Cell and Molecular Biology
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Week 06
Genetic Material in Cells
Lecture - 22
Organization of Genome (Part 1)

Hello everyone, this is Dr. Vishal Trivedi from the Department of Biosciences and Bioengineering at IIT Guwahati. In the current module, we are discussing genomic DNA or genomic genetic material, actually. So, if you recall from the previous lecture, we discussed the different types of experiments and how people have figured out which biomolecule has the potential to carry information from one generation to the next. Continuing this discussion, we are now going to talk more about genetic material, its makeup, and how it is actually packed within the cells. So in today's lecture, we are going to discuss the genetic material and how it is actually packed into the tiny structure called the nucleus in eukaryotic cells, while in prokaryotic cells, it is packed into a non-nucleus structure as well.

So when we talk about the genome or the genetic material, what is the genetic material? The first question arises: What is the genetic material? So, the genetic material is a complete set of DNA comprising nuclear and mitochondrial DNA in an organism. And that is collectively called the genetic material. This is definitely not an acceptable definition as far as prokaryotic cells are concerned because prokaryotic cells do not contain mitochondria. So we are actually going to discuss prokaryotic structures, but in general, the most acceptable definition of the genome is that it is a complete set of DNA comprising the nuclear and mitochondrial DNA, which means it is actually the DNA or the complete set of DNA that is present inside a particular type of cell, irrespective of whether it is a prokaryotic cell or a eukaryotic cell.

It is hereditary material that is present in an organism. So the main purpose of the genome is to carry information from one generation to the next. But it is actually the hereditary material that is present inside an organism, irrespective of whether it is a prokaryotic cell or a eukaryotic cell. In the previous lecture, if you recall, we said that it could be DNA or it could be RNA, because in the case of so many organisms, it could be DNA or it could be RNA. The genome is the totality of chromosomes unique to a particular organism or any cell within that organism.

Each genome contains all the information needed to build and maintain that particular organism. So these are also very important points. Actually, there are two important

points here. One is that it is actually hereditary material that is present inside an organism, and the second is that it actually contains all the information needed for an organism to build and maintain itself. Which means it is actually going to have all the information, even about the developmental stages and how the organism will go through the different developmental stages; it is actually going to have that kind of information as well.

So that the organism will actually have the required changes in the body, and so on. For example, in human rights, you are actually born as a baby, and then you go through different developmental stages. After that, you reach puberty, and post-puberty, you become an adult. Even before birth, there are many developmental stages you will go through, and all these stages are completely governed by the genome present inside the organism. Now, the first question that arises is how the genome and the genotype are different.

Right. So there are many times when a student gets confused about what the genome is and what the genotype is. Right. So, the genome is actually the hereditary material or the total hereditary materials. What is present in an organism is called a genome, whereas a part of the genome is called a genotype. The information contained within the chromosome, or I would say a part of the chromosome, has actually been called the genotype.

For example, you can have the genotype for tallness, the genotype for dark skin, the genotype for brown eyes, the genotype for gray hair, and so on. So these are some of the properties that are actually going to be localized within a small portion of the genome. They are not going to be completely there will be no genome for that particular thing, right? So a genome is a collection of genotypes, and a genotype is a subset of that particular genome. Now the question arises: What are the different types of genomes present in different organisms? So we have the four categories of the genome, which are according to the organisms: you can have prokaryotic organisms, such as bacteria, and eukaryotic organisms, such as animals and plants. And then you can also have specialized types of organisms such as viruses.

So in the case of bacteria or, in general, prokaryotic organisms, you can have the prokaryotic genome, which is actually going to be double-stranded DNA, a circular chromosome, and it is also going to have the nucleoid. All of these we will discuss in detail. Whereas in the case of the eukaryotic genome, which is present in animals, you will have double-stranded DNA, you will have linear DNA, and it will actually be present in the form of many chromosomes. And all these are actually going to be present inside a confined structure called the nucleus. So this is the nucleus that is present, right? And

then, in the case of plants, you are going to have the main genome that is present inside the nucleus, just like in an animal cell.

And then you are also going to have the organellar genomes, which will be present in the mitochondria and the chloroplasts. And then we have the viruses, so we can have the viral genomes. So viral genomes could be single-stranded DNA, double-stranded DNA, or RNA. It could be circular, or it could be linear; then it is segmented or non-segmented. And then it is actually going to be monopartite or multipartite.

