Molecular Biology
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Module - 05
Replication
Lecture-21 Replication in Prokaryotic System (Part 1)

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT Guwahati and what we were discussing we were discussing about the different aspects of molecular biology in this particular MOOCs course. So, so far what we have discussed we have discussed about the cellular structures, we have discussed about the structure and functions of the different types of biomolecules where we have discussed about the DNA, RNA, protein and enzyme and in the previous module we have also discussed about the central dogma of molecular biology. So, we have discussed that how the different types of cellular activities are dependent on the protein synthesis and how the protein synthesis is being regulated by the level of production of RNA and then by the production of RNA from the DNA. So, when we were discussing about the central dogma of molecular biology we discussed about that that it is actually a regulated event of the three different types of activities where you are actually going to have the production of DNA from the pre-existing DNA and this process is known as replications whereas, the DNA is actually going to produce the RNA and this process is known as transcription and the third is the once the RNA is available then it is actually going to be translate into the protein and all these events are being catalyzed by the different types of enzymes.

So, replication is been catalyzed by the DNA dependent DNA polymerase whereas, the transcription is been catalyzed by the DNA dependent RNA polymerase and the translation is being done by the ribosomes. So, in today's lecture we are going to start discussing about the first event and then the first event is the synthesis of the new DNA from the pre-existing DNA. If you recall when we were discussing about the cell division we discussed that during the S phase the DNA is going to be synthesized and then the you are going to have the two copies of the same DNA and then these two copies of the same DNA is actually going to be shared between the cells and that is how the cell is actually going to replicate and cell is going to divide. So, synthesis of the new DNA molecule from the pre-existing DNA molecule is known as replications.

Now, replication is a important event because it allows the synthesis of so replication means the synthesis of the DNA from the pre-existing DNA this means this is going this you can imagine that this could be a DNA of the parents and this could be a DNA of the offsprings. And you can understand that if there will be any this whole process has to be

done with lot of precision with lot of accuracy. So, that you are not going to carry the bad information right. So, you want to carry the same information what is present in this particular DNA and all the other hand it has to be complete in a given time period. So, replication has the two task one is the synthesis of the new DNA considering old DNA as template.

And the second is it should be accurate. So, there should be so it should be almost the exact 100 percent replica of the older DNA there should be no mutations or there be no substitutions there should be no alterations and all that. So, to achieve this the machinery has to be very robust machinery has to be very you know should have the components. So, that it should be able to do this job do the synthesis job, but at the other hand it should also have the components. So, that it should be able to do a quality checking and the replication is also related to the genome right.

So, prokaryotic genome and the as well as the eukaryotic genome if you recall in the previous lecture we have discussed about the prokaryotic genome and the eukaryotic genome and there is a significant difference between the prokaryotic genome and the eukaryotic genome right. And as a result the machinery is also different between the prokaryotic system and the eukaryotic system. So, in the prokaryotic system we have the different types of components which are required for synthesis of the genome whereas, in the eukaryotic systems because the eukaryotic genome is more complex you require the different types of machinery. So, because the machinery is different I have you know splitted this particular thing into two components. So, that you are we are going to discuss first about the prokaryotic replication and then we are actually going to discuss about the eukaryotic replication.

Because when we will discuss this in separate or when we are going to discuss about the prokaryotic versus eukaryotic you will understand that how the machinery is been adopted from the prokaryotic system to the eukaryotic system. So, that it is more efficient and it is actually bringing the more accuracy in the things. So, the first question comes what is replication? We already discussed right. So, DNA replication is a biological process that helps to transmit the DNA or the genetic information from the generation to generation by producing the two identical DNA strands of the daughter cells from the double standard parental DNA. This anyway we have discussed.

What is the importance of replications? Application is a way of duplicating all the genetic information from the all living parent organism to the daughter organisms thereby helping maintain the genetic materials intactness and the organism survival. Now, if this is such an important event and it may be depend on the type of DNA you would like to do the replications and so on. It is important to understand the genetic

material of the prokaryotic system because we are going to start the replication of the prokaryotic system first. So, prokaryotic genetic material remember that when we have discussed in detail about the prokaryotic replications prokaryotic genetic material. So, prokaryotic genetic material is a single chromosome and it is mean mostly been found in the cytoplasm right.

So, prokaryotic genome can be found in the cytoplasm. They are negatively super coiled and circular. Generally they can be found into a singular number. However, exceptions exist for example, the Vibrio cholera has the two copies of the genome right. So, two genomes prokaryotes such as E.

coli contains a self replicating extracellular genetic material which is also called as plasmid. So, you have the circular genome right or circular chromosome. So, it is called chromosome right. So, see you have a single copy of the chromosome which is negatively super coiled and it is a circular DNA. And mostly there are only one copy of that circular genome is present in the prokaryotic system, but there are exceptions that in the Vibrio cholera you have the two genomes.

