Cell and Molecular Biology
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Central Dogma of Molecular Biology (Part 2)
Lecture - 37
Replication (Part 3)

Hello everyone, this is Dr. Vishal Trivedi from the Department of Biosciences and Bioengineering, IIT Guwahati. And in this particular module, we are discussing the DNA applications. So far, what we have discussed is DNA replication in prokaryotes. And while we were discussing DNA replication in prokaryotes, we discussed how the initiation complex is going to be formed, how the different types of components are required for DNA replication in prokaryotes, and how DNA replication in prokaryotes is different and requires special machinery for its DNA synthesis. Now in today's lecture, we are going to discuss the eukaryotic replication.

Because eukaryotic replication is different from prokaryotic replication in terms of the components or enzymes required, the properties of these enzymes are very, very different. As the name suggests, DNA replication is the mechanism by which the cell duplicates its genetic material, ensuring that each newly formed cell receives an accurate copy of the original DNA. So, this kind of objective is also being met even when we are doing DNA replication in eukaryotes as well. The process is essential for the growth, development, and transmission of genetic information from one generation to the next, and DNA replication involves various enzymes and proteins working together to unwind and separate the double-stranded DNA molecule, synthesize the new complementary strand, and ensure the fidelity of the copied genetic material.

The basic principle of eukaryotic and prokaryotic re-replication is the same. However, there are some notable differences. Now, one important point is that eukaryotic replication is much more complicated than prokaryotic replication, and there are many reasons why it is so. So why is eukaryotic replication more complex than prokaryotic replication? Because of the simple reason that eukaryotic genomes are quite complex. They are larger than bacterial DNA.

Remember that bacterial DNA is very small, even in plasmid DNA. It has a complex structure because the bacterial chromosome or bacterial genomic content is not associated with protein molecules, and it is not as complex compared to the eukaryotic system. Remember that when we were discussing the eukaryotic genome, we discussed how it is actually associated with the histone proteins and how the histone octamers form the nucleosomes, which are then further assembled to give you the chromosomes. So, the

chromosome is mostly present in the eukaryotic structures. But apart from these differences or the complexity, the replication process in both the prokaryotic and eukaryotic systems actually involves some of the basic steps.

For example, the formation of the replication fork is common to them. Primer synthesis is also common among them. Once the primer job is over and it has to be removed by the DNA ball in the prokaryotic system, that is also common. Then you are also going to have the Okazaki fragment, whether it is the prokaryotic system or the eukaryotic system. Then the replication mode 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 both the prokaryotic as well as the eukaryotic system.

So, the replication fork is going to be bi-directional in the case of the prokaryotic or eukaryotic system. It is also required for gap bridging between the newly synthesized DNA fragments with the help of DNA pol 1 and DNA ligase. So apart from these kinds of similarities, there are significant differences in terms of the machinery and other kinds of requirements. So apart from these similarities, there are significant differences between the prokaryotic and the eukaryotic replications. We have discussed many of these differences, but I thought I should remind you so that it will be easy for you to follow up on the eukaryotic replications.

So, what is the difference? The important difference is between prokaryotic and eukaryotic replication. Eukaryotic DNA is larger than prokaryotic DNA, which is a very important difference because the purpose of replication is to duplicate the DNA, and if the amount of DNA is large, then different machinery will actually be required. The movement of the fork is slower in eukaryotes than in prokaryotes because the nucleotides have to disassemble so that the DNA becomes available to the DNA polymerase. So, this means that eukaryotic replication is going to be slower. It is going to take a longer period of time to complete.

And that is why you remember that the life cycle of the eukaryotic cells is much more complex than the prokaryotic system. For example, E. coli completes its life cycle or its duplication in 18 minutes. Compared to that, a simple mammalian cell, for example, the HEK 293. completes its duplication in approximately 16 to 17 hours.

So that is because of the simple reason that DNA replication is very, very slow in the case of the mammalian system compared to the bacterial system. So here everything is getting done in 18 minutes, whereas the same thing requires 16 to 17 hours. Then it has a distinct packaging of eukaryotic DNA in terms of chromatins. So chromatin structure is a very high-order organization in the case of the prokaryotic system, whereas it is not in the

case of the eukaryotic system. Now, let us take an example, such as what the replication rate is.

So, the replication rate in the case of prokaryotes is approximately 1000 base pairs per second, whereas the replication rate is 10 times slower than that of prokaryotes. So, it is only 50 nucleotides per second. So, it is very, very small, and it is small because of the simple reason that the nucleotides have to disassemble before the DNA becomes available to the DNA polymerase. 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 details of the different processes of DNA application in eukaryotes, let us first understand the machinery behind DNA applications. So, there are different types of polymerases because these are the important components of the replication. So, you have the five different types of DNA polymerases: alpha, beta, gamma, delta, and epsilon. And the localization of most DNA polymerases is in the nucleus, except that DNA polymerase gamma is present in the mitochondria. Then, what is the biological function? The biological function of the alpha is replication initiation, beta is involved in DNA repair, gamma is involved in mitochondrial DNA replication, and delta and epsilon are required for the replication of both the lagging and leading strands.

