Molecular Biology
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Module - 05
Replication
Lecture-23 Replication in Eukaryotic System

Hello everyone, this is Dr. Vishal Trivedi from department of biosciences and bioengineering IIT Guwahati and this particular module we are discussing about the DNA applications. So, far what we have discussed we have discussed about the DNA replication in prokaryotes and while we were discussing about the DNA replication in prokaryotes we discussed about how the initiation complex is going to be formed and how the different types of components are required for the DNA replication in prokaryotes and how the DNA replication in prokaryote is different and required the special machinery for its DNA synthesis. Now in today's lecture we are going to discuss the about the eukaryotic replications because eukaryotic replication is different from the prokaryotic replication in terms of the components or the enzymes what is required and the property of these enzymes are very different. So, as a name suggests the DNA replication is the mechanism by which the cell duplicates its genetic material ensuring that the each newly formed cell receive an accurate copy of the original DNA. So, this kind of objective is also being met even when we are doing the DNA replication in the eukaryote as well.

The process is essential for the growth, development and transmission of genetic information from the one generation to the next generation and the DNA replication involves the various enzymes and protein working together to unwind and separate the double standard DNA molecule synthesize the new complementary strand and ensure the fidelity of the copied genetic material. The basic principle of eukaryotic and prokaryotic replication are the same however there are some notable differences. Now the one important point is that the eukaryotic replication is much more complicated than the eukaryotic replications and there are many reason why it is so actually. So, why the eukaryotic replication is more complex than the prokaryotic replication because of the simple reason that the eukaryotic genomes are quite complex they are larger than the bacterial DNA.

Remember that the bacterial DNA's are very small even in the plasmid DNA also and it is a having a complex structure because remember that the bacterial chromosome or bacterial genomic content is not been associated with the protein molecules and it is not that much complex compared to that the eukaryotic system. Remember that when we were discussing about the eukaryotic genome we discussed that how it is actually been

associated with the histone proteins and how the histone octamers are forming the nucleosomes and then nucleosomes are further you know further assembled and give you the chromosomes. So, the chromosome is mostly been present in the eukaryotic structures. But apart from these differences or the complexity the replication process in the both the prokaryotic and the eukaryotic system actually involves some of the basic steps. For example, the formation of the replication fork is common between them, primer synthesis is also common between them.

Once the primer job is over and it has to be removed by the DNA pol I in the prokaryotic system that is also common then you also going to have the okazaki fragment whether it is the prokaryotic system or the eukaryotic system then the replication mode it is going to be semi conservative whether it is a eukaryotic or prokaryotic system and the movement of the replication fork would be bi-directional in the both the prokaryotic as well as the eukaryotic system. So, replication fork is going to be bi-directional in the case of prokaryotic or eukaryotic system and it is also been required for gap bridging between the newly synthesized DNA fragments with the help of the DNA pol I and the DNA ligase. So, apart from these kind of similarity there are significant difference in terms of the machinery and other kinds of proper requirements. So, apart from these similarities there are significant difference between the prokaryotic and the eukaryotic replications. We have discussed many of these differences so but I thought I should remind you so that it will be easy for you to follow up the eukaryotic replications.

So, what is the difference? The important differences between the prokaryotic and the eukaryotic replications. Eukaryotic DNA is larger than the prokaryotic DNA that is a very very important difference because the purpose of the replication is to you know to duplicate the DNA and if the DNA amount is large then it will actually going to be required the different machinery. The movement of fork is slower in eukaryote than the prokaryote because the nucleotide have to disassemble so that the DNA becomes available to the DNA and polymerase. So, this means the eukaryotic replication is going to be slower it is going to take longer period of time to complete and that is why you remember that the life cycle of the eukaryotic cells are much more you know much more than the prokaryotic system. For example, the E.

coli completes its life cycle or completes its duplication in the 18 minutes. Compared to that a simple e coli simple mammalian cells for example, the H e k 293 completes its duplication somewhere around 16 to 17 hours. So, it that is because of the simple reason that the DNA replication is very very slow in the case of mammalian system compared to the bacterial system. So, here everything is getting over by in 18 minutes whereas, here that same thing required the 16 to 17 hours. Then it has a distinct packaging of the

eukaryotic DNA in terms of the chromatin.

