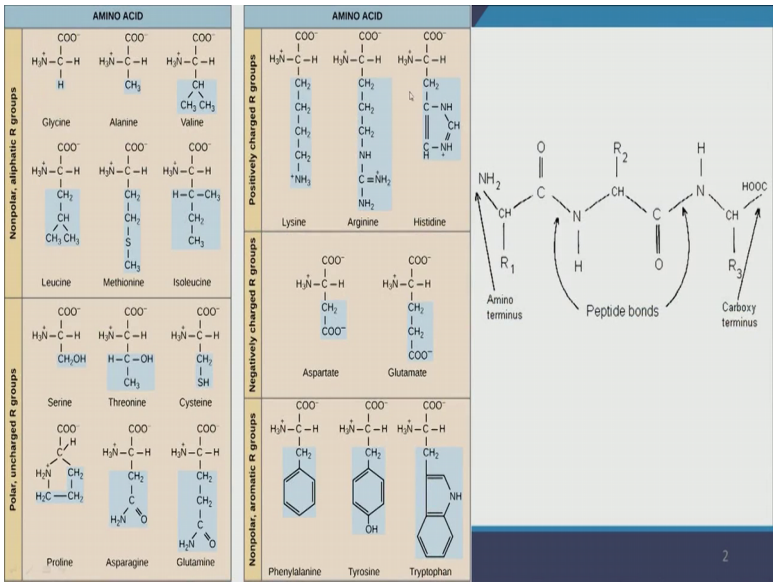
Applied Environmental Microbiology
Dr. Gargi Singh
Department of Civil Engineering
Indian Institute of Technology, Roorkee

Lecture - 06
Central Dogma II

Dear students, in the previous lecture I talked about central dogma of molecular biology and give you information about the 3 steps of molecular biology, using which the cell replicates and survives. Does it is day to day metabolic activities. Today we are going to go a little bit more intab into proteins and see what proteins are made of, and remember, last time in the last lecture I talked about amino acids, which are these beautiful compounds that are attached to each other using poly peptide bonds. And together they make these chains of amino acids attached with peptide bonds which form proteins. So, today we are going to talk more about what these amino acids are. And I am thinking why not to show you of nice picture of amino acids.

(Refer Slide Time: 01:07)



So, here we have we have on the left side of the slide we have 20 one amino acids listed here. And they are grouped according to their chemical behaviour. So, on the top left we have non-polar aliphatic R groups. So, they usually have chains that are non-polar for example, the glycine has hydrogen alanine has methane valine has this 3-carbon chain and so on and so forth. On the left part of we have polar uncharged R groups. These are

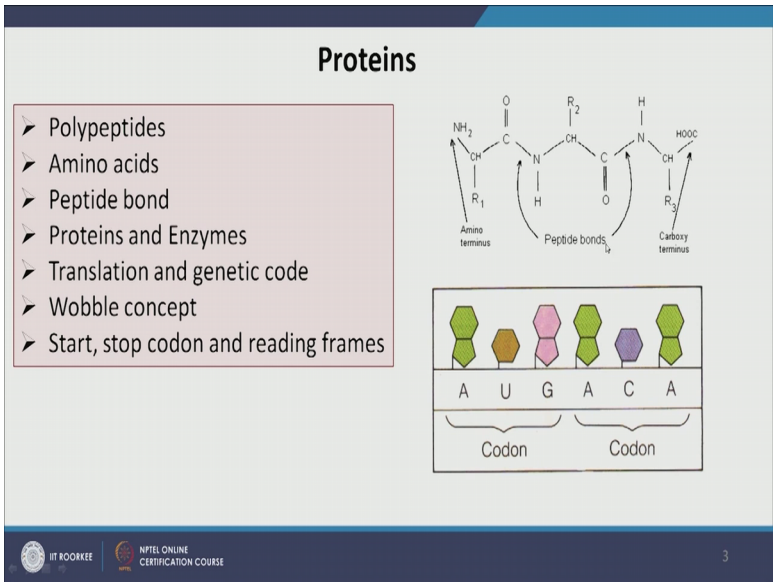
polar they have nitrogen or oxygen or sulfur attached to them and thus are polar, but they are uncharged they do not have a particular charge from them. And then on the right panel we have positively charged R groups. So, they have amine for example, Lysine arginine, histidine, then they can be negatively charged.

So, they will have astatine group C double O with the negative charge on it aspartate and glutamate and on the bottom, right we have non-polar aromatic R groups. So, here there is an essential amino acid attached to a non-polar aromatic compound in the examples are to Tyrosine and phenylalanine. Now these amino acids can attach to each other by forming peptide bonds.

So, what would be a peptide bond? How would it look like? Go let us take a look at the right side of the slide. So, we have a this this is one amino acid. This is one amino acid with it is R group. So, our group can be non-polar chain such as this one or can be aromatic such as this one or any of these ones. And it is attached to another amino acid with R 2. So, R 2 could be also either of these, and using a peptide bond this is the peptide bond C double O N. So, they attach and they form a long chain of amino acids.

