Molecular Biology
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Module - 05
Replication
Lecture-22 Replication in Prokaryotic System (Part 2)

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 the molecular biology in the course in this particular course. So, in this particular module we are discussing about the central dogma of molecular biology and in this context so far what we have discussed? We have discussed about the replications and so we have divided the discussion about the replication that it is going to be first we are going to discuss about the replication in prokaryotes and then followed by that we are going to discuss about the replication in eukaryotes because replication the sole purpose of replication is that it should synthesize the genomic content of that particular organisms in a limited time period so that it should be able to divide and should be able to multiply its number. So, what we have discussed so far? We have discussed about the replication in the prokaryotic system and what we have discussed that it is going to have the origin of replications and original replication is going to be a AT reach sequences so that it is easy for the helicases and other enzymes what is been involved into the active into the replications and they should be able to melt the DNA and should be able to unwind the DNA very easily. And then followed by that we have also discussed in extensively about the machinery what is required for the DNA replications in the prokaryotes. We have discussed about the DNA pol 1, pol 2 and pol 3.

We have discussed about their structures functions and all other kinds of features and then apart from that we have also discussed about the helicases, DNA B, SSP structures, topoisomerases and other kinds of enzymes. So, in the previous lecture we have discussed all these aspects. Now in the current lecture we are going to discuss about the special type of replications and special type of replications what is been found into the prokaryotic system and how what are the different what are the differences and what are the different machinery what is required for performing these type of this type of replications in the organisms. So, we have discussed about the replications and we have discussed about the replication in prokaryotes.

Now we are going to see the special mode of replications what are been available or what are been found into the prokaryotic system and how what kind of adaptation is happening into the bacteria and how the different type of cellular machinery is involved. So, replication if you see the replications replication has the very distinct three steps

right. You are going to have the initiation followed by the elongation and followed by the termination. So, if you recall we have discussed that the within the initiation the cellular machinery will identity first identify the origin of replications and there would be only one single origin of replication into the prokaryotic system whereas, there are going to be multiple origin of replication into the eukaryotic system. And once they have identified the origin of replication then the initiation complexes are going to be formed and the helicases and other kinds of enzymes will go and sit on to the initiation site and that is how they are actually going to unwind the DNA.

While they are unwinding the DNA they will also going to ensure that the DNA what they are unwinding and preparing it for the replication is methylated if the DNA is hemimethylated or unmethylated then the they will not actually going to initiate the replications. After that it will enter into the elongation phase where the DNA polymerase will go and sit and then start adding the nucleotides as per the borson-cirque base pairing structures and so on. And then it will enter into the terminations termination and the termination is going to termination is being done by the terse sequences which are going to interact with the termination machinery and that is how it is going to terminate. So, these are the some of the basic structures or basic mechanism what is happening, but in some of the bacterial system you are actually going to have the replication in a special mode. So, one of the such special mode is called as the rolling circle replication or rolling circle model.

So, in a rolling circle model it is been found in the archaebacteria, bacteriophage, plasmids and the viral DNA such as the HHV, HPV, Gemini virus and the viral RNAs. In a rolling circle model what you have is you have a double standard circular DNA and this is actually going to have the so there will be a nick what is going to be formed onto the DNA and then the machinery will actually going to utilize the information from the inner strands and that is how there will be a synthesis of the DNA on one end. And it will be utilizing this information keep rolling like this and that is how they are actually going to start synthesizing. Once they will synthesize the one copy of genome then it is going to be cut and then this is actually going to serve as a template to synthesize the second strands. So, what are the different enzymes what are going to play a crucial role into the rolling circle model.

So, you are going to have the RepA. So, RepA is going to initiate and nick the double standard origins or DSO. Remember that this is the first event that you are going to have you have to first form a nick structure. So, that the machinery will enter into this structure and it is going to utilize the nucleotide what is present onto the inner circle and that is how it is they are going to synthesize the this strand and that is how it is actually going to make the multiple copies. Then you require the PCRA or plasmid copy reduced

and this is going to be a helicase which moves the nicks strands.