So, chromatin structure is very very higher order organizations in the case of the eukaryotic system whereas, it is not in the case of eukaryotic system. Now, let us take an example like what is the replication rate. So, replication rate in the case of prokaryote is approximately 1000 base pair per second whereas, the replication rate is 10 times slower than the prokaryote. So, it is only the 50 nucleotides per second. So, it is very very small and it is a small because of the simple reason that the nucleotide have to disassemble before the DNA become available to the DNA polymerase.

And apart from that in the prokaryotic system you do not have the chromatin structure. So, chromatin structure has to you know disassemble the DNA has to be free from the chromatin structures and so on. So, before getting into the detail of the different processes of the DNA replication in the eukaryotes let us first understand the machinery about the DNA replications. So, there are different types of polymerases because these are the important component of the replications. So, you have the 5 different types of DNA polymerases like alpha, beta, gamma, delta and the epsilon.

And the localization most of the DNA polymerases are present in the nucleus except that the DNA poly gamma is present into the mitochondria. Then the what is the biological function? The biological function of the alpha is the replication initiation, beta is involved in the DNA repair, then the gamma is involved into the mitochondrial DNA replications, then the delta and the epsilon is required for the replication of the lagging as well as the leading strands. Then you require the number of subunits. So, as far as the structure is concerned the alpha is tetramer, beta is monomer and gamma is the homotetramer and the delta is dimer and the epsilon is not known. Then 3 prime exonuclease activity, 3 prime exonuclease activity is absent in most in the alpha and beta, but it is present in the gamma, delta and epsilon.

Then primase binding, so primase binding is present in the case of alpha, but it is absent in the all other DNA polymerases. Then you require the molecular weight of catalytic site. So, it is going to be 160 to 185 and so on. Then you require the Km for the dNTPs. So, Km for the dNTPs is in the range of 2 to 5, 10 and 0.

5 and 2 to 4. So, if you cannot be able to understand what is Km, you should you know be able to understand this by I think we have discussed very briefly in one of the lectures where we were talking about the enzyme. So, we have discussed about the Km and so Km is the Michaelis Ventum constant and it actually indirectly says what will be the affinity of the enzyme for the dNTPs. Sensitivity to arabinol CTP, so it is going to be very high in the case of the alpha and delta, but it is going to be low in the case of beta

and gamma and sensitivity to Fe2 code line. So it is going to be high in the case of alpha and the delta where and the epsilon as well, but it is going to be low in the case of the beta and gamma. Now, let us talk about the some of these DNA polymerases.

So, we will start with the alpha. So DNA polymerase alpha is localized into the nucleus and it is a tetramer. So it is going to have the 4 subunits, you are going to have the PolA1, PolA1 regulatory, PolA3 primase activity and PolA4 it is a primase activity. So for all the 4 subunits are different. So A1 is going to have the catalytic activity, A2 is going to have the regulatory activity and A3 is going to have the primase activity and A4 is also going to have the primase activity.

Now that means you are going to have the Pol alpha and Pol primase activity together. This means it is actually going to synthesize the primer and as well as it is going to sit on utilize that primer for the DNA applications. So initiation of the replication on both the leading and lagging strand synthesis is the function of the DNA polymerase alpha. Then we have the DNA polymerase delta. So DNA polymerase delta is localized into the nucleus, it catalyzes the synthesis of the lagging strands.

It has the high processivity when interacting with the PCNA or PCNA or the polyfluidating cell nuclear antigen. PCNA is a important factor what is been having a very critical role in the DNA damage and repair as well and it is also associated with the helicase activity and it improves fidelity or replication by a factor of 10 to power 2 due to its proofreading actions. It has a 4 subunit, it has 1, 2 and 3 and 4. So large subunit catalyzes the 5 prime to 3 prime catalytic activity whereas small subunit catalyzes the 3 to 5 prime exonuclease activity or the proofreading activity. Then we have the DNA polymerase epsilon.

So it is localized into the nucleus. DNA polymerase epsilon catalyzes the repair mechanism. It also catalyzes the removal of primer and filling the primer gap in the Okazaki fragment. So it is very much close to what the function what you have seen in the case of prokaryotic system as DNA pol I. So it is actually going to have the same kind of role that it is going to remove the primers and it is also going to fill the gap in the between the Okazaki fragments.