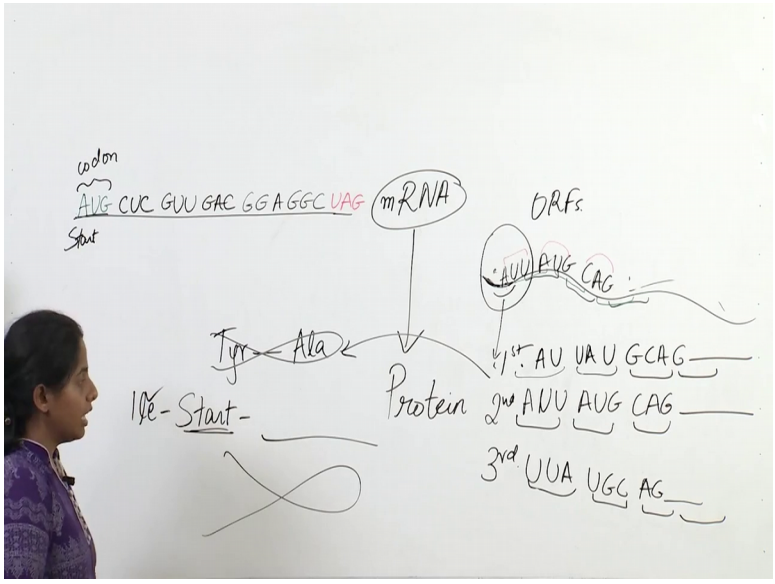
Now, they do not stay linear as it as is suggested in this particular diagram. They contort and they make very important stereo structures.

(Refer Slide Time: 03:09)



So, together when these amino acids are attached with peptide bonds have referred to as polypeptides. And they are what is called as proteins which also serve as enzymes, most of the enzymes are proteins, and they are a result of translation of the genetic code. Now in the previous lecture I talked about genetic code, how it is transcribed from DNA into messenger RNA which is used as a template for translating into protein. Now genetic code and here I have an example of genetic codes, let us take a look here. Let us say this is a messenger RNA that has been transcribed from DNA. So, our transcription assembly did a very good job and they got this messenger RNA.

(Refer Slide Time: 03:50)



Now the question is how will we convert this message into enzyme or into a protein. And the first step is this goes to the ribosome, in a ribosome together with transfer RNA and translation factors and get together at work. And what ribosome does is it starts reading it from one end. So, it let us say it was start reading from here.

So, and I mentioned last time the ribosome reads in 3 nucleotides at a time. Together they are called codon. So, it will read 3 first 3 nucleotide and they are called codon. Let us say for some reason RNA let us say there is. So, this is called codon. Now when it reads AUG? It means a particular information for ribosome. In this case AUG implies start.

So, this is in a way informing the ribosome that, this is the start of the gene. So, you should start translating from here. So, ribosome will say wow this is great they start from here. And then it will read C U C G U U G A C and so and so forth and translate them.

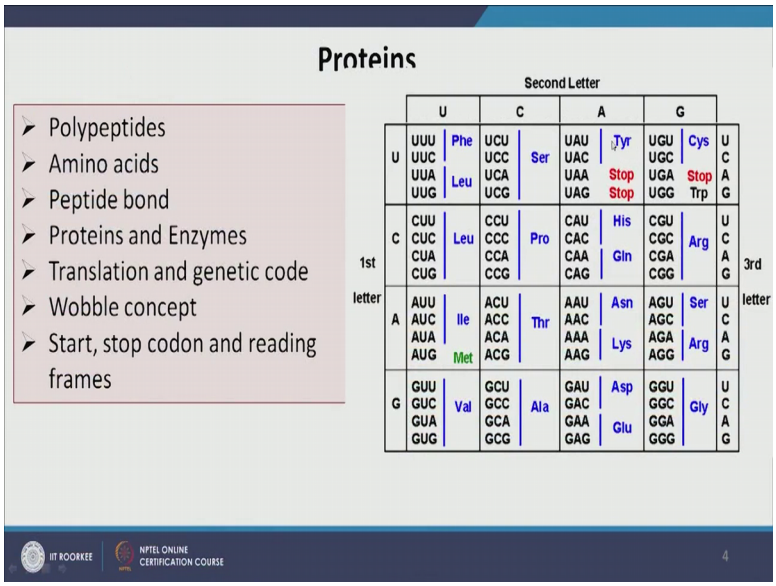
Now here I want to take a break and mention something called open reading frames ORF's. Now this is important, and I think it is relevant also. So, when DNA is transcribed into messenger RNA, usually they do not all start with the start codon at times there is there is a long chain of genetic material AUU AUG CAG and so on and so forth. And the ribosome will attach here and it will read. Let us say it reads nothing A U. It reads blank for some reason ribosome decided; that this extra backbone that I am seeing is a missing nucleotide.

So, it can read AUU and this will be it is first message. It may not know what AUU mean. A sorry, nothing A U mean and then it will read U AUG C a and so on and so forth. This is one possibility. The other possibility is that the ribosome does not start reading from nothing but it starts reading from a in that case it will read AUU AUG CAG and so on and so forth. What ribosome can also do is skip the first nucleotide and start reading from here.

So, U U AUG CAG and so on and so forth, possibly we have 3 different messages that RNA can listen can read. So, RNA could read nothing AUU AUG CAG and so on and so forth. So, this is one codon another codon another codon and so forth. This is the first reading frame. The second reading frame could be it reads from A. So, it reads AUU AUG CAG and so forth. It might also read from it might skip a altogether and start reading from U. So, U U AUG CAG and whatever else was there so on and so forth. So, ribosome has to do a very good job at reading it correct, because each of these codons AUU AUG CAG U U AUG CAG and so and so forth, are codes for a very specific amino acid.

So, I would like to show you in your in this slide these codes so that you can relate with them. So, U A U is a code for this particular amino acid. So, if the ribosome reads from in the first case, then it will read ask for amino acid t by r and followed by GCA which is code for alanine here.

(Refer Slide Time: 07:58)



So, we will get a protein that will look like this. We will have Tyr, amino acid bonded by alanine Ala. So, this will be the result of the first reading. The second reading will have AUU meaning a particular protein.

