Cell and Molecular Biology

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Week 02
Cellular Structure
Lecture - 06

Prokaryotic Cells

Hello everyone, this is Dr. Vishal Trivedi from the Department of Biosciences and Bioengineering, IIT Guwahati. If you remember when we were discussing the origin of life, we talked about the potential ways in which organisms could have originated on Earth, focusing on the primitive cell and how that primitive cell was formed within the primordial oceans. So that primitive cell is the only existing species that further evolved into the first unicellular organisms, then into multicellular organisms, and then became further specialized into organisms with tissues as well as organisms with organ systems. So in today's class, we are going to start discussing this particular cell, how the cell is actually functioning, the different types of tasks, what the different organelles are that are present within the cell, and what the structure of the cell is. So when we talk about the cell, as you can see, higher eukaryotes have multiple organs to perform specific functions, such as the liver, kidney, and heart, and these organs have specific tissues, with each tissue composed of cells.

So whatever functions are happening in higher organisms like humans, they have different types of organs; for example, we have the liver, we have the kidney, we have the heart, we have the lungs, and all these organs have their specialized functions. These organs are also made up of tissues, and those tissues are made up of cells. So whatever function we say is actually being performed by the cell that is present in that particular organ, and that's why the cell is considered to be the structural as well as the functional unit of the organism. So whatever function you see from that particular organ could be performed by that particular cell as well, which means a cell is the smallest unit that can actually perform all the functions.

For example, in a human body, we have different types of organs to perform different types of tasks. Like the heart is there for circulating the blood, the liver is there for detoxification, the kidney is there for the excretion of byproducts, and the lung is there for respiration, the cell, which is actually the structural and functional unit, can perform all these functions on its own because it has all the necessary infrastructure to do so. Based on the cellular structures, cells are classified either as prokaryotic cells or eukaryotic cells. So what you see here is a prokaryotic cell, which is actually a bacterial cell. I have taken two examples of the eukaryotic cell.

I have taken the example of the plant cell, and I have taken an example of the animal cell. Based on the cellular structure, the cells are classified into prokaryotic and eukaryotic cells. In most cases, prokaryotic cells are single-celled, whereas eukaryotes can be either single-celled or part of a multicellular tissue system. So before getting into the details of the structure of prokaryotic and eukaryotic cells, let's discuss the differences between prokaryotic and eukaryotic cells. So that you will understand what the differences are between what is going to happen and how the eukaryotic cell has evolved from the prokaryotic cell.

So, what you see here is a table where I have listed the differences. So these are the properties of the prokaryotic cell and this is the property of the eukaryotic cell. So the first criterion is the size, and the size is very small. So prokaryotic cells are mostly in the micrometer range, whereas eukaryotic cells can vary in size. They could be up to 40 micrometers in diameter.

So they could be several sizes: RBCs, macrophages, Kupffer cells, and all those kinds of things. So they will be very different. As far as the genetic material is concerned, it is circular in the case of the cytosol and is present as free material, which means it is not present in bound form. Whereas the DNA in the form of a linear chromosome is present in the well-defined double submembrane nucleus, there is no direct connection with the cytosol. So, in eukaryotes, the DNA is present in the form of chromosomes, which are contained in a well-defined structure called the nucleus, and that nucleus is not directly in contact with the cytosol.

Then the replications, as far as the replication is concerned, mean how you are actually going to make another copy of your genome, right? So, the replication is done by a single origin of replication present in prokaryotes, whereas in eukaryotes, it has multiple origins of replication. As far as the genes are concerned, the genes are the functional part of the genome that are actually responsible for the production of different types of products or different types of proteins. So, for the gene, there is no intron present, whereas in the case of eukaryotes, introns are present. So, don't worry about these particular terminologies because they will be clear when we discuss replication, transcription, and translation in subsequent modules. Then, in the prokaryotic system, there are no membrane-bound organelles, whereas membrane-bound organelles with well-defined functions are present in eukaryotic cells.