Apart from this chromosome you are also going to have the extra chromosomal self replicating genome and that is called as plasmids. And when we talk about the genetic material we are actually going to talk about the submission of the chromosome and as well as the plasmids. Now, as far as the plasmids are concerned plasmids are of small size DNA it is only in the 1500 to 2000 kB. Therefore very small compared to the actual prokaryotic DNA they contains the origin of applications and it replicates independently. So, plasmid does not require any kind of help from the chromosome they are independent self replicating extra chromosomal genetic material.

Now let us first ask the questions how you can actually be able to have the replications right. So, there are three different modes which are being proposed as far as the replication is concerned. So, replication can be done in the in the three different mode one is called as the conservative mode, the second is called as the semi conservative mode and the third is called as the dispersive mode. So, in a conservative mode conservative mode produces that or if it is conservative mode then it produces that two DNA helices from the one single original DNA helix. One helix contains the downright prior ends DNA while the other contains the entirely new DNA.

Accepted postulate, but does not have that much significance. So, what is mean by the conservative mode is that from the parental DNA you are going to have after replications. So, if you are going to do the replications you are going to generate. So, for example, from the two strands you are going to have the four strands right. So, four

strands are actually going to be segregated and as a result you are going to have for example, here you have 1 and 2.

So, that 1 and 2 will go into the parents whereas, the newly synthesized 3 and 4 will go into the daughter. So, as this is called as the conservative mode. In a conservative mode there will be no mixing of the content from the parents DNA. So, there that is why in a second round of replications you are going to have the parent DNA and you are also going to have the daughter DNA separately. This means there will be no mixing of the previous copy and it is actually going to have the pure DNA present in the parent DNA.

Then the second mode is called as semi conservative. So, one of the two helices forms each containing the one new and the one parental strands according to the Watson and Crick one strand serves as a template during the replication mode. The newly accepted postulate as the DNA polymerase needs a strand to form a new complementary strand. So, what is mean by the semi conservative mode is that from the parent DNA for example, you have the parent DNA as 1 and 2 it is actually going to do the replications and once you do the replications it is actually going to form the four copies right 1, 2, 3 and 4. So, 1, 2 is the original copy 3 and 4 is actually going to be the newly synthesized copy because the one from the one you are going to have the copy number 3 from the two you are going to have the copy number 4.

Now in the semi conservative mode the one is actually going to make a pair with 4 and the two is actually going to make a pair with 3. So, this is actually going to be 1, 4 and 2, 3 right. This means there will be a dilution of the genetic material of the parents and that is how the parents are actually going to share the 50 percent copy with the offsprings. That means, the two offsprings what are going to be produced after the first replication or the two DNA molecule what is or going to be formed after the first replications are actually going to have the mixture. It means going to have the 50 percent from the parents and the 50 percent of the newly synthesized DNA.

And this continues because even if I have a second replication this continued right and you are going to have the that is kind of dilution right. So, in the first replications you are going to have the mixing of the DNA from the parent DNA. So, you are going to have the two strands of the parents which are going to be diluted. Now, the third method is called as the dispersive. So, this would generate the DNA helices with the alternating pattern of the old and the new DNA segments and it does not have any kind of biological significance.

So, what this mean is that it is in the dispersive what we it says is that you are going to have the parental DNA and after the replication 50 percent portion is going to be

replicated with the original DNA and the 50 percent portion is going to be replicated in the with the new DNA. This means you are going to have a mixture of the both the strands and even the both components. This is not acceptable because it is not possible because when you have the copy number 1 and 2 you are going to have the generation of the 3 and 4. You are not going to have the 1 and followed by 3 and followed by 1 followed by 3 like that. So, that kind of scheme is not possible because once the DNA polymerase will sit and start synthesizing the DNA it will not come off and if it comes off then it is actually not going to have this particular type of pattern.

So, this is the most unacceptable method. Now, the question is we have the three different modes right. We have the conservative mode, we have the semi conservative mode and we have a dispersive mode and these are the three different types of model what has been proposed how the replication is going to happen right. But the question comes how scientifically we can be able to prove this. So, to prove this the there is a simple experiment and the classical experiment what is being done by the some of the scientists.