The next AUG is a start codon. So, it tells ribosome oh this is where things are getting interesting start translating from here. So, it might reject this one, and start translating further, but look here the first case it did not even encountered start codon. So, when it does not even encounter start codon there is a very good chance it would not make any protein at all. Even in third case it was encounter start codon it will make protein and this is what brings us to the importance of start codon.

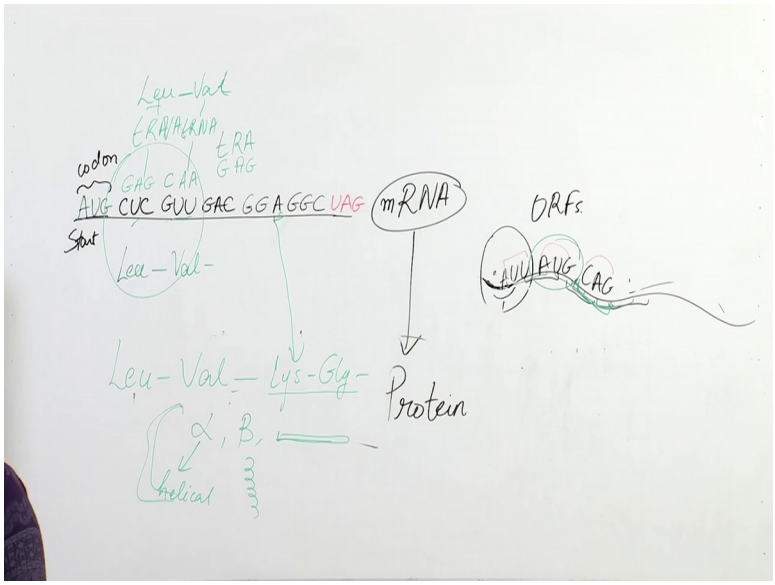
So, coming back to our messenger RNA which looks like AUU AUG CAG in this particular instance, not in this one which we will get 2 very soon. Ribosome will start reading from nothing A U and say does not mean anything. AUU still does not say me start. It is waiting for the command start. It reads AUU it says nothing and then it proceeds forward and it says AUG AUG means start this is where my genes begin.

So, it will automatically jump into action and say this is my right reading frame I got it. This is my start codon it is says proceed forward. So, this is where my reading frame begins. So, I am going to read in triplets from here onwards. So, in this particular case I have simplified the matter for ribosome, and started with the start codon itself. So, it

encounters AUG which is universal start codon and it says start. So now, it will start converting.

Now, let us what I want to do is work you through this conversion process. So, AUG means start. So, it would not actually code for any protein, but we will just start coding translating. So, CUC, CUC stands for this particular amino acid.

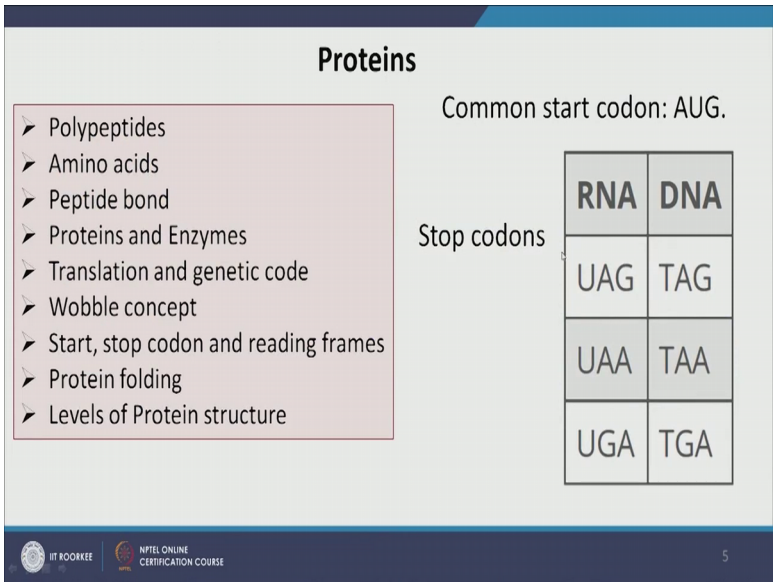
(Refer Slide Time: 10:05)



GUU will stand for another amino acid. So, now what will happen? Or the amino acids would not automatically appear here when the ribosome reads here here is the ribosome, it is reading CUC the transfer RNA that has a perfect match for CUC which would be GAG. So, transfer RNA that has GAG on it will come here and sit here GAG. This is a transfer RNA, and the transfer RNA will have the right amino acid attached to it Leu and similarly once the matching has happened, this Leu will stay here and all this can go.

