Cell and Molecular Biology
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Week 10
Central Dogma of Molecular Biology (Part 2)
Lecture - 36
Replication (Part 2)

Hello everyone, this is Dr. Vishal Trivedi from the Department of Biosciences and Bioengineering, IIT Guwahati. In this particular module, we are discussing the central dogma of molecular biology, and in this context, so far we have discussed the replications, and we have divided the discussion of replication into different parts. First, we are going to discuss the replication in prokaryotes, and then we will discuss the replication in eukaryotes. Because the sole purpose of replication is to synthesize the genomic content of that particular organism in a limited time period. So that the organism can divide and multiply its number.

So what we have discussed so far is that we have talked about replication in the prokaryotic system and that it is going to have the origin of replication, which is going to be an AT-rich sequence. So that it is easy for the helicases and other enzymes involved in the replication to melt the DNA and unwind it very easily. And then followed by that, we have also discussed extensively the machinery required for DNA replication in prokaryotes, so we have discussed DNA Pol I. We have discussed their structures, functions, and all other kinds of features, and apart from that, we have also discussed helicases, DNAB, SSB structures, topoisomerases, and other kinds of enzymes.

So, in the previous lecture, we discussed all these aspects. Now in the current lecture, we are going to discuss the special types of replications found in the prokaryotic system, the differences between them, and the different machinery required for performing these types of replications in the organisms. So, we have discussed the replications and we have discussed the replication in prokaryotes. Now, we are going to see the special modes of replication that have been available or found in the prokaryotic system, how adaptations are occurring in bacteria, and how the different types of cellular machinery are involved. So replication, if you see the replications, has three very distinct steps.

You are going to have the initiation, followed by the elongation, and followed by the termination. So if you recall, we have discussed that within the initiation, the cellular machinery will first identify the origin of the applications, and there will be only one single origin of application in the prokaryotic system, whereas there will be multiple origins of application in the eukaryotic system. And once they have identified the original

replication, the initiation complexes are formed, and the helicases and other types of enzymes will go and sit on the initiation site, and that's how they are actually going to unwind the DNA. While they are unwinding the DNA, they will also ensure that the DNA they are unwinding and preparing for replication is methylated. If the DNA is hemimethylated or unmethylated, then it will not actually initiate the replications.

After that, it will enter the elongation phase, where the DNF polymerase will go and sit, and then start adding the nucleotides according to the Watson-Crick base pairing structures, and so on. And then it will enter into the termination, and the termination is being done by the ter sequences, which are going to interact with the termination machinery, and that is how it is going to terminate. So these are some of the basic structures or basic mechanisms that are happening, but in some of the bacterial systems, you are actually going to have replication in a special mode. So, one of such special modes is called the rolling circle replication or rolling circle model. So, in a rolling circle model, it has been found in archaebacteria, bacteriophages, plasmids, and viral DNA such as HHV, HPV, Gemini virus, and viral RNAs.

In a rolling circle model, what you have is a double-stranded circular DNA. And this is actually going to have a nick that is going to be formed in the DNA, and then the machinery will actually utilize the information from the inner strands, and that is how there will be a synthesis of the DNA on one end. Right, and it will be utilizing this information. Keep rolling like this, and that is how they are actually going to start synthesizing. Once they synthesize one copy of the genome, it will be cut and then will actually serve as a template to synthesize the second strands.

So, what are the different enzymes that are going to play a crucial role in the rolling circle model? So you are going to have Rep A. Rep A is going to initiate and address the double standard origins or DSO. Remember that this is the first event that you are going to have to form a nick structure so that the machinery will enter into this structure and utilize the nucleotide that is present in the inner circle, and that is how they are going to synthesize this strand and how it is actually going to make multiple copies. Then you require the PCRA or plasmid cobi reduced, and this is going to be a helicase that moves the nix strands. Then you require the DNAPol3, which is going to have the 5' to 3' polymerase activity.

And it is going to be a male replicase. Then you require the DNA Pol I and the DNA Pol I; the function of the DNA Pol I is to remove the RNA primers, and you also require the DNA ligase. DNA ligase is going to join the ends to make the strand circular. Now, these are some of the steps that you are going to perform. So, in the initiation, elongation, and termination, right? So in the initiation, the Rep A is actually going to recognize the

double standard origin.

So in this case, you are going to have the, for example, you are going to have the circle, and then suppose this is the place, right? So, this is going to be a place where you will have the double standard origin or DSO. So, this is actually going to go and allow the sitting of the repa, and after recognition, it is actually going to make a nick, right? So, it is going to make a nick in the upper strand. So, what is meant by the nickname is that it is actually going to make a break. Then in the elongation phase, the rep A stays attached to the nicked 5 prime phosphate end. So it is actually going to create a cleavage, and it is going to hold the DNA.