So, the Meselson star or Matthew Meselson and Stahl actually performed the experiments where they have asked what would be the mode of replication in the bacterial system. So, what was the aim? The aim was to establish which method is applied into the prokinetic replication is it the conservative mode or semi conservative mode or the dispersive mode right. So, to ask these questions they have prepared the they have designed an experiment right and these are the requirements. So, you require a media you required a media to grow the E. coli for several generations and the media is having the two different types of media.

You are going to have the media with the standard form of nitrogen that is the N14. So, I will say this is the normal nitrogen that is the N14 nitrogen and then you also require a medium with the rare and the heavy form of nitrogen and that is called as N15. So, these are the two different types of isotopes what you can actually use. One is the normal normal nitrogen or the heavy nitrogen and then you can actually be able to you require the analytical reagents right. So, you can actually require the techniques so that you can be able to separate the DNA what is being formed by the N14 versus N15.

So, you require a cesium chloride gradient to get the separate DNA based on their density. Remember that this is heavy and this is light. So, it is actually going to have the different types of densities and that is why N14 can be separated from the N15 it is if it is present in the same mixture. So, if you have the pure DNA it is of the N14 or the pure DNA of N15 it is going to be formed the different bands into the cesium chloride gradient mixture. And if you are not sure about the gradient centrifugations remember

that we have already discussed that technique while we were discussing about the cell cellular fractionations and we have discussed about the density gradient centrifugations.

So, there we I think we have taken an example of the sucrose, but here we are they are using the cesium chloride. So, that is the only difference and you know that the gradient can be up in the upward direction or the downward directions. And we have also discussed in detail about the principles and how the things are getting separated when you are running them onto the gradient. So, gradient is actually going to separate the molecule based on the their density. So, this is the procedure what you are going to follow right.

You are going to grow the culture of E. coli N15 isotope media for several generations. So, that the heavy isotope is incorporated into the purine and pyrimidine bases remember that both the most of the nitrogenous bases are made up of the N15. So, you when you grow them into N15 media all your purine and pyrimidines are actually going to be get labeled means all the nitrogen what is present as N14 is actually going to be replaced by the N15. So, all the DNA is going to be heavy DNA.

And then some of the E. coli from the heavy isotope media is taken and transferred into the normal media containing the N14 as the nitrogen source. So, now what will happen is that when it is going to synthesize the new DNA molecule it is has to utilize this N14 right. So, based on the density gradient the DNA band will be generated into the centrifuge tubes. For equilibrium density gradient analysis the DNA are collected from the media and put it into the 6 molar cesium chloride. DNA sample in heavy salt gradient are taken into centrifuge for 50 to 60 hours at 1 lakh g rotations.

So, this is what exactly going to happen when you are actually going to have the E. coli into the N15 media you are going and when you run them onto the onto the cesium chloride density gradient what you will see is that it is actually going to form a separate band and this band is for the N15. So, yes when you are going to transfer that into the N14 you are going to have the bands which is of the intermediate density. So, this is the band this is the region where you are going to get the band for the N14 and this is the region for the N15. So, when you are going to grow the media when you are going to grow the bacteria into the normal media after N15 media it is actually going to have the N15 band into the intermediate spin right intermediates position it is not going to be related to the N15 or the N14.

Now if you grow the further right if you go for several more generations then what will happen is that you are actually going to start getting the band which is corresponding to the N14 which means there will be some DNA what is going to be served as the template

of the N14 and that is how you are going to have the intermediate DNA, but you also going to have the N14. Now this will continue and that is how this is going to be the final product. So, if you are only growing the bacteria into the heavy media you are going to have the single band at the N15, but you if you are running it at the into the N14 media. So, the DNA is initially been N15. So, N15 DNA is been transferred into the N15 labeled DNA containing bacteria when you put it into the normal media it is going to have the 50 percent N14 and 50 percent N15 and as a result after the first generation you are going to see a band which is intermediate So, this is actually N14 by N15 media right and this is going to be the intermediate DNA.

Now if you continue the for the several more generations then it is actually going to form the N14 dimers and as well as the N14 and N15 intermediate. So, it is actually going to form the intermediate DNA band and as well as the N14 light bands and this will continue because this is the actual thing right. So, this remember that when we were talking about the semi conservative mode what we said is that if you have this DNA right and you have the two strands at one and two and after the replication what will happen is that it is actually going to form the four strand right it is going to form two strands and it is going to form the four strands right. So, one is going to make a pair with four and two is going to make a pair with three this means the imagine that this is N15. So, if this is N15 so this is going to be N15 this is going to be N15 right.