Then you require the DNA pol III and DNA pol III is going to have the 5 prime 3 prime polymerase activity and it is going to be male replicates. Then you require the DNA pol I and DNA pol I the function of the DNA pol I is going to be that it is going to remove the RNA primers and then you also require the DNA ligase. DNA ligase is going to join the end to make the strand circular. Now these are the some of the steps what you are going to perform. So, in the initiation, elongation and termination.

So in the initiation the RepA 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 in this suppose this is the place. So, this is going to be a place where you are going to 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. So, it is going to make a nick into the upper strands.

So, what is mean by the nick is it is actually going to make a break actually. Then in the elongation phase the RepA stay attached with the nicked 5 prime phosphate end. So, it is actually going to make a cleavage and it is going to hold the DNA. Whereas the 3 prime end is 3 acting as the DNA pol III primers and the DNA pol III act in a 5 prime to 3 prime direction produces the multiple concatamer multiple single standard copies of the original DNA series to increase the efficiency of all 3, 1 replicates PCRA is incorporated before it unwind to the double standard DNA. And then in the termination state you are going to have the RepA 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 the RNA polymerase or the primer synthesis RNA polymerase and DNA pol III copies the single standard origin and elongates after replication the DNA pol I remove the RNA primer and put the correct bases there and then the DNA ligase will come and it will join the nicks to give you the double standard circular DNA molecule. So, this is exactly what is going to happen. You have this is the single standard original replication and this is going to be the double standard original replication. So, when you have the so in the initiation stage your RepA is actually go and come and bind to this particular portion and then it is actually going to make a nick. After making a nick the RepA is actually going to bind the 5 prime end whereas the 3 prime end is going to be free.

And then on the 3 prime end the pol III is actually going to bind and then it is actually going to utilize this portion as a primer and that is how it is actually going to start synthesizing the DNA and that is how it is actually going to make the same copy, the

single standard same copy right. So, it is actually going to make the single standard copy so it is going to run like this And that is how it is actually going to make the single standard copy. So one strand is going to be synthesized fully. So for example, it goes like this and one single copy is going to be synthesized and then Rep A is going to cut the strand and DNA ligase is going to join the links. So it is going to have the double standard and it is 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 the similar kind of steps.

So it is very clear that in the double standard origin of applications the Rep A 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 III and then the DNA pol III is going to synthesize and that is how it is actually going to make the single standard concatamer. These concatamer are going to be cut right once they will one round is over right and that is going to be double standard simply by the DNA pol I and that is how it is actually going to have the double standard circular DNA molecule the daughter DNA molecule and the other molecule is also going to be utilized. So in this one you are going to have the two strands right this strand and this strand. This strand is going to serve the template to synthesize the outer strands and afterwards once the application is over then 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 example of the ruling circle model is that it is going to be present in the some of the E.

coli species right. So you are going to have the donor cell you are going to have the recipient cells and once they will 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 ruling circuit model right and because of that its genome is going to be replicated and it is going to have the concavitabra right. So this single standard 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 standard plasmid is produced by the new cells. So this single standard circular DNA is going to be then utilized as a template by the recipient cells and it is going to make the double standard 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. So for example if this bacteria is ampicillin resistance right and this bacteria is ampicillin sensitive then this bacteria is actually going to provide that resistance through the ruling circuit model and provide the DNA responsible for that and this DNA is going to provide the ampicillin resistance even into the donor molecule also.

So apart from the ruling circuit model you are going to have the another kind of replication mode that is called as D loop formation or the D loop replications. This replication is found in the small circular and organellar DNA for example it is present in the chloroplast and mitochondria. Remember that the chloroplast and mitochondria although they are present within the eukaryotic cell but they are not eukaryotic in origin right they are already they are being prokaryotic in origin. So they follow many of these features what is present in the bacterial cell right for example they have the circular DNA they have their own DNA. So chloroplast and mitochondria are actually going to follow the mechanism what is being known for the or whatever we have discussed so far into the prokaryotic replication system right.

So where one triple standard structure called a displacement loop is going to be formed and mitochondrial DNA it is actually the 16.6 kB which is consist of two strands heavy strand and the light strand. So this is the heavy strand the inner strand and the outside is the light strands. It comprises a lengthier NCR or the non-coding region and act as the regulatory region. In this region the mitochondrial DNA has its promoter for the transcription.

