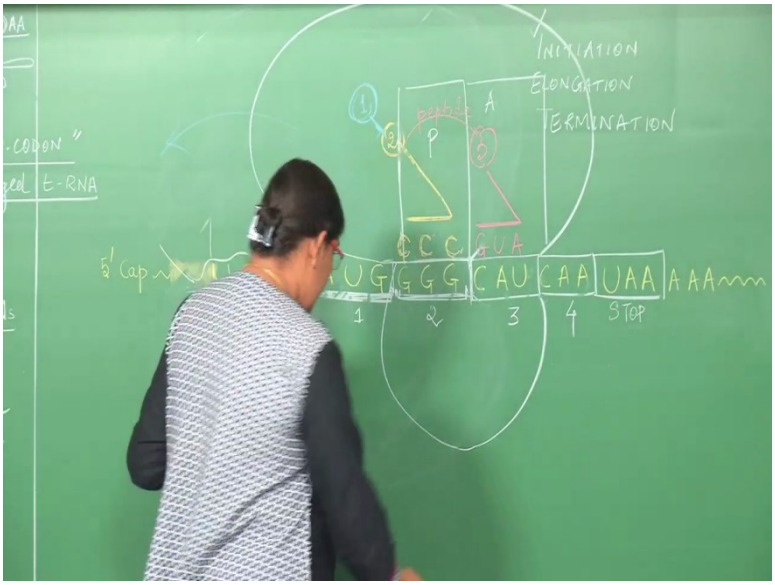


So now it has, methionine has been passed on and methionine is now linked to this tRNA. Now this entire ribosome shifts and what happens is now the ribosome small subunit is sitting here, and the large subunit is sitting here. So what was the A-site in the first round now becomes the P-site. AUG is the exit site, so now the tRNA is empty; it has donated its methionine. So from here the tRNA which had brought in the methionine is free, so this tRNA will go out from the E-site.

This becomes the P-site and now this is the codon which is now ready to be read against sitting at the A-site. So now at this site the third tRNA will come which will read the codon through its anticodon and will bring the third amino acid. Again there will be a peptide bond formation between these 2 and then this tRNA becomes empty and then this tRNA also moves out of the E-site. So this is how the process of translation happens and the ribosome keeps sliding one codon at a time and along the mRNA template and reads it, brings in the

corresponding tRNA and every time the charged tRNA comes, it then forms of peptide bond with the previous tRNA and hence the polypeptide chain keeps on getting elongated.

Now this keeps on happening till the ribosome encounters the stop site. I hope you have understood how this is happening. There are some very nice videos on you tube, I will give you links to those, it will make it even clearer to you. So by the time it reaches the stop codon what has happened is, the ribosome is sitting.

This becomes the A-site in the ribosome; this is where is the peptidyl site. So there will be a corresponding tRNA which would have its fourth amino acid which was connected to the third amino acid which in turn was connected to the second amino acid and then the first amino acid which was the methionine. So you are getting a chain of amino acids getting synthesised and now the ribosome has bumped into a position which is the stop codon.

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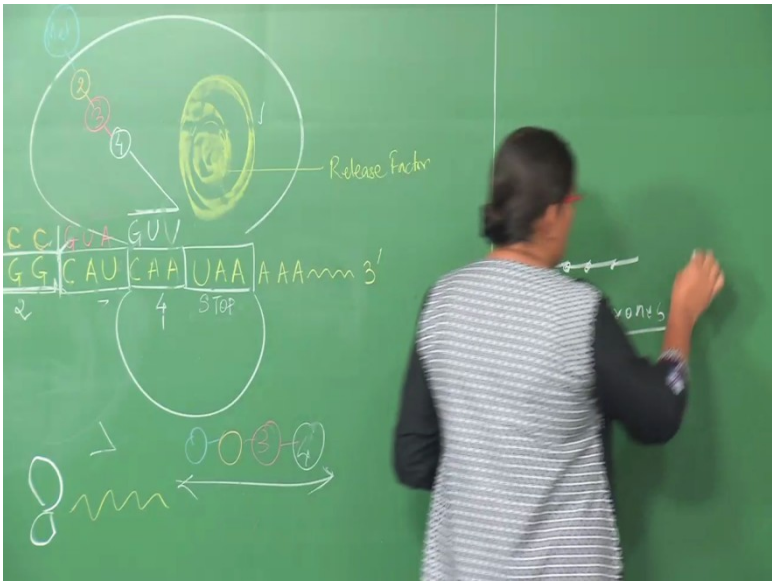


Now there is no tRNA for the stop codon. So instead of a tRNA whenever the ribosome hits this stop codon, what is called as a “release factor” comes, a release factor, it is a protein which comes and binds to this A-site, it is called as the release factor. And once this release factor comes and binds to the A-site, it basically causes the dissociation of this entire complex. So at the end of it everything gets dissociated, the small subunit, the large subunit, the ribosomes come apart, the mRNA comes apart, the tRNA becomes free again, the last tRNA and then you are left with just the polypeptide chain.

So you have the methionine, the second amino acid, the third amino acid, right, and the final the fourth amino acid. Now you have got a peptide chain. This is a polypeptide. So this has

happened as what you have seen here is, you started with the start codon on the mRNA, assembly of the ribosomes, coming in of methionine, coming in of a second tRNA again through base pairing with anticodon. And then the amino acids keep on getting added onto this growing chain of polypeptide.