Now here the new DNA what is going to be formed is not going to be N15 because there is no N15 available into the media right. So, what will happen is that the N1 strand which is going to be N15 the other strand is going to be N14 right and same is true here right N15 and N14 and that is why it is actually going to give you a intermediate density. Now if this will go for another generation then this N14 is going to replicate this N14 is going to replicate and N15 will again going to have another copy. So, this is actually what happen is that when you are going to have another replication then N14 and N14 and you are going to have the four DNA molecule right.

So, you are going to have the N15 as an N14 right. So, two copies of this and you are going to have the two copies of this right. So, he this is actually going to give you a band which is corresponding to the N14 and this is your this is going to give you a band which is for the intermediate. So, that is all you are going to get this actually here in the subsequent generations. So, what you can conclude from this is that whenever you there will be a DNA applications going to be impure it is going to give you a DNA which is impure. So, it is going to have the original copy and it is also going to have the new copy.

So, out of these three proposed replication mode the semi conservative mode is the

selected mode that can be observed into the prokaryotic system and as well as the eukaryotic system. Now let us talk about the replication machinery. So, first is what is replicon? So, replicon is the region of the DNA what is going to be participate into the replication reactions. So, replication replicon or the small stretch of DNA which is going to be involved into the replication is a DNA segment of the spore period that undergo replications. Replicants what is there in the replicon? So, replicon is going to have the origin of the application and it also going to have the terminations regions.

For example, the E. coli has one replicon on its genome. So, it is going to be mono replinonic which means it is going to start from one side and it is going to end on to the one side. So, it is going to have the mono replinonic whereas, eukaryotes have the multiple origin of replication hence they are multi replinonic. So, this means if it is a equal if it is a eukaryotic region eukaryotic genome then you may have the multiple origin of replications and why it is so, because the eukaryotic genomes are big compared to the prokaryotic genome. So, they can actually afford to grow with go with the single origin of replication.

So, that by the time the replication is going to be over they are also going to have the synthesis and the other kinds of preparations. So, let us first talk about the origin of replications. So, origin of replications as the name suggests one the origin of replication represent the starting point of the replication in the prokaryotes. It is approximately 245 base pair 80 rich regions cis acting sequences. What is mean by the six acting sequences? Six acting sequences is that they can affect only the molecule of the DNA in which they reside.

So, cis acting means they are actually going to affect within that molecule. Second question is why it is 80 rich? So, 80 rich sequence is preferred because 80 rich is easy to melt. We know that A is making 2 base pair with T whereas, G is making 3 base pair with C. So, if you have the G into GC regions then it is you are supposed to break the 3 hydrogen bonding and it is difficult to break the 3 hydrogen bonding compared to the 2 hydrogen bonding. So, for melting or unwinding of duplex DNA less energy is required to break the hydrogen bonding than the GC sequences.

Melting of duplex DNA is ATP hydrolysis dependent as the energy released helps break the hydrogen bonding between A and T. Above mentioned 80 rich sequence is recognized by the enzyme which is called as DNA helicase to initiate the unwinding process. So, this is the typical original applications where you are going to have the 200 base pair 245 base pair long stretch and it is going to have the 80 rich region in the in the region and it is going to have the DUE and DOR. DUE means the duplex unwinding element whereas, DOR is called as DNA oligomerization.

So, the origin of the application in E. coli is known as the ODC. It contains the 2 short repeat motifs like 5 copies of the 9 mer sequences spread throughout the origin of the application DOR site serve as the DNA binding site of the DNA A which is a replication initiation protein and the 3 copies of the 13 base pair or 13 mer 80 rich repeat which is called as DO site where the DNA starts unwinding. So, upon binding of DNA A at the 9 mer region the 13 mer region starts melting. So, origins ORE of C contains the 11 copies of 5 prime GAT C 3 prime repeat methylated on that on both strands and only complete methylation can lead to the initiation of applications. Hemimethylated origins cannot initiate the replication until it is fully stored or methylated.

So, this is important that the methylation is also going to control the replications because remember that the methylation is the defense mechanism. We are going to discuss about some of these enzymes. So, which are I think going to be used in the molecular cloning. So, restriction enzyme restriction methylate system is a kind of a defense system and that is how the machinery is not going to initiate the replication until the DNA is fully methylated. If the DNA is not methylated, hemimethylated or unmethylated then it is not going to be replicated because it is considered to be host it is going to be considered to be DNA of the external origins.

Then we have the replication forks. Replication fork is going to be formed and that is how it is actually going to initiate. So, once the DNA is been melted at the origin of replication it is going to form the replication fork. So, the Y shape structure is generally found when the DNA starts melting and opening up. As the DNA open up bi-directionally at the origin of C2 replication forks are generated. Extension of the two oppositely directed replication fork leads to a replication bubble.