So you have the different types of organelles: you have the nucleus, the mitochondria, the chloroplast, the endoplasmic reticulum, and so on. So, that's all we are going to discuss in this particular module: the cell wall. There is a definite, very complex cell wall that is present in the prokaryotic system, whereas in the case of eukaryotes, except for fungi and plants, eukaryotic cells are devoid of a thick cell wall, which means animal cells are devoid of a cell wall, whereas fungi and plants have a cell wall. Then the ribosomes. Ribosomes are the protein machinery, and they are actually going to be 70S.

This is the kind of parameter. So that's 70S, whereas in the case of the eukaryote, it is the 80S. Then we have the transcription and the translation. So transcription and translation occur simultaneously in the case of prokaryotic cells, whereas in the case of eukaryotes, transcription occurs in the nucleus, and translation happens within the cytosol. As we said at the beginning, the genome is present in a well-defined nucleus, which is very, very far away from the cytosol, and translation is not happening simultaneously.

Transcription is happening in the nucleus, and translation is happening inside the cytosol. Let us start with the discussion about prokaryotic cells. So, the simple prokaryotic cell shown here, right, is a bacterial cell, and the structure of the prokaryotic cell is very simple and smaller than that of the eukaryotic cell. As we said, prokaryotic cells are in the range of micrometers, whereas eukaryotic cells are very, very big compared to prokaryotic cells. One of the classical differences between prokaryotic cells and eukaryotic cells is that prokaryotic cells have no membrane-bound organelles, including the nucleus, so what you have is a cell where all the organelles are present within this cytosol.

What you have here are the different types of organelles: the flagella, the genomic DNA or genome of the bacteria or prokaryotic cell, a well-defined cell wall, the plasma membrane, and a protective capsule, which actually provides strength and protection. And then it has the ribosome, which is called the protein machinery; it also has the food granules and all other kinds of things, and it has the pili, then it has a cytoplasm and the plasmids. So let's discuss all the substructures that are present in the prokaryotic cell. The first substructure is the flagella. Flagella are present in those bacteria that are actually motile.

Flagella are present in bacteria, and they are required for motion within the bacteria. So you can see that if bacteria are present in a drop, they can actually use these flagella to swim around. So, flagella attached to the bacterial capsule is a central feature of most prokaryotic cells, especially motile bacteria. It provides motion or locomotion to the bacteria and is responsible for their chemotaxis. I am sure you are probably not aware of this terminology, which is called chemotaxis.

What does chemotaxis mean? Chemotaxis means the attraction of an organism towards chemicals. For example, if there is a sugar crystal, right? Then what are the bacteria going to see? It is actually going to move towards this sugar crystal because it is looking for that particular sugar crystal it wants to eat, so, that directed motion of a bacterium towards a particular chemical is known as chemotaxis, and how it will move is that it is actually going to use this flagellum, which is attached to the capsule. Movement of bacteria towards a chemical gradient is known as chemotaxis, which means once you have a sugar molecule here, it is actually going to be dissolved in the water, and it is actually going to have a gradient. So, because the bacterium can sense this particular gradient, it will move towards that particular food source. It could be glucose; it could be any other molecule as well.

So, the flagellum is a part of the cell wall, and its motion is regulated by the motor protein present inside the cell. So, the flagellum is attached to the cell wall, and inside it actually has motor neurons just like in humans, where we have muscles. It's also simply attached to the cellular machinery so that it can have motor proteins, and those motor proteins can actually change the flipping movement. So, the flagellum is actually going to have a flipping moment.

It's actually going to move like that, and the flagellar motion is an energy-consuming process governed by the ATPase present at the bottom of that particular shot.

It is made up of the protein called flagellin, and the reduction or separation of the flagellar protein reduces bacterial infectivity and the ability to grow. So some of the bacteria also use the flagellum for accessing different types of hosts, and that's how they can use this to reach the host, right? They can also use it to reach the host, and that's how they can be infectious. So if you actually reduce the production of this flagellant protein and if somehow you compromise the flagellar movement, you are actually going to make the bacteria non-mortal, and that's how the bacteria will lose their ability to infect, and that's how they will not cause the disease. Now the second is the bacterial surface layer, so as you can see, the bacteria have a very complex surface layer because bacteria possess three anatomical barriers to protect the cells from external damage. Since the bacteria do not have membrane-bound organelles, it is very susceptible to hypotonic lysis because bacteria are mostly present in water or hypotonic solutions.