So all of these are the summaries of the properties of this genome. And the genome is actually organized inside a particular organism. For example, in a prokaryotic cell, the genome is distributed or present within the cytosol. Whereas in the case of eukaryotic cells, the genome is either present inside the organelles or is actually located inside a well-defined structure called the nucleus. So, how is the genome organized? So, the genetic organization of the genome is like this.

So in the cell, each DNA molecule is associated with the protein molecules, and each DNA molecule and its associated protein are called the chromosome. In any organism, the DNA is actually associated with protein molecules, and this particular structure is called chromosomes. This organization is actually going to hold true for prokaryotic or eukaryotic cells. In the eukaryotic cell, there are many types of chromosomes, and in the prokaryotic cell, the genome is very small, so it actually has a single chromosome. So this is just a classic example.

You are going to have the eukaryotic cell; you are going to have the nucleus. Within the nucleus, there is DNA. So, this is the DNA, and this DNA is actually going to be associated with different types of proteins. And that's how the DNA is actually going to be condensed in the form of different levels of organizational condensation. And then ultimately, it is going to form the chromosomes.

This is a similar kind of organization even in prokaryotes. The only difference is that prokaryotes will have only one chromosome, which is a circular chromosome. And the packaging of DNA into the chromosomes. So the DNA is actually going to be packed into a dense material, and that is going to be called a chromosome. The chromosome is actually going to have DNA, and it is also going to have proteins.

And protein actually plays a very crucial role in packaging DNA into the form of chromosomes. So, the packaging of DNA into the chromosome serves several important functions. Chromosomes are a compact form of DNA that readily fit into the cell, right? This is the way we are going to discuss it in detail. Then it protects the DNA from

damage, and only packed DNA can be transmitted efficiently to both daughter cells when a cell divides. Since it is a packet, you can actually share this packet between the daughter cells.

Very precisely, if it is loose DNA, then there is a possibility that you may actually share 50%, 75%, or 80%, and so on. But if it is a packet, you will either share the complete packet, or you will not share it. So that actually provides regular flexibility as well as regulation, ensuring that the DNA is actually going to pack into the form of a chromosome. Now the question is: why? There is a need to pack the DNA. So why is the packaging of DNA required? So DNA is packaged into a form of chromosome, and then this packaged DNA is actually required for many reasons.

Number one, it is actually going to be required for DNA computation. It has actually been required for DNA protections. It has actually been required for the regulation of gene expression. It is also required to facilitate DNA replication and repair. Then ensure accurate chromosomal segregation, and lastly, it is also required to enable the regulatory interactions.

So what is meant by DNA compaction? DNA computation by packaging the DNA into compact structures such as nucleosomes and higher-order chromatin fibers significantly reduces the physical size of the DNA molecule. You know that, for example, the human genome, right? The human genome is approximately the size of one meter of fiber, isn't it? So if you have a 1-meter fiber and you know that the size of the right cell is approximately 30 micrometers, right? So if you have a cell of 30 micrometers and a genome of 1 meter of fiber, it cannot fit into this, right? It cannot be fit into this, so to fit it, you are actually required to compact it, to pack it into such a dense material that it will actually fit into this particular size. So, that is the purpose of packaging DNA into chromosomes. Then the second point is that it is actually going to provide protection for the DNA. So, the second point is that it is actually going to provide protection to the DNA.

So the densely packed chromatin structure shielded the DNA from exposure to potentially harmful agents, such as chemicals, radiation, and enzymes. It also helped prevent the DNA from becoming tangled or broken during the cellular processes. So you can imagine that if I have DNA, which is loose DNA, right? If it is DNA that is loose, it is actually accessible to all sorts of damaging materials. For example, if you are exposed to free radicals or if you are exposed to hydrogen peroxide, it will actually have direct access to the DNA, and we will discuss this in our subsequent module. When we discuss DNA damage and repair, there are several types of DNA damaging agents.

One is free radicals, and the other could be alkylating agents, such as the drugs you are taking. And if the DNA is not properly packed, it will be exposed, and that's how it is actually going to get damaged. It's going to be damaged because there are modifications to the nucleotides and so on. This is completely being protected by if you have the DNA. And this DNA is surrounded by protein molecules.