So, when the replication fork moves in this direction and as well as in this direction it opens the DNA in both the direction and as a result it is actually going to form a bubble like situation. Replication fork is going to move in both this direction and this is going to be called as replication bubble. And this replication bubble will move in both the direction where it is actually going to start synthesizing the new DNA. So, this is going to serve as a template for the this machinery and this is going to be served as the machinery for the this replication. So, you are going to have the one machinery which will run in this direction and they were going to have another machinery which will run in this direction for and that for it is actually going to have the replication of the both the origins, both the sites.

So you are going to have the replication in this direction and you are going to have the replication in this direction. To maintain the single standard situation the single standard

DNA binding proteins are going to code the single standard DNA to prevent the rewinding of the double standard DNA. So, these are the some of the components what are going to be present within the DNA structure. So, you are going to have the replication original replications and then you it is going to form the replication forks. Apart from this you also require a battery of the machinery what is been formed or what is been assembled on to the replication fork to start the replications.

So, these are the some of the important enzyme what is been found into the prokaryotic replications. So, first enzyme is the DNA helicase or helicase right. So, it is called as DNA B it melts or open up the DNA at the replication forks. So, it is going to be the first enzyme which is going to be sit at the original replications and then it is actually going to start the opening of the DNA. Then the second is the single standard DNA binding proteins and these are the they will prevent the unwinding of the single standard DNA to the double standard DNA.

So, you can imagine that as soon as the bubble is going to be formed the single standard DNA binding protein will go and sit on to the nucleotides. So, that they should not have any kind of interaction remember that these two molecules are complementary to each other. So, as soon as they get open they are supposed to be remain like that. So, that the other molecules will come and sit and do their jobs, but if you do not do that then they will come and stick to each other because your every A is going to have a you know complementity to T and every G is going to have the complementity to C. So, this complementity can only be break if you have a molecule which is sitting on to this right.

So, that is how you are going to have no interaction between these two and that is how they are going to be remain separated and then the other machine G will come and sit and start the replications. Then you also have the topoisomerase or DNA G. Topoisomerase works at the region ahead of the replication fork to prevent the super coiling. And then you also have the DNA pol I or it is also called as the Kornberg enzyme because the Kornberg is the first scientist who discovered the DNA pol I.

So, the first DNA polymerase to be discovered in E. coli by the Nobel prize winner Arthur Kornberg and the gene what is actually coding that DNA pol I is called as pol A and it is a monomeric protein of 928 amino acid or the 109 kilo Dalton and it has three different types of activity. It has a 5 prime to 3 prime polymerase activity, it has a 3 prime to 5 prime exonuclease activity and it also has the 5 prime to 3 prime exonuclease activity. So, do not worry about these activities because that anyway we are going to elaborate or going to discuss when we are going to talk about the other events. So, these three activities are really important. This activity is important for the DNA synthesis and other two activities are required for the proofreading and as well as the other kinds of

activities.

DNA polymerase I or the Kornberg enzyme is having the 5 prime to 3 prime exonuclease activity is independent of the other activities. Then we have the protease cleaves polymerase I into the two fragments right. So, if you treat the DNA pol I with the protease like trypsin then it is actually going to generate the two distinct fragments. And you are going to have the larger C-terminal fragment or it is called as Clino fragments which contains both the 5 prime to 3 prime polymerase activity and 3 prime to 5 prime exonuclease activity. And then you also going to have the smaller fragment which has the proofread active proofreading activity that is the 5 prime to 3 prime exonuclease activity.

So, Clino fragment is also very important and very popular in terms of the in vitro replication such as the PCR. So, sometime people are using the Clino fragments rather than the complete enzyme because the with the complete enzyme you have the always a danger of you having the 5 prime to 3 prime exonuclease activity and that may actually have the interference in terms of the DNA replication under the in vitro conditions. So, it has a low processivity which means only the 200 nucleotides can be processed then it also has the low polymerization rate. So, it has around 20 nucleotide can be added at the per second and the apart from these you also going to have other kinds of activities with associated with the DNA pol I that is the RNA primer removals, gap filling and the DNA repairs. These three are actually going to be discussed in detail when we are going to talk about the elongations and terminations.

And it is actually a metalloenzyme. So, it is actually a zinc dependent enzymes. Then we also have the DNA pol II. So, monomeric protein with pol B as a structural gene which is a size of the 90 kilo Dalton. DNA pol II having the two activities 5 prime to 3 prime polymerase activity and 3 prime to 5 prime exonuclease activity. It has a low polymerization that is the about 40 nucleotide per second and it also has the low processivity rate such as the 1500 nucleotides.