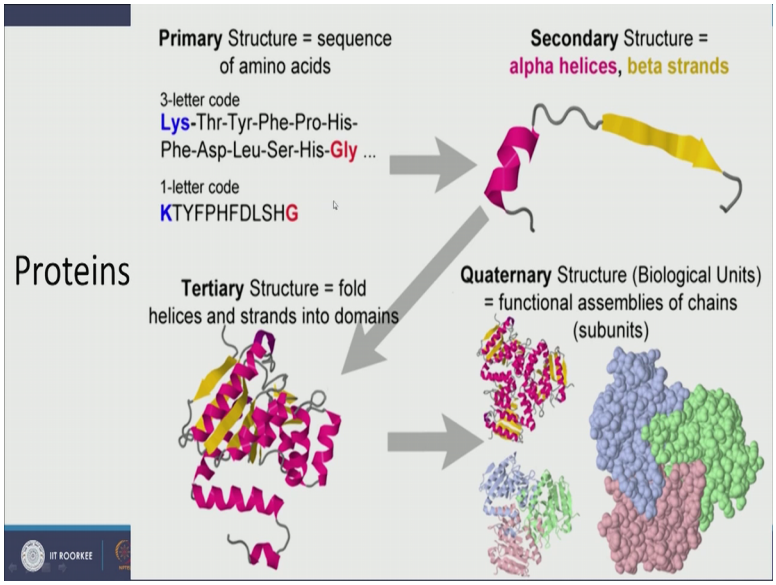
Similarly, the transfer RNA that has a complement for GUU which would be CAA again transfer RNA with a particular amino acid. Now what will happen is, Leu and wall wave form a peptide bond between them. So, we will start getting an amino acid chain that would look like this and so on and so forth. So, this is the primary structure of the protein that we have made. And in this particular slide I have listed down the typical start codon AUG.

(Refer Slide Time: 11:23)



And the often you stop codon. So, there are multiple stop codons for messenger RNA there U A G U A A and UGA in DNA they look like T A G T A and T G A.

(Refer Slide Time: 11:35)



And I was talking about primary structure. So, when we have amino acid like this, and they are usually abbreviated in 3 letter code as Lys Thr Tyr Phe and so on so forth.

Or in one letter code if we remember here, each of these proteins they have a 3 letter code in a 2 letter code. Gly would be glycine, Lys will be Lysine, a k would be also Lysine. So, Lysine can be represented by Lys or by k. So, so here we have k t y. So, this

is also your protein code. So, this structure this sequence of amino acids. The Leu Val I am just giving an example Lys glycine these this is called the primary structure. So, the linear structure of amino acid is referred to as primary structure usually what will happen is that this linear structure will either coil around along each other along itself to make alpha strands beta strands or other structures.

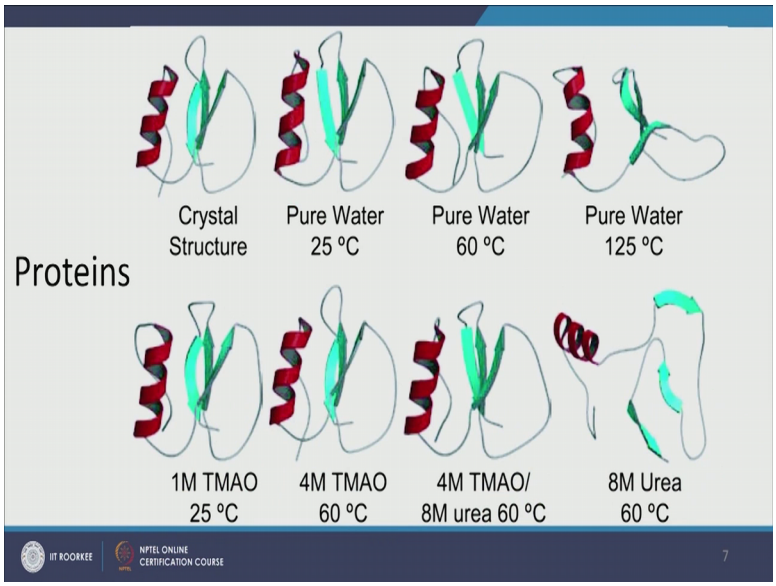
So, this is called secondary structure. They can either cone up like this like a spring make alpha helix. So, if this is a helical structure, or it can make beta strands; so beta strands would be trust bets strand. Now these alpha and beta this coils and these long-elongated portions of the amino acid chain polypeptide chain, will assemble with each other and make of tertiary structure, which these tertiary structures get together to make a coronary structure. And the stereo all the spatial adjustment and the position of these this squadronary structure is what determines, the property and activity of protein with any change in a single amino acid.

For example, there is let us say there is a mutation here. And instead of GGA it reads something else, and it encodes the wrong protein wrong amino acid. So, this wrong amino acid will affect it is primary structure definitely it will also make a small dent on it is secondary structure. And thereafter on tertiary and finally, on the quaternary structure, and it is possible that the protein will be ineffective it would not work. So, in this slide we have primary structure here amino acids join together with poly peptide the peptide bonds.

And they make either alpha helixes, or beta strands and see they are connected with each other with poly peptide bonds. And their directional usually both alpha and beta strands. And they can assemble together fold into each other to make domains. This is tertiary structure. And this tertiary and remember, this is very chemistry sensitive. Any change in charge, any change in ph, and this will change. The structure the folding would change. And then this will form the quaternary structure. So, many domains like this one here and other domains will get together and make a functional assembly of chains. A functional assembly this together is one protein. So, when I talk about ribosome as is being part of proteins. So, think about many many molecules of amino acids.

Who are connected with poly peptide bonds, who have folded into secondary a tertiary and quaternary structures and now are acting together as ribosome.

(Refer Slide Time: 14:57)



Now, briefly in the previous lecture I mentioned about denaturation for DNA, which is when we increase the temperature, the molecules start vibrating at a higher frequency with higher energy. And thus, the double strands of the DNA, DNA will separate from each other this is called denaturation. A similar is similar observation is experienced in terms of protein. So, here we have a crystal structure of the protein on top left, and you can see how intact and well-arranged it is. And the arrows here depict the direction of the strands. So, we have alpha helix here, and we have beta strands here in blue.