It is going to have the 4 subunit. You are going to have 1, 2, 3 and 4 and 5 prime to 3 prime polymerase activity, 5 prime to 3 prime exonuclease activity and 3 prime to 5 prime exonuclease activity is present in the DNA polymerase epsilon and it is required for the different types of activities. For example 3 to 5 prime to 3 prime exonuclease activity is required to remove the RNA primer whereas 3 to 5 to 5 prime exonuclease activity is required for the proofreading. Now what is the replication factor RFA or

replication factor protein RFA? So it plays a significant role in stabilizing the single standard DNA region that are exposed during the DNA replication and repair mechanism. RPA prevents this single standard region from forming the secondary structure and protect them from the degradation allowing the other enzyme and factor to perform their function accurately.

So RPA is actually going to do the same job what you have understood in the case of SSP actually into the prokaryotic system. And then we have the PCNA. PCNA is important for the DNA synthesis and the repair and we are going to do discuss in detail about its role into the DNA repair when we are just going to discuss about the DNA repair mechanisms. The molecular weight of the molecule is 35000 kilo Dalton it is a multimeric protein and it is found into the large amount to the nuclei of the polyfluidating cells. And what is the function? So PCNA act as a clamp to keep the DNA polytheta and delta from dissociating off from the leading strand and PCNA help both hold the DNA polymerase epsilon to the DNA.

Replication factor C or RFC also known as clamp holder or to the matchmaker. So this is the PCNA which is going to be a clamp and then it is going to be a RFC so it is going to make a complex with each other. So binding of PCNA and RFC is going to make a complex and this complex is going to have the affinity for the DNA. So binding and hydrolysis of ATP once this is formed it is actually going to bind the ATP and it is going to hydrolyze the ATP and that actually is going to bring the structural changes into the PCNA and clamp holder RFC. And once it there will be a structural changes into the RFC it is actually going to have the affinity for the DNA and that is how it will go and bind to the DNA.

And then that actually is going to load the PCNA onto the DNA and once it binds to the DNA then it is actually going to form a complex with the polytheta and there will be hydrolysis of the ATP and the DNA polymerase delta is going to be recruited onto the DNA poly on the DNA and that is how it is actually going to help in the initiation stage of the DNA applications. Now one of the important aspect of the DNA replication in the eukaryotes is that it is actually the DNA is not freely available compared to the prokaryotes where the DNA is freely available and it is only required to locate the origin of the application and then all the machinery is going to assemble onto the original replications and then it is actually going to start the synthesis. Compared to that here first you have to bring the free double standard structure and then you are actually going to unwind the DNA and then you are actually going to do all that what you have discussed in the prokaryotic system. So, the first thing is you have to dismantle the chromatin. So, that a chromatin should be available for further that so, that the free DNA is available for all these kind of activity.

So, histone dissociation and then associations and all these events has to be reversed once your replication is done otherwise this free DNA which is not covered with the protein would be vulnerable for the different types of DNases and other kinds of enzyme what is present inside the nucleus. So, DNA replication is sandwiched between the two additional steps in the eukaryotes dissociation of the histones on the synthesis of histone. So, methylation at the fifth position of cysteine residue by the DNA methyl transferase appear to be functioned by loosening up the chromatin structures. This allows the DNA access to the protein and enzyme needed for the DNA applications. Remember that the DNA and the histones are attached with each other by a positive negative interaction.

So, it because of this electrostatic interactions. So, once the histones once the cytosine in the DNA is going to be methylated by the transferases it is actually going to bring or it is going to loosen the interaction between the histone and the chromatin structures and that is how it is going to allow the DNA access to the protein and enzyme needed for the DNA applications. Then it occurs simultaneously with the DNA. So, synthesis of the histone it is occurs simultaneously with the DNA applications. So, these are the sequential steps into the DNA applications you are going to have the first step is the formation of the reinitiation complex.

The second step is the initiation the third step is the elongation the fourth step is the termination and the fifth step is the telomerase function. So, that you can actually be able to have the completion of the telomeric regions. So, the first start with the pre initiation the pre initiation the pre initiation step is the crucial step that prepare the DNA for the actual replication process. This is steps primarily occur at the origin of replication which are specific DNA sequence where the replication begins. The process of identifying these sequences is known as the replication replicator selections which occurs into the G1 phase.