It mainly serve as the alternate DNA repair polymerase. Therefore it can be replicate DNA if the template is damaged. It does not require ATP for any type of this activity. Then you also have the DNA pol III. So, DNA pol III is a primary DNA replicase with the structural gene pol C and it has a 900 kilo Dalton. It is a multimeric protein complex of 10 different protein polypeptides such as alpha, epsilon, theta, z, tau, y, sigma and all that.

It has the high polymerization rate about 1000 nucleotides per second and it has high processivity that is the 50000 nucleotides. And DNA polymerase III serves as a

holoenzyme during their applications and holoenzyme refer to the multi protein complex whose catalytic activity is associated with the extra components. Then we have the DNA pol III. So, this is the structure of the DNA pol III and you can see that all these components are assembled to form this particular enzyme.

And there are four essential components. You have the two copies of the catalytic core that is the alpha subunit and the epsilon subunit. Alpha subunit has the 5 prime to 3 prime polymerase activity whereas the epsilon subunit has the 3 prime to 5 prime exonuclease activity whereas the theta subunit is for increasing the efficiency of the of the epsilon subunit. Two copies of the dimerization component that is the tau and the two copies of the homodimer of beta subunit ring for the processivity component and the one copy of the clamp loaders and these are the enzymes these are the subunits what are present in the clamp loaders. And how it is actually going to do the DNA polymerizations? So, the clamp loader links the two catalytic cores and the two beta clamps increases the processivity of the DNA pol III holoenzyme and the loading of clamps are done by the clamp loaders and the dimer component helps the catalytic cores to function at the same time.

Then we have the DNA polymerase IV and DNA polymerase V. Both are the Y family polymerase that do not have the 3 prime to 5 prime exonuclease activity. It has the low catalytic efficiency and a low processivity and fidelity. It is involved in the transition synthesis and replication damage DNA by the bypassing the nucleotide that can block the progression of a replication fork and it is been in size by the structural gene like the din B and the u mu d 2 c or the pol V. Then we also require the DNA primase. So DNA primase is required for the synthesis of RNA primers complementary to the strands then you also require the DNA ligase seal the DNA fragment gap into the strands.

Now there are 3 major events what is going to happen when you are going to have the DNA applications. So you have the first stage that is called as the initiation the second stage which is called as the elongation and the third stage is called as termination. So the first stage is going to have the recognition and starting of the replications then the elongation you are going to have the replication fork leads the dNTP synthesis and the proofreading and the third is termination where you are going to have the stopping of the replications. So E coli chromosome DNA is circular with no free end and it is replicated bi-directionally. So it resembles the Greek letter theta hence this replication mode is also known as the theta replication.

It can be seen in the gram negative bacteria such as proteobacteria some commonly used plasmid like Col-E1, RK2, F and P1 bacteriophage as well. So this mode of replication which is called as theta replication is been found into the prokaryotic system and as well

as the plasmids like Col-E1, RK2, F and P1 bacteriophage as well. So these are the some of the steps into the initiation. So initially the 2 to 4 DNA A protein using the ATP binds to the 9 mer DOR region in the ORAC performing the initiation complex. Once this is done it is going to enter into the second stage where the DNA coils around the DNA A multiple opiates which leads to the topological stress and once the topological stress is been generated it is actually during the presence of the in the presence of ATP the DNA A influences the 13 mer ATRH DUE region to start melting and once the DUE region is start melting the further melting is carried out by the recruiting hexamer protein which is called as helicase or the DNA B.

DNA B helicase clamps around each of the 2 single standard strands of the DUE site of the original C or C and the clamping of DNA U is supported by the clamp loader DNA C they make the DNA B DNA C complex and that is how the it is going to have the initial melting of the DNA at the original replication and that is how it is actually going to form the replication fork and the replication bubbles. Then we have the DNA C which is going to open up the DNA B ring and helps in placing the ring around the single standard DNA at the origin. While DNA B moves forward with the help of the ATP hydrolysis the single standard DNA binding protein covers the single standard DNA to prevent the unwinding and single standard DNA bind cooperatively in a sequence dependent manner sequence independent manner. So, single SSBs are actually going to bind the nucleotides and that is how they are actually going to destroy the affinity of the complementary strands and that is how it is actually going to keep the strands into the single standard DNA. Then we have the next is DNA B recruits the DNA G which is called as RNA primase to synthesize the RNA primer on both the both the strands which is called as leading strand or the lagging strands.