So, this is pure water is 25-degree celsius. So, I have added water. And it is in there is slight change in configuration, but nothing much you can see how these 2 beta strands have separated from each other. How the alpha helix is coiled little more on the top and gone away. So, just adding pure water that is a hydrogen bond liquid we will make at least some difference in the structure. So, we can see when this. And then we increase the temperature we see more differences. The alpha helix starts moving away, the beta strands move differently. And finally, after boiling the entire protein collapses. And this is if you have seen an omelette being cooked.

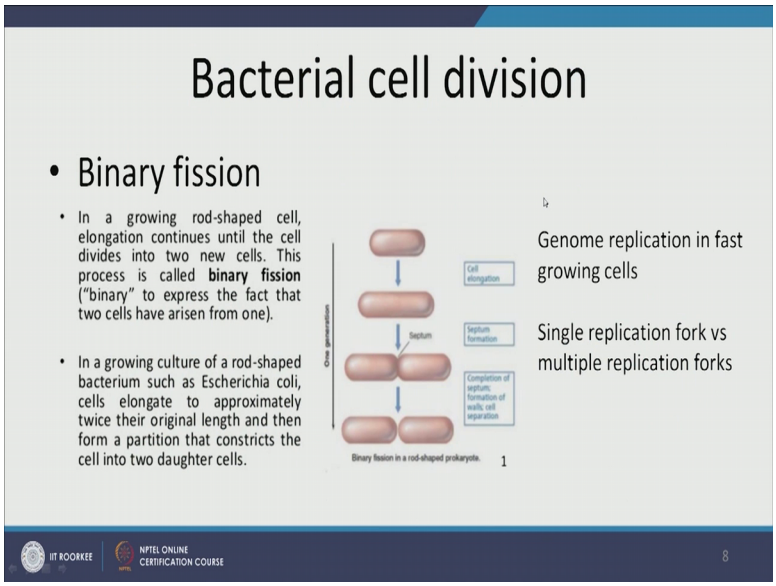
That is protein denaturation at work. First, we have liquid protein that are beautifully in right structure right shape for life to begin, but as the temperature rises you rises denature. And when they get denatured they solidify and we have an omelette ready. The TMAO this is a tri methyl amine compound that is used for preventing denaturation of

protein. So, here I added the crystal structure to pure water and because I added TMAO one molarity; there is a very high concentration. The structure has not collapsed as much as it would collapse without it. Then I add more well I increased temperature. So, you remember notice here, to go from 25 to 60-degree celsius and prevent collapse I need 4 times more TMAO.

And then that 60-degree Celsius to see the effect of TMAO we add urea. Urea is a denaturing agent, it denatures proteins it also denatures DNA nucleic acids. So, when I add urea things start breaking away, and denaturation begins and then if I even at 60-degree Celsius without going further high in temperature. I had 8 molarity of urea without any TMAO of and the entire the entire protein has collapsed. And this is important that we do not need to always raise temperature to denature proteins or nucleic acid we can use chemical reactions.

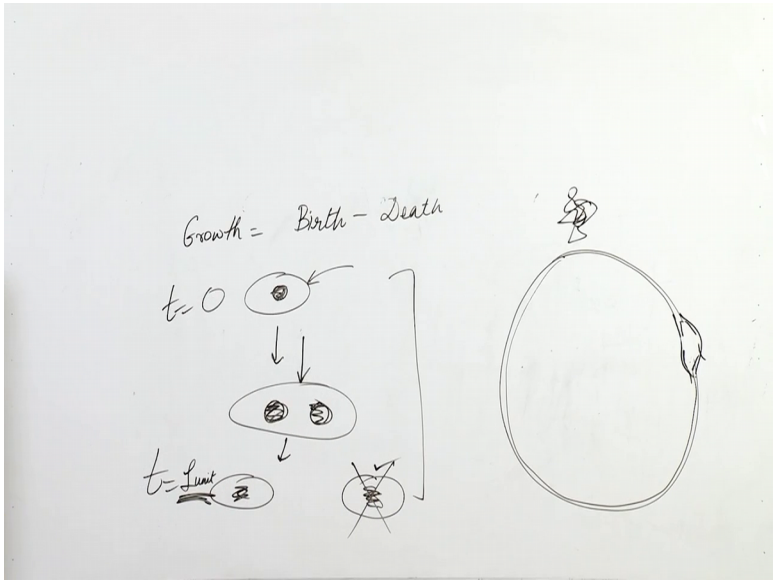
Now, now that we know about proteins you sort of know what MRNA which is messenger RNA and about DNA let us talk about the basic cell activity which is growth.

(Refer Slide Time: 17:52)



In biology growth is defined as increase in total number of cells; so if there are 2 cells to begin with and both cells make 4 daughter cells. So now, we have 4 members, part 2 of them die. So, we are still left with the net population of 2 bacterial cell this is net 0 growth. So, in other words growth in biology means net growth.

(Refer Slide Time: 18:21)



Which is it implies for total number of new cells. So, this is a rate of replication or we can call it birth in laymans term minus by death.

Now, it is important at this time to understand the mechanism of prokaryotic cell growth, most prokaryotes thread or prokaryotes grow by what is called as binary fission. So, we have one cell. And this is American basketball American football sized shaped cell. What it will do it will take as many nutrients as it can grow in size do DNA replication. So, earlier it had only one DNA, but now to grow in size, and now it will have only one chromosome now it will create 2 copies of chromosome. Everything is twice the amount of cytoplasm is twice the amount of surface areas also nearly twice, the volume has increased to twice. And then it will undergo binary fission and it will make 2 daughter cells. By the way this is not nucleus, this is genetic material and usually is very coiled hyper coiled.