This process leads to the assembly of multi protein complexes at each replicator in the genome and the origin activator only occurs after cell enter into the S phase and trigger the replicator associated protein complex to initiate the DNA unwinding and the DNA polymerase recruitment. So pre initiation complex is come is a is a is been formed on to the origin of the applications. So the combination of the ORC MCM2 to 7 and CD66 and CDT1 along with the other regulatory protein factors form the pre initiation complex at the origin. This complex serves as a platform for the initiation of the application. So, imagine that if this is the origin or applications then the ORG will go and bind and once the ORG will go ORC will go and bind then the CDC6 and CDT11 is actually going to bind to this particular ORC.

And once these are going to bind then you are going to have the binding of the MCM2 to 7 and these are the once these are going to bind it is actually going to make the pre initiation complex and these pre initiation complex job pre initiation complex is that it should allow the recruitment of the DNA polymerase so that it will actually going to start the DNA synthesis. So, these are the some of the crucial step into the pre initiation complex formation. Now you are going to have the initiation. So, it involves the coordinated action of various protein complexes and enzyme to ensure the accurate and faithful duplication of genetic material. This process ensure that the each daughter cell receives a complete copy of the genome during cell divisions.

So, you are going to have the autonomous replicating sequences or the ARS or the replicators. For example, each contains approximately 400 automatic replicating sequences. So, these automatic replicated sequences are the independent sequences they are actually going to have their own origin or application. So, they can be able to you know start the replications and remember that in the case compared to the prokaryotic system in the eukaryotic system you are going to have the multiple origin or application that is how you are actually going to be complete the duplication of the genomic DNA at multiple points. So, you are going to start like the DNA replication will start from one end of the DNA and then it is going to over start from there and then it is going to finish by the end of the DNA.

No, it is not like that. In the case of eukaryotic system the DNA replication is going to be start at multiple points and that you know because the replication rate is very low compared to the prokaryotic system. So, it is actually required the multiple points at which the DNA replication is going to start. The second point is because the DNA size is very big it needs the multiple machinery to replicate the things. So, a specific site for the initiation of the DNA replication is the AT-rich sequences which is highly conserved 11 base pair sequences then you also have the flanking sequences and then you also require the 100 to 150 base pair long the 3 prime ends sequences. So, it may be the AT-rich sequences are actually the original replications the sequences or the site where the pre initiation complex is going to assemble and then the initiation is going to start.

The multiple origin of replications are spaced from the 300 to 300 kB apart which means for example if you have a DNA then you are going to have the multiple origin of the applications. So, all these original applications would be somewhere around 300 kB which means from this particular original application one fork will run in this direction and another fork will run in this direction and that is how it is actually going to complete the replication or the duplication of this amount of DNA. So, for example if it happens up to this so this is at this from this origin of the application it is only going to give you a DNA until this. But then you are going to have another original replication that also is

going to run in this direction and this direction and that also going to synthesize this amount of DNA. So, in a same amount of time this original replication will give you the DNA number 1 this will go actually going to give you DNA number 2 and this again this will going to give you the DNA number 3 and so on and that is how these all are actually going to assembled later on and it will give you the complete synthesis of this particular whole stretch.

So, that is what the adaptation or that is what the stretch D is what going to be adopted by the eukaryotic system because the eukaryotic genomes are very very large compared to the bacterial genome. The sequence between the 2 original replication is known as the replicons. So, this is actually a replicons this is the one replicons which is actually going to be you know participate into the replications this is another replicons this is another replicons. So, these are the multiple replicons what are going to be formed into the eukaryotic system. The AT rich also known as the ARS or the automatic replicating sequences similar to is similar to AT rich 13 mer present in the E coli OEC.

It is also called as the ORE or the origin replicating elements. The flanking sequences consist of the overlapping sequences that include the variant of the core sequences. So, ORE or the ORC so, ORE is called as origin replicating elements and ORC is called as origin replication complexes. So, ORE which is a 111 base pair sequence in the core sequence bind to a set of proteins for example, DNA pol alpha helicases DNA pol delta RFC, PCNA SSB, RFA and that all are going to assemble on to the origin recognition complexes and all these are going to make the origin recognition complexes which is a multimeric proteins. And initiation of the replication in all eukaryotic require this multimeric protein which binds to the several sequences.