It has a very well-defined anatomical barrier so that it can actually withstand this, so what are the different layers? You have a bacterial capsule, which is the outermost layer, and it is made up of high molecular weight polysaccharides. So what you see here is the bacterial capsule, and this capsule is required because it provides protection; this is the outermost layer, and it is impermeable to water and other aqueous solvents, and it is responsible for the antigenicity of the bacterial cell. Then you have the cell wall, which is present in the middle layer, and the cell wall is giving its response to the Gram staining, and the third is the plasma membrane. So you have the three layers: one is the capsule, the outermost layer; then you have the cell wall, and then you have the plasma membrane. And why the bacteria have such a complicated system is that bacteria have always been present in harsh environmental conditions; they could be present in water, in a strong acid solution, in an alkali solution, or in a solution where a lot of chemical toxicants are present.

So because these things are there, it actually is protecting itself by using all these layers. So the capsule is a very thick layer that is not going to allow these chemicals to get inside the cell. Then we have the cell wall. The cell wall composition in gram-negative and gram-positive bacteria is different. The cell wall has different constituents and is responsible for their reactivity towards the gram stain.

So we have two different types of bacteria: one is called gram-positive bacteria and the other is called gram-negative bacteria, and both of these bacteria have different types of cell wall compositions. And because of that, they will be differentially responsible for one of the classical stains, which is called the Gram stain, and because of the Gram stain, they are classified either as Gram-positive, which means the Gram-positive are actually going to give you the staining, whereas they will be Gram-negative if they do not give you the Gram staining. Based on this, only the gram staining classifies the bacteria as gram positive or gram negative, so let's see what different components are present in the outermost layer of the cell wall. What you have is a peptidoglycan layer, so this is the peptidoglycan layer you see here. The peptidoglycan layer is very thick in the case of gram-positive bacteria, and you see that multiple layers are present in gram-positive bacteria.

whereas it is very thin in the case of gram-negative bacteria, and because of this, it actually has a differential response to gram staining. Peptidoglycan is a polymer of NAG and NAM. NAG is N-acetylglucosamine and NAM is N-acetylmuramic acid. So NAG and NAM are actually the sugar molecules that are present, and these sugar molecules are connected to each other by a beta 1, 4 linkage. So these are the sugar molecules that have been attached to each other by a beta 1,4 linkage, and alternatively, you have the NAM block, which is connected to a NAG block, and then it has a NAM block like that.

So you have, you see, that it has one layer, then you have a second layer, you have a third layer, you have a fourth layer, and a fifth layer, and then these layers are being attached by the peptide chains. which are composed of the amino acid L-allylene, D-glutamic acid, L-lysine, and D-alanine, which means it is actually a combination of the L and D amino acids. These are the two different types of amino acids, and you know that the L-amino acids are more abundant in nature compared to the D-amino acids. So the peptide chain present in one layer cross-links to the next layer to form a meshwork that is responsible for the physical strength of the cell. So what you have is the NAM and NAG blocks, and then the second layer also has the same structure, and these layers are actually connected by peptide chains, and that's how they give tensile strength to the cell wall.

And that's why they are very, very robust, or they are very, very rigid in terms of accepting outside molecules, and the peptidoglycan synthesis is targeted by antibiotics such as penicillin. Whereas the lysozymes actually degrade the peptidoglycan layer by cleaving the glycosidic bond connecting the NAG and NAM to form the polymer, you have two options if you want to destroy the cell wall: you can actually use antibiotics. So if you put in the antibiotics, what the antibiotics are going to do is actually target the peptidoglycan synthesis. One of the classical examples is penicillin. The other option is that you can use an enzyme called lysozyme, which is actually going to degrade the linkage between NAM and NAC.

Lysozyme is a very important enzyme that is present in our tears as well as in saliva, and that's how tears and saliva actually protect humans from bacterial infections, because as soon as bacteria enter, irrespective of whether they are gram-positive or gram-negative bacteria. A lysozyme is actually cleaving the bond between the NAG and NAG, and that's how they are destroying the cell wall; once they destroy the cell wall, these bacteria are very, very susceptible to osmotic damage. So they will be very susceptible to the water, and they will be very susceptible to the tear-like conditions, and that's how they are actually going to get lysed and how they will die. So this is one of the strategies, and that's how people are trying to develop many antibiotics that are actually going to work on peptidoglycan synthesis. Apart from that, the cell wall also has lipoteichoic acid, so in addition to the peptidoglycan layer, you also have lipoteichoic acid present in the cell wall.