Whereas the 3' prime end is free, acting as the DNA pol III primers, and the DNA pol III acts in a 5' to 3' direction, it produces multiple concatamers and multiple single-stranded copies of the original DNA sequence. To increase the efficiency of all three, one replicates PCRA that is incorporated before it unwinds to the double-stranded CNA. And then in the termination state, you are going to have the rep A, which is attached, cut the leading strand to stop the replication of that strand. Therefore, the second strand is to be left to synthesize. To replicate the second strand, RNA polymerase or the primer synthesis, RNA polymerase, and DNA Pol III copies the single-stranded origin and elongates.

After replication, the DNA pol I removes the RNA primer and puts the correct bases there, and then the DNA ligase will come and join them to give you the double-stranded circular DNA molecule. So this is exactly what is going to happen, right? You have, this is a single standard original application, and this is going to be the double standard original application. So when you have the initiation stage, your rep A is actually going to come and bind to this particular portion, and then it is actually going to make a nick. After making a nick, the rep way is actually going to bind the 5 prime end, whereas the 3 prime end is going to be free, right? And then, on the free prime end, the ball 3 is actually going to bind. And then it is actually going to utilize this portion as a primer; this portion as a primer, and that is how it is actually going to start synthesizing, and that's how it is actually going to make the same copy, the single standard same copy, right? So it is actually going to make a single standard copy.

So it is going to run like this, and that's how it is actually going to make the single standard copy. So one strand is going to be synthesized completely, right? So, for example, it goes like this. And one single copy is going to be synthesized, and then RepA is going to cut the strand, and DNA ligase is going to join the links. So it's going to have the double standard, and it's going to have the other double standard DNA molecules, and the single standard DNA molecule is produced, which will be synthesized in a double

standard molecule using similar kinds of steps. So it's very clear that in the double standard original application, the repe is going to bind, and then it is going to make the nick, and that nick is going to serve as a primer for the DNA pol 3, and then the DNA pol 3 is going to synthesize, and that's how it is actually going to make the.

Single standard concatamer and these concatamers are going to be cut right once one round is over, and that is going to be double standard simply by the DNA pol 1, and that's how it is actually going to have the double standard circular DNA molecule, the daughter DNA molecule. the other molecule is also going to be utilized. So, in this one, you are going to have the two strands: this strand and this strand. This strand is going to serve as the template to synthesize the outer strands, and afterwards, once the application is over, the outer strand is also going to be utilized to synthesize the inner strands, and that is how it is actually going to complete the cycle. One of the classical examples of the rolling circle model is that it is present in some of the E.

coli species. So you are going to have the donor cell, you are going to have the recipient cells, and once they are going to form, there will be a conjugation, and then after the conjugation, they are going to exchange the genetic material. So how they are going to exchange genetic material is that the donor cell is actually going to have the rolling circuit model, and because of that, its genome is going to be replicated, and it's going to have the concavator. Right, so this single-stranded DNA is going to be transferred to the next recipient DNA, and that is going to be present to the recipient cells. And then the circular and the double-stranded plasmid is produced by the new cells, so this single-stranded DNA, which is actually a single-stranded circular DNA, is going to be utilized as a template by the recipient cells, and it is going to make the double-stranded DNA, and that is how you are actually going to have the plasmid. And that is how you are actually going to have the exchange of genetic material, and that is how you are going to exchange the phenotypic feature.

For example, if this bacteria is ampicillin resistant, right? And this bacterium is ampicillin sensitive, then this bacterium is actually going to provide that resistance through the rolling circle model and provide the DNA responsible for that. And this DNA is going to provide the ampicillin resistance even in the donor molecule as well. So apart from the rolling circle model, you are going to have another kind of replication mode that is called D-loop formation or D-loop replication. This replication is found in the small circular and organelle DNA. For example, it is present in the chloroplasts and mitochondria.

Remember that the chloroplast and mitochondria, although they are present within the eukaryotic cell, are not eukaryotic in origin; they are prokaryotic in origin, so they follow

many of the features present in bacterial cells. For example, they have circular DNA; they have their own DNA. So, scleroplasts and mitochondria are actually going to follow the mechanism that is known for the prokaryotic replication system or whatever we have discussed so far. So, where a triple standard structure called a displacement loop is going to be formed. And the mitochondrial DNA is actually 16.

6 KB, which consists of two strands: the heavy strand and the light strand. So this is the heavy strand, the inner strand, and the outside is the light strand. It comprises a lengthier NCR, or the non-coding region, and acts as the regulatory region. In this region, the mitochondrial DNA had its promoter for transcription. One is the light strand promoter and the other is called the heavy strand promoter or HSP.

Conserved sequence MOCs, CSBs, and the termination-associated sequences are also present. How the replication terminates at the task is still not known. So, NCR also contains the origin of the leading or heavy strand. So, this heavy strand is called the leading strand, whereas the light strand is also called the lagging strand. The origin of the leading strand OH is on this heavy strand, whereas the origin of the lagging strand is patterned onto the light strands.