And we have genetic material here. So, we have 2 daughter cells. 2 prokaryotic daughter cells. And let us say out of these cells one of them dies. So, because we from one we made 2, but one of them died we still have one left. So, at time t equal to 0, we had one at time t equal to 1 unit. And we will talk about what this one unit is they still have one cell left and thus there is no effective growth. So, in this is important to understand what growth in biology means. The other thing I want to mention is what is the unit of time.

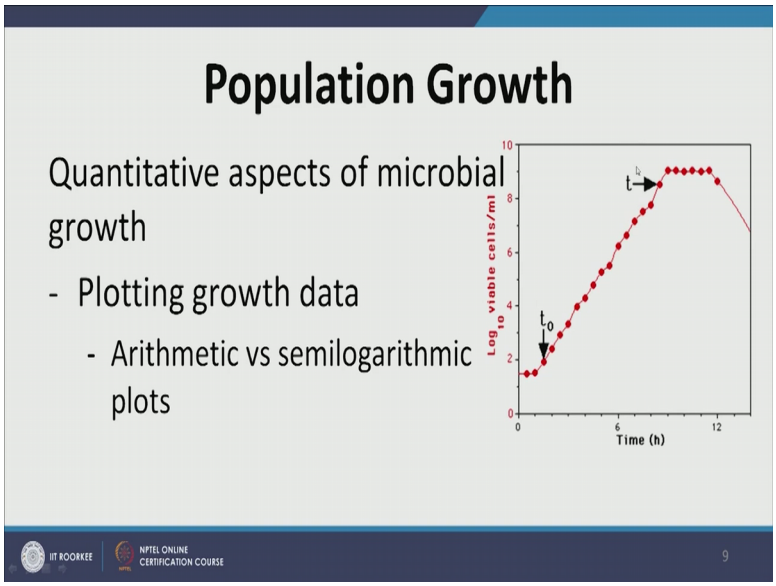
How much time does it take for one cell to break into 2 it is called generation time. So, this is one generation from parent to daughter cell.

Now so, in this figure you have cell a rod-shaped cell which looks like e coli. And it and it grows twice in size makes 2 of everything. And then this is septum formation. This is where the cell starts breaking down into 2, and now we have 2 cells. So, where there was one, now there is 2. Now there is this there are multiple kinds of e coli escherichia coli which is a model organism for biological studies you want to experiment anything or see toxicity of anything and to clone some gene we use e coli, more often than not. Now some e coli have a generation time or replication time of few hours.

Some of them have replication time of 20 minutes. So, how does the, how does it work out on a cellular level? How does a cell determine? How fast it can grow and split into daughter cells? One of the rate limiting step is replication of DNA. So, remember I told you DNA of prokaryotes super coiled circular DNA. So, to make things easier to see here, this will look like this. A double stranded helical structure, I am not making helical structure, but just trying to make a double stranded structure. Now if we can uncoil this this is how to look.

So, our beautiful topoisomerase comes here it takes these 2 apart and it starts making new copy of these. And then this is a replication fork. So, we have nice replication fork, and there is a replication going on. So, if we have one replication fork, the e coli will split or will undergo growth at it is standard time. But in the case of superfast e coli that has super-fast replication time what it uses is multiple replication forks. And those multiple replication force allow it to make 4 copies of DNA then it could only make 2. And that is why it grows faster. Now let us look at quantitative aspects of microbial growth, now that we have some idea about binary fission in microbes.

(Refer Slide Time: 22:38)



It's important to know from an applied perspective, how can we quantify microbial growth. And even before we go to how to quantify population growth, let us look into why to quantify population growth.

And a very simple day to day example would be souring of milk. So, I have milk and because the bacteria do not undergo spontaneous generation we talked about it in the first lecture. So, I have milk and it is sitting overnight, and I want to know when will it go sour this is a very practical application. From an applied environmental microbiological perspective, I have waste water and I want to know how fast anaerobic conditions will set in and they said the reason why I want to know how microbes grow. And when can I expect them to reach a particular number. And what resources will be utilized.

For them to reach a particular number and that is why scientists ever since we discovered microbes they were very interested in finding on microbial growth, because the studies in microbiology were pioneered by people who are interested in public health. And even now are we know that understanding microbial growth will also help us understand public health. For example, let us say I had a mosquito bite me. A plasma and now the malarial plasmodium is inside my body. I want to know how fast this parasite will grow in my body, and then when I actually start showing the symptoms. So, microbial in other words microbial growth is a very important and relevant concern for everybody interested in environmental or public health engineering.

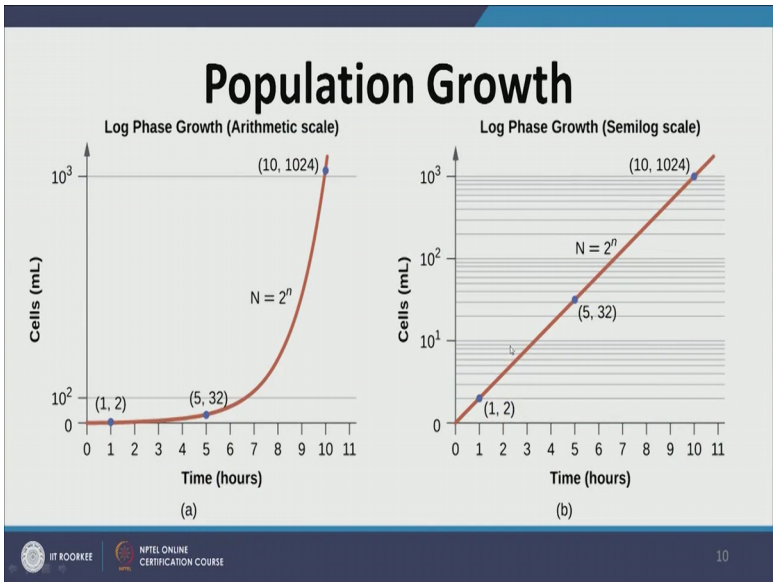
So, what we can do is we can usually plot our observations. So, if there is a way in which we can find out number of cells. And this is a challenge and I will talk about it why now why it is a challenge. If we can plot number of cells, bit time we will get we will get some pattern. And we can use this pattern to understand last time we did this experiment, it took microbes 6 hours to reach nearly 10 to power 6 number of viable cells. So, if this time we have microbes growing for 6 hours we can expect a similar result. Now the question arises how do you count cells. I think it was the second lecture when I talked about different kinds of microscopy that allow us to see cells.