So lipoteichoic acid is only present in the gram-positive bacterial cell wall and it is an important antigenic determinant. So that's why, for lipoteichoic acid, our immune system is actually going to work, and that's how it is actually going to produce the response. Then we have the lipopolysaccharides or LPS. Lipopolysaccharides are only present in the gram-negative cell wall, and they are important antigenic determinants. So, compared to the lipoteichoic acid, which is only present in the gram-positive bacteria, in the gram-negative bacteria, you have the lipopolysaccharide, and that lipopolysaccharide is a very, very important antigenic determinant because that is actually going to induce the immune response in humans.

This is I have just given you a write-up so that if you are interested, you can actually read about Gram staining. Gram staining is a staining technique that was developed by the Danish scientist Hans Christian Gram. And as I said, Gram staining is for gram-positive bacteria, which take up the gram stain, whereas gram-negative bacteria do not take up the stain. We are more interested in reading the gram staining; you can go through this publication, and I have also given you a small write-up so that you can go through this particular write-up as well.

Let's move on to beyond the cell wall. So apart from the cell wall, they have the cytosol and the other organelles. So prokaryotic cells do not contain any membrane-bound organelles. The organelles present in the cytosol include the ribosomes, which are the 70S ribosomes, and the genetic material, whereas the electron transport chain and complexes are embedded within the plasma membrane. So within the plasma membrane, you have the electron transport chain; you will see the description of the electron transport chain when we talk about the mitochondria. Apart from that, the genomic material is present in the chromosome as well as in the extra chromosomal DNA.

So, prokaryotic cells contain genetic material in the form of a circular DNA known as the bacterial chromosome. So, the bacterial chromosome is different from the eukaryotic chromosome that is present in eukaryotic cells. It contains the genetic elements for replication, transcription, and translation. Bacterial chromosomes follow a rolling circle model of DNA replication. The genes present on the chromosome do not contain the non-coding regions which are called introns, and they are co-translated to the protein.

Besides main circular DNA, bacteria also contain extra chromosomal or extra circular DNA known as plasmids. So what you see here is actually a plasmid. These plasmids are called extra chromosomal DNA, which means they are actually important for the bacteria, but they are present as extra chromosomal DNA. The presence of a plasmid containing resistant genes confers resistance to the known antibiotic. Exchange of extra chromosomal DNA between the different bacterial strains is one of the mechanisms responsible for the spread of antibiotic resistance across bacterial populations.

So the plasmid is very important because it is the only genetic material that has actually been exchanged between different bacterial species, and that's how they are able to exchange their properties with neighboring residues. For example, if a bacterium has about 200 copies of a plasmid that actually provides resistance against an antibiotic, such as penicillin, it will give some of these plasmids to another bacterium that is sensitive to the antibiotic. So once these sensitive bacteria actually receive these plasmids, they will also be resistant to the antibiotics. That's why it is important that when people are working in laboratories or in bio-pharma industries, these plasmids have to be the plasmid-bearing bacteria that people generate while doing recombinant DNA technology, which has to be destroyed properly. So that the genetic pool of this plasmid does not go into the environment, and that's how there will be an exchange of genetic material or plasmid between the two bacteria; it is actually going to spread antibiotic resistance even in natural bacteria.

And that is one of the mechanisms through which the bacteria are acquiring resistance, and they acquire the resistance very quickly because of the exchange of plasmid material, and that's why it is important to study plasmids. So a plasmid is a circular DNA, and there are different forms of plasmids that are present when you are going to use the plasmids for the different types of treatment. For example, the bacterial plasmid is a double circular DNA molecule, and it exists in three different forms. If both strands of the circular DNA are intact, it is called covalently circular DNA.