Now, what are the different key players that are involved in the D-loop replications? So, you are going to have the DNAPol gamma. So, it is a main replicase or the polymerase. It has two subunit polymerase A, or PolA. It is from the DNAPol of the BA family. It shows the proofreading activity in the 3' to 5' exonuclease activity, and it is highly accurate.

That is the one error in the 1-billion-base-pair. Then we have the PolB, which has the add-on subunit to improve the interaction between the PolA and the DNA templates. It increases both catalytic activity and processivity. Then you also require Twinkle. So Twinkle is a hexamer helicase that needs the fork structure to be loaded and to start unbinding; then you require the mitochondrial single-stranded DNA binding protein.

So ssbs bind with the newly formed single-stranded DNA to protect it from nucleases and maintain the single-stranded structures. Then you also require the pol RMT so mitochondrial RNA polymerase synthesizes the RNA primer onto the displayed strand as it cannot work on the single-stranded DNA as a template. Then you also require DNA ligase 3. So DNA ligase joins the nicks in the new DNA strands. Then you also require RNase H1 and MGM1.

So mitochondrial genome maintenance exonuclease 1 helps with primer removal after replication. So, these are the steps of the D-loop formations and D-loop replications. So

you start at the OH site. The PolRMT starts synthesizing the primer onto the H strand to synthesize the entire H strand. So, you are going to have the genomic content in the form of two circular strands, right? You are going to have the H strand, which is the blue one, and you are going to have the L strand, which is the red one.

So in the first part, on the OH side, which is present on the H strand, Paul RMT is going to sit, and it is going to start synthesizing; it is going to start, you know, replicating that. The whole parental displaced H strand is covered by the single-stranded DNA binding protein. The binding of the SSB stops the polar empty for random RNA synthesis onto the H strands. So this is what is going to happen. Then the twinkle, the helicase comes before the pol gamma to unwind the double-stranded DNA to move the fork while the SSB maintains the single-stranded DNA, leading to the formation of the replisome.

So, the replisome is going to be formed on the DNA strand. After two-thirds of the genome is synthesized, the replisome passes through the OL site, or the origin of replication, onto the light strand, or L strand, actually. There the parental single-stranded H strand forms stem-loop structures. So, this is a stem-loop structure that is going to be formed. The stem loop structure stops SSB from binding; thereby, the one short stretch of the single-stranded DNA in the loop becomes accessible for Paul RMT to make the RNA primers.

So, once you have this, it is actually going to stop the binding of the SSBs, and on the other hand, it is actually going to allow the PolRMT, or RNA polymerase, to synthesize the primer for the L strand as well. And then, after 25 nucleotides of the stem-loop structure, the polymerase gamma replaces the PolyRMT at the 3' end of the primer, which results in the synthesis of the L strands. H and L strands are replicated continuously until they reach the termination sequences, forming a triple-strand displacement loop (D-loop), and then the two DNA strands are formed. So this is what is going to happen here: you are going to have the L strand and the H strand, and in the initial region, the L strand is on the L strands of the origin of the application, which is the OH side. For RMT, it is going to bind, and that is how it is actually going to start the applications, whereas on the L side, first it is actually going to have the stem-loop structure, and this stem-loop structure is going to stop the binding of the SSBs.

And it is also going to serve as a primer, and that is how it is actually going to allow the binding of the pole RMT, and it is going to synthesize the primer, and that is how it is actually going to initiate the synthesis of the L strands. And ultimately, both the L strand dimer and the H strand are actually going to be formed, and that is how you are going to have the two double-stranded daughter DNA. So, from this parent DNA, you are going to have the two daughter DNAs at this end. So, this is all about the DNA replication in the

prokaryotic system; what we have discussed so far includes the origin of replication in the prokaryotic system. This origin of application has the classical features of ATDG regions, and it is going to be recognized by the cellular machinery; that is how the helicases and other cellular proteins are actually going to bind and unbind the DNA.

And then it is going to enter the elongation phase, and in the elongation phase, the DNA polymerase is going to synthesize the DNA strands, joining the nucleotides onto the incoming 3 prime site. And that is how it is actually going to synthesize, and once it reaches the termination sites, it is going to be terminated by the ter sequences, and so on. And in the current lecture, we have also discussed the special mode of DNA replication. So where we have discussed the rolling circle model, which is very, very common in the bacterial system. And then we also discuss the D-loop formation, which is more common in the organellar DNA such as mitochondrial DNA and chloroplast DNA.

So, with this small discussion about the prokinetic replications, I would like to conclude the lecture here. In our subsequent lecture, we are going to discuss the DNA replication in the eukaryotic system. Thank you.