So now what you have is that if you have even these molecules, they will actually interact with the proteins rather than the DNA. So the drug will go and interact with the DNA because the DNA will not be accessible, as it is surrounded by different types of proteins. And that's how DNA is protected. Now, the third point is the regulation of gene expression. You know that gene expression is a very tightly regulated process.

So if gene regulation is not being done, then it is actually going to have very significant negative effects on the health of that particular cell. For example, you took the food. And you have taken the meal correctly; you have taken the food, and it has produced glucose, right? And the glucose in the blood has increased the blood glucose level. Now, if I have to tackle this problem, what I have to do is secrete and synthesize a large quantity of insulin.

Right. That means as soon as this occurs, I have to perform gene expression profiling. I have to change the gene expression profile in the pancreas. And as a result, what will happen is that the pancreatic beta cells are actually going to start secreting the insulin that is going to affect some of the effector organs, such as the liver and muscles, and that's how they will convert the glucose into glycogen, right? And that's how they are actually going to protect the body from the harmful effects of having very high levels of blood glucose. Now, this is temporary, right? This effect is temporary because, after some time, the blood glucose level will reach a normal level. And then if this process continues, it will actually go down to that level.

Right. For example, if the blood glucose level is 80 milligrams per deciliter, which means 80 milligrams per 100 ml, then it is the normal level; however, as soon as you have eaten food, the level will go up, for example, to 200, and from 200, it will return to 80. But if this process continues, it will continue to come down, right? It will come down to 50, right? And it will come down to zero if this continues because the insulin does not know that there is enough glucose, right? So there is a regulation of gene expression required, isn't there? As soon as this reaches 80, the blood glucose level will actually indicate to the pancreatic cell that no more insulin is required, and that is how it will change; again, it will change the gene expression profiling, and that is how there will be no secretion of insulin. And that's why it is very important to maintain the normal physiology of an organism. So, the regulation of gene expression and DNA packaging

can influence the accessibility of the gene to the cellular machinery involved in gene expression, such as transcription factors and RNA polymerase. By compacting or loosening the chromatin structure, cells can control which genes are accessible for transcription and thus regulate gene expression patterns.

So we have discussed how gene expression profiling is going to have a significant effect on the overall physiology of that particular organism, and it is completely influenced by whether the DNA is present in a compact structure or not. Because if the gene is present in a compact structure and is not accessible for the cellular machinery to perform transcription and translation, then it will not be transcribed. On the other hand, as soon as you would like to have the downregulation of a particular gene expression, you just put that gene into a tight, compact structure. And that's how it is actually going to control the overall gene expression of that particular protein. The fourth point is that it is actually going to facilitate DNA replication and repair.

This is anyway we are going to discuss in detail when we talk about DNA damage, repair, and replication. So, during the DNA replication and repair process, the packaging of DNA into the nucleosome must be temporarily loosened to allow the necessary proteins and enzymes to access the DNA strands. After replication or repair, DNA is repacked into nucleosomes and higher-order chromatin structures. Hence, proper genome packaging ensures accurate replication and repair of DNA. So this is very, very important that we have the packaging and unpackaging of the chromosome so that some amount of DNA is open and then that DNA is replicated.

Point five is that it actually ensures the accurate chromatin segregation. So, during cell division, the genome must be accurately divided between the daughter cells. The compact organization of the DNA into chromosomes facilitates this process. This, anyway, we have discussed in detail, haven't we? If you have a single chromosome, you will divide it and make two chromosomes, correct? And then you are actually going to divide these chromosomes equally, right? You are going to give one to the sister or daughter cell, right? And one you are going to keep along with the parents, right? If it is not compact, if it is DNA, then the division could be 70-80%; it could vary because some amount of DNA you will put into sister or daughter cells, and some amount you will put into parent cells, and so on. So, there is a possibility that the daughter cells will have 1.

25 copies of the genome and the parents will be left with only 75%. So this kind of possibility should not exist. That's why the DNA is actually going to be packed into packets. So you will take one packet for yourself and then you are actually going to give one packet to the daughter cell. And that's how there will be an equal division of genetic material between the two daughter cells.

Then we have the enables the regulatory interactions which is the next point. The 3D organization of the genome within the nucleus allows for the specific regulatory interaction between the different regions of DNA. This special organization facilitates the long-range interaction between the enhancer region of DNA. So this is all about why DNA is required to be packed into a compact structure to form chromosomes. Now let's see how you can have this kind of packaging in the different types of organisms.