So, ORE located adjacent to the approximately AT base pair AT rich sequence that is very easy to unwind the binding of ORC to ORE causes the unwinding at the DU that is the DNA unwinding elements. Now, events in the replication fork the DNA synthesis is initiated by the ORC and ORE the replication forks move bidirectionally and replication proceeds simultaneously as many as 200 forks which means you are going to have the 200 origin of replications or replicons simultaneously for working at together. Then the formation of the replication forks the replication fork in the eukaryotic consists of 4 components that forms in the following sequences. Sequence number 1 the DNA helicase and DNA pol alpha unwind the short segment of the parental DNA at AT base pair AT rich sequences called DU or the DNA unwinding elements. Then the DNA pol alpha initiate the synthesis of the RNA primer which is going to be a 110 base pair RNA primers.

Then the daughter strand synthesis is initiated by the DNA pol epsilon and the DNA pol

delta in the leading strand respectively. So this DNA pol epsilon and DNA pol Hidah is going to have the initiation of the DNA in the leading strands SSB and RFA binds to the single standard DNA and prevents its reannealing. So DNA pol epsilon and DNA pol delta is going to have the initiation strand is DNA synthesis going to initiate into the leading as well as the lagging strands. Then the 2 additional factors which play important role into the replication of eukaryotes are the PCNA and the RFC. So PCNA is actually going to be proliferating cell and nuclear integers and it act as a clamp to keep the DNA pol delta to keep dissociating of the leading strand and thus increasing the processivity of DNA pol epsilon.

Whereas the RFC is going to work as a clamp loader or matchmaker and its function is that it assists the DNA pol delta to form the clamp between the DNA and the PCNA and it helps in setting up a link between the DNA pol delta and DNA pol epsilon so that the leading and lagging strands synthesis can take place simultaneously. So this is the one of the examples where the fork is running in the both directions and this is the situation in the how the replication fork is going to be formed and so the replication initiation complex is going to be assembled on both the side what you see here is one side one fork is one initiation complex is going to be assembled on this side and another one is going to be assembled on this side and that is how it is actually keep you know removing the association of the DNA from the nucleosome and that is how the this will run in this direction and this will run in this direction. Rate of the replication fork movement so the rate of replication fork movement in eukaryote is approximately 50 nucleotide per second which is only one tenth of the eukaryote replication rate replication of human chromosome proceeds bi-directionally from the multiple origin spaced 300 to 30 to 300 kBBS pair apart and completed within an hour and average chromosome contain nearly 100 replicons between and thus the replication proceeds simultaneously as many as 200 replicons. So this is all about the pre initiation complex and the initiation once the initiation is done then it is actually going to enter into the next phase and that is called as the elongation. So elongation during elongation an enzyme called DNA polymerase add the DNA nucleotide to the C prime end of the newly synthesized poly nucleotide strands the template strand specify which of the four nucleotide that is ATGC is going to be added at the position along the new chain.

So you know that the wherever you in the template is the template has A then it is actually going to add the T if the template has G then it is actually going to add the C. So it is always going to follow the Watson-Crick base pairing rule and that is how it is actually going to add. Only the nucleotide complementary to the template nucleotide at the position is added to the new strands. For example when the DNA polymerase meet an adenosine nucleotide onto the template strand it adds the thymine to the C prime end of the newly synthesized strand and then move to the next nucleotide on the template

strand. The above process will continue until the DNA polymerase reaches add to the end of the template strands.

So these are the some of the events what is going to happen in the elongation into the eukaryotic DNA applications. You are going to have the assembly of the DNA pol delta and the epsilon onto the leading and the lagging strands and that is how you are going to have the synthesis of the leading and the lagging strands. So you are going to have the recruitment of the polymerase and primases onto the both strands right. So this is going to be leading strand this is going to be lagging strand and then this clamp is actually going to keep sliding into this direction and that is how it is actually going to be keep unwinding the DNA and same is true for this one also right. And in this one you are going to have the synthesis of the lagging strands and this side you are going to have the synthesis of the lagging strand whereas on this side you are going to have the synthesis of leading strands.