So what you see here is covalently circular DNA. Whereas if one of the strands has a nick, then it acquires the conformation of open circular DNA. So if you are actually going to put the nick in one of the strands, like, for example, here, then it is actually going to acquire another conformation, which is called the open circular DNA or the OC form. This is called the triple C form. This is called the OC form, and the third is called the supercoiled form. During the isolation of the plasmid DNA from the bacteria, covalently circular DNA loses a few turns, and as a result, it acquires supercoiled transformations.

Interchange between these forms is possible under in vitro or in vivo conditions, such as when the DNA gyrase produces additional terms in the circular DNA to adopt the circular conformation. So, the bacterial plasmid is actually acquiring all these three conformations under in vitro or in vivo conditions, and that's how the different enzymes are working. For example, if you take the circular DNA and put the DNA gyrase, it is actually going to create the turns in this, and that's how it will generate the supercoiled DNA. But if you take the supercoiled DNA and treat it with topoisomerase, it is actually going to reduce the turns, and that's how it is going to be turned into covalently closed circular DNA.

Let us see one of the plasmids. These are the bacterial plasmids that have been very commercially available or widely used in recombinant DNA technologies. So plasmids are widely used for the cloning of foreign DNA into the bacterial system as a host strain. And this is the plasmid that has different types of components. One of the things that you have here is the origin of applications. So the origin of the application is a place from where the bacteria are actually going to start their replication.

Then it also has the antibiotic resistance genes; for example, here you see it has the antibiotic resistance genes for ampicillin. So if this plasmid goes to any bacteria, it is actually going to give resistance against ampicillin, the antibiotic. So, the antibiotic resistance genes or the enzymatic genes are responsible for causing the phenotypic changes in the host after the entry of the plasmid. Apart from that, what you see here is an enzyme that is also present within the plasmids. And because the plasmid has the origin of replication, it has antibiotic resistance and all these components.

They are independent, and that's how they are very, very, you know, dangerous because they can be independent; they don't depend on the nucleus for its replications or early activities. And that's why they can independently go to the new bacteria, and the new bacteria is also going to have the additional features that this plasmid is actually acquiring. Let's see how we can isolate the plasmid from the prokaryotic cell.

The plasmid isolation is a multistep process. It has many steps. So in step one, what you have to do is collect the bacteria. So first you have to take the bacteria, and you have to transform that bacteria with the plasmid, or suppose the bacterial plasmid is present in the bacterial cell. The first thing you have to do is grow these cells so that you have a sufficient number of bacteria. Then you have to do step one, you have to do a centrifugation, and then you have to re-suspend these bacterial cells in solution one. The first solution actually contains 50 mM glucose, 35 mM tris-HCl, and 10 mM EDTA.

So the method that we are discussing is called the alkaline lysis method. In step two, you are going to perform alkaline lysis; you will do this with the help of 0.2 normal NaOH and 1% SDS, which will lyse the cells and denature the DNA. Ultimately, you are going to do the third step, which is called renaturation. So the renaturation is going to be performed by the potassium acetate solutions and the glacial acetic acid solution.

And what will happen is that in this step, there will be renaturation. So that renaturation is actually going to renature the plasmid DNA, but it will not renature the chromosomal DNA, and because of that, the chromosomal DNA is actually going to be discarded in the next step. So when you are going to do the centrifugation of the chromosomal DNA, since it has not been renatured, it will precipitate and will be present in the form of a pellet, whereas the supernatant will contain the plasmid DNA. Then in step 4, you are going to just do the purification of this plasmid. So you are going to do the deproteinations, and that will result in the isolation of the plasmids.

That deproteination you are going to do with the help of the chemical called phenol-chloroform-isoamyl solution is actually going to remove the protein so that you can make very high purity plasmid DNA. In step 5, you are going to resuspend that plasmid in alcohol from the solutions. So that's what you are going to get in step 4; you are actually going to have the plasmid as well as the protein, and then what you are going to do is collect the supernatant, which will contain the protein. After that, you are going to add the alcohol to the supernatant, and once you add the alcohol, the plasmid will precipitate, and that's how you are going to isolate the pure plasmid. That pure plasmid can be used for different types of applications, which we are not going to discuss.