We'll start with the prokaryotic organisms. So first, we are going to discuss how the genome is organized into the prokaryotic structures. So the genome organization in a prokaryotic cell is typical, where you have the cell wall, the capsule, the plasma membrane, and inside the plasma membrane, you have the cytosol and the nucleoid. The nucleoid is the region where all the genomic content is present, and you also have the ribosomes and plasmids. And you are going to have all this kind of flagella and all that, right? So, they have the small bodies and a small genome right. So, you know that the chromogenomic bacteria are very small, right? So, they are going to have smaller genomes.

They do not contain a nucleus or any other membrane-bound organ. This is all we have discussed when we were talking about the cellular structures. Then they have a small circular DNA molecule that is present inside the nucleoid region. So, this is the nucleoid region where the genomic content of the bacterial cell is going to be present. They have a single chromosome that floats within the cytoplasm.

The genome size ranges between 10 to the power of 4 and 10 to the power of 7 base pairs with a high gene density. Apart from this single chromosome, some bacteria have extra chromosomal DNA, which is called plasmids. We have also discussed the plasmids when we were talking about the bacterial cell, and we have also discussed how you can isolate the plasmid from the bacterial cell, and we have shown you a demo video on how to do that. In a prokaryotic cell, you are going to have the chromosomal DNA as the genomic content. The second is that you're going to have the extra chromosomal DNA, which is also called plasmid.

So let's discuss the plasmids. So prokaryotes frequently carry one or more smaller independent extra-chromosomal DNA molecules, which are called plasmids. Plasmids are not genomic DNA. They are accessory DNA molecules, smaller circular DNA molecules that have the ability to self-replicate. Unlike the larger chromosomal DNA, plasmids are typically not essential for bacterial growth. So, TP plasmids are actually required for providing specific properties, and by the plasmids, they can exchange that property between particular bacterial colonies; for example, there could be a property like

resistance against.

So, if a bacterial colony has acquired resistance to ampicillin and that property has been captured in the form of a plasmid, then it can actually share that plasmid within the colony, and that is how it can distribute that particular property among the organisms. What is the importance of plasmids? So plasmids provide advantages to the bacteria, such as antibiotic resistance, herbicide resistance, etc. So all these properties are actually due to the different types of antibiotics and different types of genes, and all these genes are actually going to be cloned within the plasmids, and that's how the plasmids are actually going to express the protein and provide the necessary resistance mechanisms. Within the cell, right, and that's how the bacteria can actually provide that particular plasmid to the colony, and that's how the colony is actually going to be resistant. In addition, unlike chromosomal DNA, plasmids are often present in many complete copies per cell.

So, unlike the chromosomal DNA, bacteria will not have a single colony or single copy of the plasmid. It could have 200 copies, it could have 500 copies, and so on, because the number of copies will decide which bacteria will have the higher resistance property. So, if you have a very high number of plasmids, you can have a higher resistance to that particular antibiotic or that particular type of phenotype. Then we will talk about the bacterial genomes.

So, the bacterial genome is very small. So, bacterial chromosomal DNA is usually a circular molecule that is a few million nucleotides in length. For example, in the case of E.

coli, you have 4.6 million base pairs. Similarly, you have H. influenzae. H. influenzae is going to have 1.8 million copies.

So it is actually a small genome that is present in the bacteria. And then, a typical bacterial chromosome contains a few thousand different types of genes. Unlike eukaryotic organisms, you are not going to have any useless genes. You are not going to have the other types of non-expression genes, actually. So bacteria only contain the genes that are going to be expressed and that actually have some meaningful effect or purpose inside the cell because, you know, their size is very small, so they do not want to retain unwanted materials. Then structural gene sequences account for the majority of bacterial DNA and the encoding of proteins.

The untranscribed DNA between adjacent genes is termed intergenic regions. And these regions are very small or almost absent in the bacterial systems. Then, since you have the DNA, you have to pack this DNA into the form of a chromosome so that you can achieve

compaction and make the structure very compact. So, in the packaging of DNA, prokaryotic cells usually have a smaller genome that needs to pack its DNA, which is still substantial.

You know that we bacteria are a few microns in size. So, their DNA size is relatively large as well. So it has to be compacted. E. coli must pack its 1 mm chromosome into a cell that is only 1 micrometer long. It is less clear how prokaryotic DNA is compacted, but it is actually packed into a small structure or within the cell.