Now for one of the important component of this whole reaction is the synthesis of the primer and that is being done by the enzyme which is called as primase. So primase all newly synthesized nucleotides strand must be initiated by the specialized RNA polymerase called as a primase. It initiate the poly nucleotide synthesis by creating a short RNA nucleotide strand complementary to the template DNA strands. The slot set of RNA nucleotide is known as the primers. Once the RNA has been extended at the template strand the primer exist and the DNA polymerase strand the new strand with the nucleotide complementary to the template strand.

RNA nucleotide in the primers are removed by the deononucleotide by the help of the DNA polymerase. Once the DNA replication is finished the daughter molecules are made entirely of continuous DNA strand with no RNA portions. The leading and the lagging strands the DNA polymerase can only synthesize new strand in the 5 prime to 3 prime direction so that the two newly synthesized strand grow in the opposite direction because of the template strand at the each replication force are anti-prolaryl. Leading strand it is synthesized continuously towards the replication fork as helicase unwind the template on double standard DNA. Whereas for the lagging strand it is synthesized in the direction away from the replication fork and away from the DNA helicase unwind.

Its synthesis this strand is synthesized in pieces known as the Okazaki fragment and each fragment began its own RNA primer. This all we have discussed when we were discussing about the prokaryotic system. Then we have the leading strand synthesis. So in the leading strand synthesis you are going to have it is initiated upon the RNA primer synthesis by the primase unit of the DNA pol alpha. Then the DNA pol alpha adds a stretch of DNA to the primers at this point the RFC carried out a process known as the

polymerase switching and RFC removed DNA pol alpha and assemble the PCNA in the region of primer strand terminus.

Then the DNA pol epsilon bind to the PSNA and carried out leading strand synthesis due to the 5 prime 3 prime polymerase activity. After the addition of several nucleotide in the total strand is removed by the DNA pol epsilon due to its 3 to 5 micro nucleus activity and gap is also filled by the same polymerase again. Then the nick is sealed by the DNA ligase and finally, the fidelity of the replication is removed by the DNA pol delta due to its proofreading activity. Then we have the lagging strand synthesis. The lagging strand synthesis of the Okazaki fragments initiated same way as the leading strand synthesis.

RNA primer is synthesized by DNA pol alpha due to its primase activity. The primer is then extended by the DNA pol delta due to its 5 prime to 3 prime polymerase activity using the dNTPs. All but one of the ribonucleotide in RNA primer is removed by the RNase H1. Then the exonuclease activity of FEN and the RTH1 complex removes the one remaining nucleotide. The gap is filled by the DNA epsilon by the 5 prime to 3 prime activity and the DNA ligase join the Okazaki fragment of the growing DNA strands. So this is all what we are going to show the lagging strand synthesis and as well as so in the lagging strand synthesis you are going to have the synthesis of the RNA, you are going to have the synthesis of unwinding the DNA and so on.

Combined activity of DNA pol delta and DNA pol epsilon. So looping of the lagging strand allow a combined DNA polymerase delta and DNA polymerase epsilon asymmetric dimer to assemble and elongate both leading and lagging strand in the same overall direction of the fork movement. And then the last portion or the last step is the terminations. So when the replication fork meets each other then the termination occur. It will result in the formation of the two duplex DNA. Even though the replication is terminated 5 prime end of the telomereic part of the new silicidase DNA molecule found to have shorter DNA strand than the template strand.

This shortage is corrected by the action of an enzyme the only actual replication is completed the enzyme is called as the telomerases. So when the replication is happening it is going to start from the center and one of the fork will go in this direction the other fork will go in this direction and that is how when they will meet with each other or they will meet the fork of the other the general replication is going to stop. But at these corners that these corners what will happen is that a synthesis is not going to be complete because the last primer what you are going to use is not going to allow the synthesis of the last portion of the DNA and these portion are actually going to be synthesized by an enzyme which is called as telomerase. And once the until the telomerase does not come

and synthesize the telomers it is very difficult to say that the DNA replication is complete because if that does not happen then this portion is going to be lost and if it is keep losing then there will be a shortening of the genomic DNA. Telomerases are the enzyme which actually synthesize the telomers or which actually are going to since complete the synthesis of the telomers.

So during eukaryotic replications telomerase play a crucial role in ensuring the accurate replication of the linear chromosome. So the genetic cell uses a semi-conserved application to replicate this DNA and this process poses a challenge at the end of the linear chromosome. Telomerases serve to overcome this challenge and maintain the integrity of the genetic information. So at the corner of the chromosome these corners are not going to be replicated because there will be a because it is going to be a problem. So a short stretch of the 5 to 8 base pair and the tandem repeats are and the G series nucleotide sequences are actually going to be a problem for telomerase.