So, and then we are going to have the RNA primer which is influences the DNA C to release the DNA B from the site right and to initiate the elongation phase the primosome of prime formation occurs and as a result it is actually going to synthesize the primers and the primosome is actually a functional complex which is going to have the DNA G, DNA B and SSB and some of the accessory proteins. So, in the initiation exactly what will happen is that you are actually going to have the binding of the some of the components at the DU site and some of the component at the DUR site. Once these two sites are actually going to be occupied by the initiation factors DNA B and other kinds of proteins then you are going to have the recruitment of the helicase And helicase is actually going to be a hexagonal protein. So, it is actually going to run in both the direction. So, it is going to run in this direction and this will run in this direction and that is how it is actually going to form the replication bubble.

And once it is going to be formed it is going to allow the binding of the single standard

DNA binding protein and that is how it is actually going to allow the binding of the is how there will be a synthesis of the primers and one replication one fork will run in both the directions and that is how it is actually going to have the one under strand which is called going to be called as leading strand the other strand is going to be called as lagging strands. And once this initiation stage is over then it will enter into the elongation stage. So what is the basics of the elongations? So mainly the DNA pol III does not does the polymerization chain elongation happens by the free 3 prime OH primers attacking the alpha phosphoryl group of the incoming ATNTPs as a product byproduct of the reaction as mentioned earlier the pyrophosphate is going to be generated the bond formed between known is the phosphodiester bonds and that is how it is actually going to start adding the nucleotides based on the base pairing information. So once you have the Watson-Crick based pairing information which is going to be available through the DNA pol III it is going to be keep adding the nucleotides and these keep nucleotides are going to be coupled with each other by the phosphodiester linkage and the pyrophosphate is going to be released and this pyrophosphate is going to be get hydrolyzed by the pyrophosphatase and that is also going to generate the energy which is going to be utilized into the process. In this case there are two replication forks generated in for the prokaryotic that moves in the opposite direction for each other replication forks proceeds bidirectionally at a speed which is 1000 base pair per second per fork both the leading and the lagging strands are replicated simultaneously.

What is the leading strand? The DNA polymerase III synthesizes the strand from the 5 prime to 3 prime direction continuously towards the replication fork. So one fork is running in the direction of the 5 prime to 3 prime the other is running in the reverse direction that is the in the reverse order and that is what is called as lagging strand. So in the lagging strands the synthesis happen in the 5 prime to 3 prime direction but the disk continuously away from the replication fork. So if you have a bubble and if this is the bubble and if the replication is going in this direction machinery is going in this direction then this is going to be the leading strand and this is going to be lagging strand because it will wait for this region to be available to get open and then only it is actually going to do the synthesis in the reverse direction and that is why this is going to be called as the lagging strand and this is going to be called as the leading strands.

So there are three different stages of the elongations right. So DNAB helicase separate the two DNA molecules binding to the lagging strands template at the replication forks and moving along according to the polarity of 5 prime to 3 prime direction. Now DNAG the prime is associated with the DNAB and synthesizes the RNA primers complementary to the associated single standard strands. Interaction between the DNAB and DNAG regulates the Okazaki fragment length. Tighter association results in more frequent short fragments whereas the loose interaction will produce a longer fronted on lagging strands.

Length of the Okazaki fragments could vary between the 1000 to 2 herm nucleotides. Formation of replisome so it contains the DNA pol III holoenzyme and the associated protein like the DNAB and the DNAJ DNAG. This replisome starts the joining of the DNA piece by forming the phosphodiester bonds. DNA pol I removes the RNA primers of both the strands by the 5 prime to 3 prime exonuclease activity which generates a gap after the primer remover in the both the strands. DNA pol I also fill in the gap between the lagging strand fragment after the primer remover. Lastly the left over nicks are sealed by the help of DNA ligase with the help of the NAD plus as a energy source.

So this is what exactly going to happen in the replications elongation stage. So in the elongation stage what will happen is that you are going to have the replication fork which is moving in this direction. So this is going to be considered as the leading strand and this is going to be considered as lagging strand. So once this portion is going to open then the synthesis is going to start from this direction. So it is going to happen in this direction. Whereas in the case of leading strand because the synthesis always occurs in the direction of 5 prime to 3 prime and so it has to wait for the 3 prime to be available for making a primer and that is how it is actually going to be in the opposite direction.

And that is why this strand is going to be called as lagging strand and this strand is going to be called as leading strands. Now what is the role of gyrase in the elongation? So due to the unwinding of with the help of the DNA helicase the double stranded DNA in front of the fork become positively supercoiled. If the supercoiling increases the fork will halt.