So, we can focus and zoom in a particular a very small particular portion of our slide, and look at the morphology of cell maybe even get an idea of how it is interacting with it is environment in that slide. But there is no way we can swipe through or we can take a look at the entire slide to get an idea of how many microbes and count them how many microbes are there in our sample. For that we have specialized techniques. And one of the most easy technique and most popular technique that you might or might not have studied in your 11th twelfth biology or will perhaps under come across somewhere s turbidity. So, what we have we can give microbes we can take.

Let us say one ml of microbial solution, and I want to know how what is the concentration of it. I can take agar or some liquid brought that is perfect for the microbes to grow. I can add that inoculum into the broth which has all the food all the nutrients that microbes can dream of if they have dreams. And as the microbes start growing the turbidity of the liquid would change. And then I can plot the turbidity. When they are usually using other quantitative methods, I can say that the plot of turbidity gives me an idea of where the microbes are in their growth phase. If it is very turbid if it is this turbid, it I know they are in exponential phase if they are this turbid I know they are dying now.

They have done their exponential phase. So, turbidity is one of the measures, and we will be talking about other measures, but for now it is important to know that what basically we do is we prod growth data versus time and try to get an idea of the kinetics of population growth; earlier when people started studying population growth of microbes.

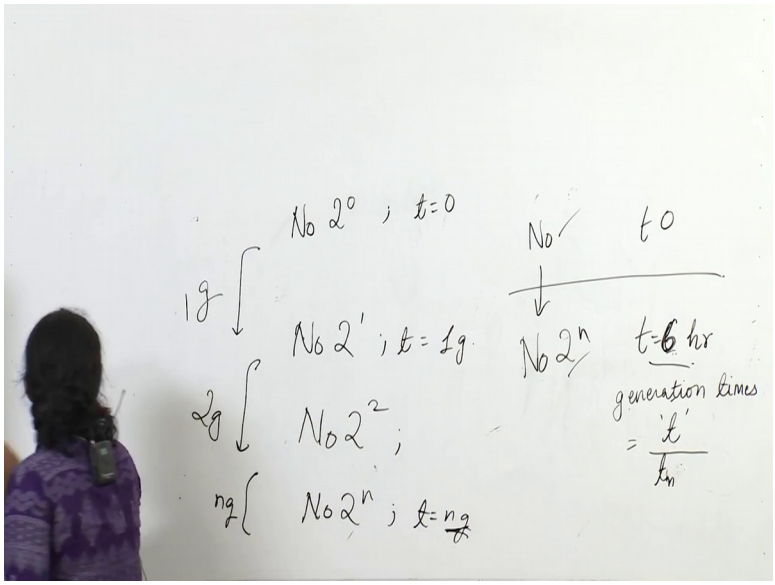
(Refer Slide Time: 26:27)



They found that when we plot arithmetically, which is simply going from 0 1 2 3 4 in a decimal system. We get something like this, an exponential growth. And then we plot it on a semi logarithmic scale, it gives us a linear plot which sort of confirmed or provided strong evidence in favour that the microbes grow exponentially. So, if it is an exponential growth on a semi logarithmic scale.

It should give us a straight line. Now let us try to understand let us try to model population growth and this is where we will end todays lecture.

(Refer Slide Time: 27:09)



So, let us say in the beginning we had N naught number of cells, right. So, in the water that I have or in the inoculum that I have there are N naught number of cells. After one generation time, how many can I expect? After one generation time N naught power 2 because each of them would have grown and split into 2. So, here I had 2 to power 0 at time 0. At time one generation time I had 2 to power 1. And then if I give them one more generation time I will have N naught 2 power 2. How would this work out? So, look here we have one cell.

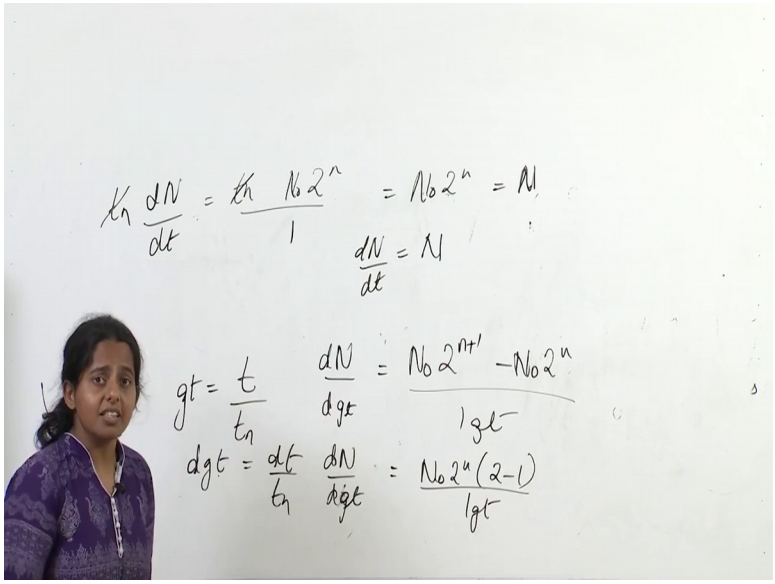
In one generation time it splits into 2 identical cells. Further each of these were split into 2 identical cells. So, we have 2 to power 0, 2 to power 1, 2 to power 4 and so on and so forth until n generations after n generation of time we have N naught 2 to power N. So, a trend is coming here we can note n generation time. So, the rate of change of microbial numbers is can derive the rate of change of microbial numbers from this.