So telomerase is actually going to fill these gaps. Now what is the end replication problem? So linear genome including those of the several viruses as well as the chromosome of eukaryotic cell force a special problem completion of replication of the lagging strand. Contamination of the RNA primer from the 5 and end of the linear molecule would leave a gap known as the primer gap and this primer gap cannot be filled by action of DNA polymerase because of the absence of the primer terminus to the extent and if the DNA does not get replicated the chromosome will shorten a bit with each round of replication and this problem has been solved by an enzyme which is called as the telomerases. So telomerases also known as the RNA dependent DNA polymerase and it is a ribonucleotide containing the RNA component having repeat of 9 to 13 nucleotides long. This RNA component serves as a template for the synthesis of the telomeric repeat at the parental DNA ends. So telomerases uses at the 3 prime end of the parental DNA strand as a primer and its template self DNA component.

So it is going to use the self DNA complaint as a template and it has a 5 prime to 3 prime RNA dependent DNA polymerase activity due to which it add the successive telomeric repeats to the parental DNA strand at its 3 prime end. This means the enzyme itself is going to have the RNA component and that RNA component is actually going to serve as a template and that is how it is actually going to synthesize the DNA complementary to that particular sequence and it is going to add that repeat on multiple occasions and that is how it is actually going to fill the last gap of that particular DNA. So regeneration of the telomerases, telomeric DNA consists of the simple tandem repeated sequence at the 5 prime end which are in the human for example you have the A GGG TT in higher plants you have the A GGG TT TT algae you have the A GGG TT TT protozoan you have the GGGG and TT TT and the yeast you are going to have the

GGG and T. So these are the repeat sequences what are being present into the telomeric regions and the telomerase is going to add these repeats on multiple occasions. Telomerase uses its RNA component as a template and parental DNA as a primer and then by its RNA dependent DNA polymerase activity it repeatedly add the telomeric sequences to the 3 prime end of the parental DNA and then it is released.

At last the RNA primers of telomerase is bound near the leading lagging strand and it is extended by the DNA polymerase. Thus the lagging strand synthesis is completed. So this is what exactly what is going to happen. So for example you once you have the this kind of situation where this portion is actually going to be need to be synthesized. So telomerase is actually going to bind to the 3 prime end of the telomer and that is complementary to the telomeric RNA and that is how it is actually going to extend.

So bases are added using RNA as a template. So it is going to have this particular type of RNA what is been already been present inside the telomerase and it is going to synthesize this. So it is that is how it is actually going to synthesize this sequence and you remember this is actually having a U. So instead of U you are going to have the A and that is how it is actually going to be keep synthesizing this and utilizing this DNA polymerase will actually going to bind a complementary DNA lagging strand and it is actually going to synthesize this strand and that is how you are going to have the completion of the synthesis of the telomerases. Elizabeth Blackburn and her colleague have provided the answer to fill up the gaps with the help of the telomerases known as the modified reverse transcriptase or telomer transferase. So that the genes are end are conserved in human the RNA template consist of AAU CCC repeats.

Examples of repetitive sequence which varies among species for example, Tetrahyena is going to have the AA triple C and Oxyrhittica you are going to have the AAA triple C. Now this is the mechanism through which sorry so this is the mechanism through which the telomerase is going to fill the gaps you are going to have 3 prime end of the lagging strand base pair with the unique region of the telomerase associated RNA. The telomerase catalytic site at the deoxynucleotide using the RNA molecule as a template and the telomerase then translocate to the new 3 prime end by pairing with the RNA template and it continue with the reverse transcription. DNA polymerase uses the newly made primer for the synthesis of DNA to fill the remaining gap.

The primer is the removed and nicked between the fragment is sealed by the DNA ligase. So this is all about the DNA applications in the prokaryotic and as well as the eukaryotic system. What we have discussed so far we have discussed about the DNA application in the eukaryotic system and how it is different from the prokaryotic system in terms of the machinery and in terms of the processivity and in terms of the processes

and you can you might have now realized that it is very complicated in the eukaryotic system compared to the prokaryotic system. 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 DNA applications. Thank you. .