One is the light strand promoter and the other is called as the heavy strand promoters or HSP. Conserved sequence MOXES CSBs and the termination associated sequences are also present. How the replication terminate at the task is still not known. So NCR also contains the origin of leading or the heavy strands. So this heavy strand is called as the leading strand whereas the light strand is also called as the lagging strand.

So origin of leading strand OH is on this heavy strand whereas the origin of lagging strand is written onto the light strands. Now what are the different key players which are involved into the D-loop replications. So you are going to have the D-N-A-pol gamma. So it is a main replicates or the polymerase. It has two subunit polymerase A or PolA.

It is from the DNA pol 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 the processivity.

Then you also require the Twinkle. So Twinkle is a hexamer helicase which needs the fork structure for being loaded and for start unwinding. Then you require the mitochondrial single standard DNA binding protein. So SSBs binds with the newly formed single standard DNA to protect from the nucleases and maintain the single standard structures.

Then you also require the PolRMT. So mitochondrial RNA polymerase synthesizes the RNA primer onto the displayed strand as it cannot work on the single standard DNA as a template. Then you also require the DNA ligase 3. So DNA ligase joins the nicks in the new DNA strands. Then you also require the RNase H1 and MgM1. So mitochondrial genome maintenance exonuclease 1 that helps into the primer removal after the replications.

So these are the steps of the D-loop formations, D-loop replications. So you start at the OH site. The PolRMT starts making the primer onto the H strand to synthesize the whole H strand. So you are going to have the genomic content in the form of the two circular strands. You are going to have H strand which is on the blue one and you are going to have L strand which is the red one.

So in the first part on the OH side which is present onto the H strand is PolRMT is going to sit and it is going to start replicating that. Whole parental displays H strand is covered by the single standard DNA binding protein. The binding of the SSB stop the PolRMT for a random RNA synthesis onto the H strands. So this is what is going to happen here. Then the twinkle the helicase comes before the Pol gamma to unwind the double standard DNA to move the fork while the SSB maintain the single standard DNA leading to the formation of the replisome.

So the replisome is going to be formed onto the DNA strand. After the two third of the genome is synthesized the replisome passes through the OL side or the origin of the application onto the light strand or L strand actually. There the parental single standard H strand forms a stem loop kind of structures. So this is a stem loop of structure which is going to be formed. The stem loop structure stops SSB from binding thereby the one short stretch of the single standard DNA in the loop become accessible for PolRMT to make the RNA primers.

So once you have this it is actually going to stop the binding of the SSB and the other hand it is actually going to allow the PolRMT or it is a RNA polymerase to synthesize the primer for the L strand also. And then after 25 nucleotides of stem loop structure the polymerase gamma replaces the PolRMT at a 3 prime end of the primer which results into the synthesis of the L strands. H and L strands are replicated continuously until they reach to the termination sequences and forming a triple strand displacement loop D loop and then the two DNA strands are going to be formed. So this is what it is going to happen here that you are going to have the L strand and H strand and in the initial region the L strand on the L strands of the origin of the application that is the O H side the PolRMT 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 PolRMT 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 the both the L strand dimer or the H strand is actually going to be formed and that is how you are going to have the two double standard daughter DNA.

So from the this parent DNA you are going to have the two daughter DNA at this end. So this is all about the DNA replications into the prokaryotic system what we have discussed so far we have discussed about the origin of the applications into the prokaryotic system and this origin of the application is has a classical features of ATDG regions and it is going to be recognized by the cellular machinery and that is how the helicases and other cellular proteins are actually going to go and bind and unwind the DNA and then it is going to be enter into the elongation phase and in the elongation phase the DNA polymerase is going to synthesize the DNA strands and it is going to join the nucleotide onto the incoming 3 prime site and that is how it is actually going to synthesize and once it reach to the termination sites it is going to be terminated by the TIR sequences and so on. And in the current lecture we have also discussed about the special mode of DNA replications so where we have discussed about the rolling circle model which is very very common in the bacterial system and then we also discuss about the D loop formation which is more common in the in the organelle DNA such as the mitochondrial DNA and the chloroplast DNA. So with this small discussion about the prokaryotic replications I would like to conclude the lecture here in our subsequent lecture we are going to discuss about the DNA replications in the eukaryotic system. Thank you. Thank you.