So, if now we know that at any given instant, we can find out the number of microbes using this particular formula. And the way it would work is this knowing the generation time. Now how will you know generation time? Let us say at time t equal to 0 at the beginning I know my turbidity was 3 is one nto or 3 nto, and then after 3 hours I know my turbidity is 7 nto or 70 nto whatever it is. I can use information here and find out what the generation time is. And the way it would work is this. N naught this is 6 hours, right.

So, because I know N naught, I know turbidity here I know turbidity here. So, I know N naught by 2 n, and I can find out this. And why? Because time how will you express time into number of generation hours? A generation time, a generation times for microbes can be defined as number of actual time that has passed divided by time taken by one step; so let us called as t, t small n time taken by one step. So, by using this we can find out generation time by having this information. And further we can also devise a kinetic model for our of microbial growth.

So, let us create a kinetic model for microbial growth. So, we know now that and any given instant we have N naught to 2 power n number of microbes.

(Refer Slide Time: 30:20)



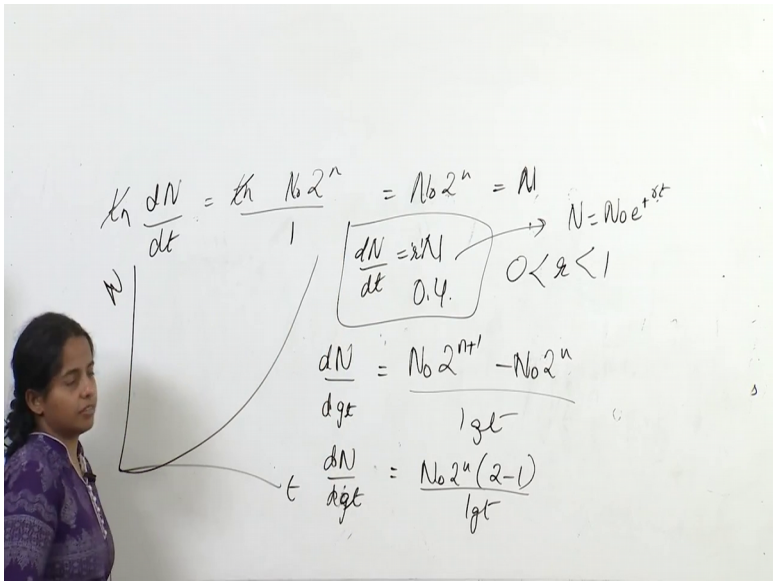
Where n is we can represent it in terms of time. After one generation time I will have N naught 2 n plus 1. So, one generation time. So, what is the rate of change of micros here. So, dN by d generation time dgt can be given by N naught 2 n plus 1 minus N naught 2 n upon one generation time right. So, here we have N naught 2 n 2 minus 1 upon 1 generation time.

Now, because generation time is written as actual time travels divided by the time it takes for microbes to replicate tn. Dgt here can be replaced and it will cancel on both sides, because it can be replaced by this, dt by t N. So, we can rewrite this equation as dN by dt tn is equal to tn N naught 2 to power n by 1. 1 unit of time, and these will cancel with each other. So, we are left by dN by dt is equal to N naught 2 to power n. Now remember what n not 2 to power n is.

It is a number of microbes that represent at are present at this particular time. So, this can be also written as N. So now, we have derived our kinetic equation as dN by dt is equal to N. This is idea scenario where every cell every parent cell within it is replication time generation time will divide into 2 daughter cells. What we often noticed though is that this replication is not always perfect. There is an inefficiency attached to it. Not all parent cells will in given time in the right generation time break into 2 cells. And thereafter we add a rate constant r which is as now a definition such as between 0 and 1;

so it never 100 percent efficiency little less than 100 percent which is called the rate of growth.

(Refer Slide Time: 32:49)



So, if it is 0.4 so, we know forty percent of microbes are replicating in one generation time. And this is our prime preliminary growth model of microbes, dN by dt is equal to rn. And before I call off the lecture, I would like to give you a small food for thought. And it consider it like your homework. If dN by dt is equal to rn is the universal microbial growth calculator, what is the major popular major limitation with it? If we plot this model, it will look something like this n t. So, to plot it you just need to integrate it, and we will get n is equal to N naught e minus rt are the rate factor.

So, it is an not minus plus. So, we get an exponential growth. What is the major limitation with this? And I will give a I will give you a hint. If only sum equals I which are very, very light. So, small we can not see. So, light we can not feel them. Grow with this particular growth rate for 2 days they will already way more than earth ways. So, in the next lecture my dear students, we will talk about another growth model, and different ways in which we measure growth in the lab; in and how that has informed our studies. And from here on we will move on to the applied and a applied portions of this course.

So, thank you very much. See you next lecture.