Now this is just a polypeptide chain, it is just a linear arrangement of amino acids but in reality the proteins are much more folded and that is possible because after this process of translation has happened, the termination will stop because of the binding of release factor. So once the translation has happened, polypeptide chain is ready.

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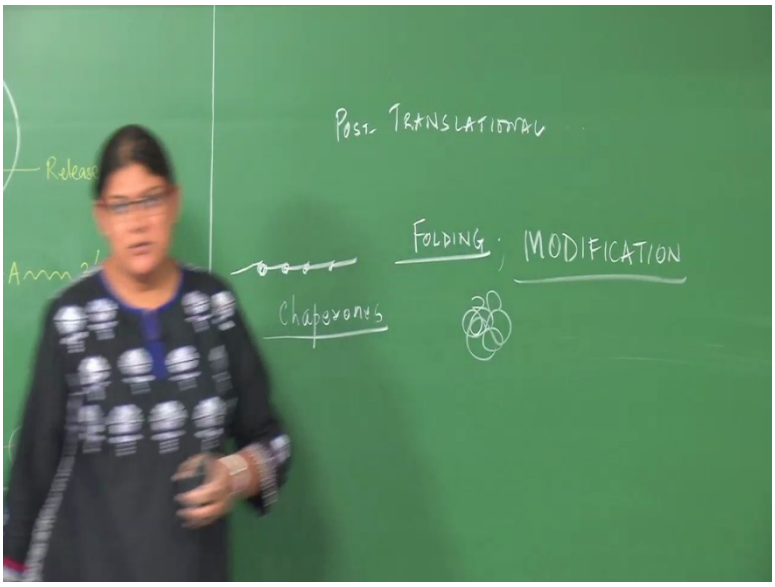


This polypeptide chain; so let us say this is now the polypeptide chain. This polypeptide chain will undergo foldin. It is like I take this thread and then try to wind it and you know form it into a ball of thread. So, that processing happens inside special structures which are called as chaperones.

So, chaperone proteins bring about what is called as protein folding. So after the amino acids have joined in the range, they have to be folded, so protein folding happens and this gets completely into a 3-D structure and if further required as it happens in endoplasmic reticulum and the Golgi complex in eukaryotes this folded protein will get further processed and then secreted or sent to its appropriate target.

So this is, this protein folding and specific other required modifications, I am not going into them because of simplicity; these modifications are what are called as post-translational modifications. So let me just wind up this video by talking about and summarising what we

studied in this video. We saw how mRNA, the script which is written on mRNA is decoded by the tRNA and the script is written into 3 letter words which are called as the codons.

The codons are read and interpreted by tRNA by the means of complementary anticodon. It is the tRNA which in response to a specific codon will bring in the specific amino acid and this entire adding of amino acid happens codon by codon in the ribosome, with the help of ribosome. And then once the ribosome and the entire translational machinery bumps into a stop codon the release factor comes every things gets dissociated, the peptide chain gets separated from the rest of the complex and this peptide chain will then undergo the process of post-translational modification.

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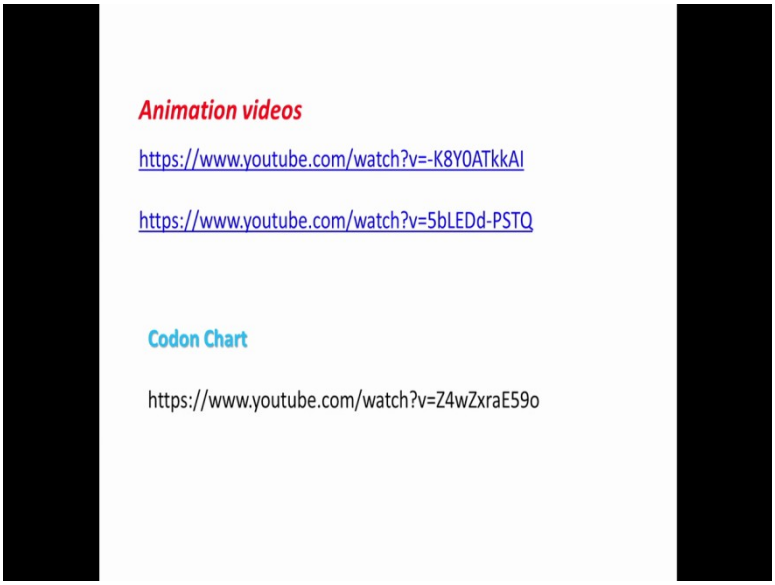


I hope this video has helped you understand the concept of how the message is decoded and I would like you to really go through these animation videos. They are really very nice. In just 7 to 10 minutes, they exactly show you how the process is happening. So whatever I could not do it on the blackboard will be easily understood by you when you watch this particular video.

And if you really want to know all the 64 codons and what they really code for and how to read the there is another video on codon chart you can have a look at that too. So thank you and hopefully you have enjoyed this series of videos; I do understand there have been a few mistakes here and there, we will try to correct them and if you have any confusions please write back to us and we will try to clarify your confusions. Thank you.

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