Therefore to overcome the halting from the supercoiling topoisomerase are needed. In E. coli the DNA gyrase the type 2 gyrase was discovered by the scientist called as Martin Gallet. And gyrase contains the two different subunits gyre A which contains cut and rejoin the DNA and gyre B which is responsible for providing the energy by the ATP hydrolysis. Then we also have the proofreading activity because when you are doing a DNA applications it is possible that the DNA polymerase could add the some nucleotide which may not be match which may not be as per the information available onto the template. And in that case there is a proofreading activity required right because when you are synthesizing a product it has to go into the into a testing stage or it is going to be get into a stage where you should test whether the product what you are synthesizing is of good quality or not. So proofreading activity is going to ensure that there is a sequence what is being produced is exactly identical of the template.

So DNA replication is amazingly accurate with one error in 1 billion nucleotides incorporated. This above mentioned tolerated mutant level is desired for the large

genome size especially. DNA polymerase carried out the process by their 3, 5 to 5 prime exonuclease activity. When an inaccurate nucleotide is incorporated the synthesis rates get reduced due to the wrong positioning of the 3 prime OH. This works like one delete key removing only the most recent error. So what happen is that when you are actually synthesizing and synthesizing it keep going and keep checking whether the attachment what I have made is actually making a pair with the template or not.

So if it is not then it is actually going to go back and it is going to do the corrections. Now the third process is called as the terminations. As there is a bidirectional replication the fork will melt at a position diametrically opposite to the ORC on the genome. Termination region contains multiple copies of 23 base pair long sequences or TIR sequences. Every TIR sequence act as a recognition site for a protein which is called as TUS.

So termination utilization substrate proteins. TUS-TIR complex allow one replication fork to pass if it is moving in a one direction but blocks the progression if it is from the opposite direction. The directionality depend on the TIRs protein localization on the DNA helix. In E. coli the orientation of the TUS-TIR complex is such that it ensure both the fork movement will be stopped at or near the same point.

After the complete replication process that the new two circles are physically interlocked or catenated. This decatenation is carried out by the topoisomerase IV to generate the separate two double standard DNA molecules. So termination is being done by the places where you are going to have the TIR sequences and these TIR sequences are going to be recognized by a protein which is called as the TUS and or the termination utilization substrate proteins and the TIRs and the TIR is going to form a complex allowing only one replication to fork to pass. But if there are two replication forks which are you know going to reach to that particular point and they will try to bypass then it is actually going to halt the replications. Now let us talk about the summary of the things what we have discussed so far right. So there will be and what will be the difference between the replication between the prokaryotic and as well as the eukaryotic system.

So in the initiation you are going to have the DNA A which is actually going to participate. So initially you are going to have the initiation reactions where you are going to have the DNA A whereas in the prokaryotes you are going to have the protein which is called as ORC. Then you also going to have the activity of helicase which is going to unwind the DNA and it is going to be DNA B then that is been done by the MCM complex in the eukaryotic system then you are going to have the helicase lower that is going to be DNA C whereas the same function is been done by the Cdc 6 and Cdt 1. You do not have to worry about all these proteins because when we are going to talk

about eukaryotic We will discuss all these. Then the single standard DNA binding proteins it is going to be prokaryotic SSBs whereas in the case of eukaryotes it is going to be RPA. Then we have the primase so it is going to be DNA G in the case of prokaryotic system whereas in the case of eukaryotic system you are going to have the pol alpha primase.

And then we have the polymerase so you are going to have the DNA pol III which is the main polymerase required for the replication in the prokaryotic system whereas in the case of eukaryotes you are going to have the DNA pol III delta and the poly DNA pol epsilon. Clamp you are going to have the beta clamp whereas in the case of the prokaryotes you are going to have the PCNA ring. Then we have the clamp loader so you are going to have the gamma complex whereas in the case of the eukaryotes you are going to have the RFC. Then we have the ligase, ligase is required for you know joining the lagging strands right.

So it is going to be a DNA ligase whereas in the case of eukaryotes going to be a DNA ligase I. Then we require the primase removal so primase removal is being done by the DNA pol I or ribonucleus H in the case of prokaryotes whereas in the case of the eukaryotes it is going to be done by the RNase H or FEN I. So these are the some of the components what are being different between the prokaryotic system and the eukaryotic system. And what we have discussed so far we have discussed about the DNA replication machinery in the prokaryotic system. We have discussed about the origin of the applications, features of the origin of the application and so on. So in a subsequent lecture we are going to discuss some more aspects related to the prokaryotic application. Thank you.