So let me give you a very real experience of how you can isolate the plasmids from the bacterial cell. So I will take you to my lab, where my student is actually going to show you a very small demo of how you can isolate the plasmids from the bacterial cell. Hello everyone, in this video we will show you how to isolate plasmid DNA using the alkaline lysis method. For the preparation of plasmid DNA, we need resuspension buffer, lysis buffer, and neutralization buffer.

In addition to that, we need isopropanol, RNase, and ethanol. Resuspension buffer contains 25 mM Tris and 10 mM EDTA, and we have to add RNase A at a final concentration of 100 micrograms per ml. Lysis buffer contains 0.2 N sodium hydroxide and 1% SDS.

Neutralization buffer contains 3 M potassium acetate, pH 6.0. For the isolation of plasmid DNA, we need at least an overnight-grown culture with an OD of 3.0. So this is already a cultured one. We have to harvest the cells by centrifugation. These vials, we have to centrifuge at 11,000 rpm for at least 1 minute to get the cells to precipitate.

Now that we have the cell pellet, we can proceed with the alkaline lysis method to isolate plasmid DNA. In the first step, we are going to add resuspension buffer, which contains RNase. Mix thoroughly until all the cells are suspended in the resuspension solution. So after the cells are completely suspended, we have to lyse the cells using strong alkaline conditions, specifically 0.

2 N NaOH and 1% SDS. Now we have to gently flip the tube in order to lyse the cells completely. We can keep it in this condition for up to 5 minutes, but not more than 5 minutes, which will degrade the plasmid DNA and also cause genomic DNA to come out, interfering with the process. In the next step, we have to neutralize the sodium hydroxide by using a neutralization buffer to prevent any further degradation. After adding the neutralization buffer, you can see that there is a white precipitate, which means all the proteins have precipitated by the neutralization buffer. You can flip the tube two or three times to completely precipitate all the remaining proteins.

Now the solution part contains our plasmid DNA, and all the precipitated material contains genomic DNA as well as proteins from bacteria. Now we have to centrifuge this lysate for 10 minutes at 11,000 g. The precipitate settled; now we have to transfer the clear white supernatant. This clear supernatant contains plasmid DNA, and now we have to precipitate this plasmid DNA with isopropanol, followed by washing with 70% ethanol.

We can see a white precipitate in the solution. Now we have to centrifuge it, collect the white precipitate, and wash it with 70% ethanol. After precipitating plasmid DNA with isopropanol, we will get a pellet of plasmid DNA. Now we have to wash the pellets. We wash this pellet with 70% ethanol.

Again, centrifuge the pellet; now we have the pellet. We have to air dry the pellet and dissolve it in deionized water or TEA buffer. We will leave it at room temperature until the ethanol has evaporated; next, we will add TEA. To ease the process of the manual alkaline lysis method, there are several kits available from commercial vendors. The basic difference between the alkaline lysis method and the kit-based method is that the kit-based method contains silica-based columns where the lysis lysate, which contains plasmid DNA, binds to these beads. After washing away the unwanted components, they will elute out, and we will elute the plasmid DNA in TEA buffer or water.

The composition of the lysis buffer is the same as the previous method, and the neutralization buffer also contains the same composition. But in commercial kits, we have one extra wash buffer that will remove any unwanted contamination and provide pure DNA. So when you see this demo, what you will see is that the students were discussing all four or five steps that we have just discussed. And after these steps, what you are going to get is actually the plasmid like this.

So what you see here are the three forms of the plasmid. You have the covalently closed circular DNA, which means the CCC forms; you are going to have the OC forms, and then you are also going to have the supercoiled form. So what you see here is actually the covalently closed circular DNA. This is the open circular DNA, and this is the supercoiled DNA. And whereas, since we have not used the RNase, you are also going to see some amount of RNA because RNA is also present in the bacterial cell.

So what we have discussed so far is the bacterial prokaryotic cells. We have discussed the structure of the prokaryotic cell. We have discussed how the cell wall is. The cell wall is thick in the case of gram-positive bacteria, whereas it is thin in the case of gram-negative bacteria. Apart from the cell wall, you also have the capsule as well as the plasma membrane, and all these anatomical barriers make the bacterial cell very resistant to environmental changes or the chemicals present in the environment.

So, with this, I would like to conclude my lecture. In our subsequent lecture, we are going to discuss eukaryotic cells. Thank you.