So, the region where it actually packs the DNA is called the nucleoid. So, the nucleoid is a primitive nucleus, or I would say it is actually a primitive nucleus, except that it is not going to have a membrane, right? So, it is not a membrane; it is a region in which the bacteria are actually going to have chromosomal DNA. A prokaryotic chromosome is circular and resides in a cell region called the nucleoid, containing only one complete copy of its chromosome that is packed into the nucleoid, with eighty percent of the DNA able to be unfolded by agents that act on RNA or proteins. The proteins responsible for condensation and maintaining the support coil structure of the DNA have not been identified, so it is still unknown how the different types of proteins are involved and what the various types of proteins are that contribute to making the structure very compact. The type of protein found in the prokaryotic chromosome, known as the nucleoid-associated protein, is responsible for compacting the DNA into chromosomal structures. DNA determines which proteins and enzymes an organism can synthesize and, therefore, what chemical reactions it can carry out.

So what is the function of the genome in any organism that actually determines the proteome of that particular organism? It is actually going to decide what different types of proteins and enzymes will be produced, and that's how it will eventually be decided. The metabolism and physiology of that particular organism are important. So, this is the micrograph of a bacterial cell where the nucleoid is actually shown. So this is actually the region where the nucleoid is present, and within the nucleoid, what you're going to have is the bacterial chromosome, which is actually super quiet. What the key features of the nucleoid are is that most, but not all, bacterial species contain circular chromosomal DNA.

A typical chromosome is a few million base pairs long. Most bacterial species contain a single type of chromosome, but it may be present in multiple copies. Several thousand different genes are interspersed throughout the chromosome, and one original replication is required to initiate DNA replication. So anyway, we are going to discuss when you will talk about the origin of applications. So the origin of applications in the case of the bacterial chromosome is singular. So it is actually going to start here, and it is actually

going to go through, and then it will come here, right? And you know that it is actually going to go in both directions.

So this is going to be the leading strand, and this is going to be the lagging strand. And that's how it is actually going to produce two copies of the genome after one cycle. One will come from this side, and the second will come from the other side. So second will come like this: So that's why it is actually going to have one original copy and one replica copy.

The short repetitive sequence may be interspersed throughout the chromosomes. The chromosomal DNA must be compacted about 1,000-fold. Remember that 1 mm will actually need to be compacted within a 1-micrometer diameter. So it actually has to be compacted to around 1,000 times. The formation of the loop domains and the number of loops vary according to the size of the bacterial chromosomes and the species.

For example, E. coli has 50 to 100 strains with 40,000 to 80,000 base pairs of DNA in each. So you're going to have a circular chromosome, and then this circular chromosome is actually going to be looped, and it is going to be compacted by doing this. And that's how it is actually going to form very strong structure. Well, let us take an example of a bacterial chromosome from a bacterial species to see how the chromosome is structured.

So, in the case of E. coli, the E. coli chromosome is compacted to one fifth of a volume, right? The determinants of nucleoid folding are. So, negative supercoiling by the topoisomerases and the condensation caused by the attachment of the nucleotide structure proteins occur. The nucleoid is highly condensed during rapid growth. RNA polymerase concentrates in the transcriptional loci, and RNA polymerase is distributed throughout the chromosome. So this is the bacterial cell where we have already discussed the different types of parameters.

So this is all about the genomic material, and what we have discussed is the importance of genetic material and the different types of genetic material present in different types of organisms. So we have taken an example of the prokaryotic structures. Then we have also discussed the eukaryotic structures, and we will also discuss the viruses. In the case of prokaryotes, you have double-stranded DNA, which is present in the cytosol in the form of a circular chromosome. And then, apart from that, you're also going to have the extra chromosomal DNA in the form of plasmids.

In the case of a eukaryotic cell, you're going to have either the genetic material present inside the nucleus or it is actually going to have the genetic material that is present inside the organelles such as the mitochondria or the chloroplasts. In the case of viruses, they

can have single-stranded DNA, double-stranded DNA, or RNA, and the viruses are unique organisms, so they actually have different types of physiology and different types of manipulation of their genetic material. Lastly, we have also discussed how the genome is organized within prokaryotic structures and how compaction occurs inside the genome in the prokaryotic cell. So with this, I would like to conclude my lecture here. In our subsequent lecture, we are going to discuss some more aspects related to the genomic material or the genomic content. Thank you.