Then you require the number of subunits. So as far as the structure is concerned, alpha is a tetramer, beta is a monomer, gamma is the homotetramer, delta is a dimer, and epsilon is not known. Then 3' prime exonuclease activity is absent in most of the alpha and beta, but it is present in the gamma, delta, and epsilon. Then primase binding is present in the case of alpha, but it is absent in all other DNA polymerases. Then you require the molecular weight of the catalytic site, which is going to be 160 to 185 and so on.

Then you require the KM for the DNTPs. So, Km for dNTP is in the range of 2 to 5, 10, and 0.5 to 2 to 4. So, if you cannot understand what Km is, I think we discussed very briefly in one of the lectures where we were talking about the enzymes. So, we have discussed the Km, and Km is the Michaelis-Menten constant, which actually indirectly indicates the affinity of the enzyme for the dNTPs, right? Sensitivity to arabinol CTP.

So, it is going to be very high in the case of alpha and delta, but it is going to be low in the case of beta and gamma and sensitivity to ephedrocordyline. So, it is going to be high in the case of alpha and delta, as well as epsilon, but it is going to be low in the case of beta and gamma, right? Now, let us talk about some of these DNA polymerases. So, we will start with the alpha. So, DNA polymerase alpha is localized in the nucleus and it is a

tetramer, right? So, it is going to have four subunits. You are going to have the Pol A1, Pol A1 regulatory, Pol A3 primase activity, and Pol A4; it is the primase activity.

So, all four subunits are different, right? So, A1 is going to have the catalytic activity, A2 is going to have the regulatory activity, 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 activities together, right? This means it is actually going to synthesize the primer and will also sit and utilize that primer for DNA replication. So, the initiation of replication on both the leading and lagging strands is the function of DNA polymerase alpha. Then we have DNA polymerase delta. So DNA polymerase delta is localized in the nucleus.

It catalyzes the synthesis of the lagging strand. It has high processivity when interacting with PCNA or the proliferating cell nuclear antigen. PCNA is an important factor that has a very critical role in DNA damage and repair as well. And it is also associated with helicase activity, and it improves the fidelity of replication by a factor of 10 to the power of 2 due to its proofreading actions. It has four subunits: one, two, three, and four.

So, the large subunit catalyzes the 5' to 3' catalytic activity, whereas the small subunit catalyzes the 3' to 5' exonuclease activity or the proofreading activity. Then, we have the DNA polymerase epsilon, right? So, it is localized into the nucleus; DNA pol epsilon catalyzes the repair mechanism and also catalyzes the removal of the primer and filling the primer gap in the Okazaki fragment. So, it is very much close to what you have seen in the case of the prokaryotic system as DNA pol 1. So, it is actually going to have the same kind of role that removes the primers and also fills the gap between the Okazaki fragments. It is going to have the four subunits; you are going to have 5' to 3' polymerase activity, 5' to 3' exonuclease activity, and 3' to 5' exonuclease activity, which are present in DNA polymerase epsilon, and these activities are required for the different types of functions.

For example, 3 to 5 prime to 3 prime exonuclease activity is required to remove the RNA primer, whereas 3 to 5 prime to 5 prime exonuclease activity is required for proofreading. Now, what is the replication factor RFA or replication vector protein RFA? It plays a significant role in stabilizing the single-stranded DNA regions that are exposed during DNA replication. The repair mechanism RPA prevents this single standard region from forming the secondary structure and protects it from degradation, allowing the other enzymes and factors to perform their functions accurately, so RPA is actually going to do the same job as you have understood in the case of SSB in the prokaryotic system. And then we have the PCNA; PCNA is important for DNA synthesis and repair, and we are going to discuss in detail its role in DNA repair when we discuss the DNA repair

mechanisms. The molecular weight of the molecule is 35,000 kilodaltons.

It is a multimeric protein and it is found in large amounts in the nuclei of the polyphenating cells. And what is the function? So, PCNA acts as a clamp to keep the DNA polymerase delta from dissociating off from the leading strand, and PCNA helps both hold the DNA polymerase epsilon to the DNA. Replication factor C, or RFC, also known as the clamp holder or the matchmaker. So, this is the PCNA, which is going to be a clamp, and then it is going to be an RSC. So, it is going to create a complex with each other.

So, the binding of PCNA and RSC is going to create a complex, and this complex is going to have an affinity for the DNA, right? So, binding and hydrolysis of ATP, once this is formed, are actually going to bind the ATP and hydrolyze the ATP, and that actually is going to bring structural changes to the PCNA and clamp holder RFC. Once there are structural changes in the RFC, it will actually have an affinity for the DNA, and that is how it will go and bind to the DNA, which will then load the PCNA onto the DNA. And once it binds to the DNA, 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 to the DNA, and that is how it is actually going to help in the initiation stage of DNA replication. Now one of the important aspects of DNA replication in eukaryotes is that the DNA is not freely available compared to prokaryotes, where the DNA is freely available. It is only required to locate the origin of replication, and then all the machinery is going to assemble onto the original replication, and then it is actually going to start the synthesis.

Compared to that, here you first 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 you have discussed in the prokaryotic system. So, the first thing is you have to dismantle the chromatin. So, that chromatin should be available so that the free DNA is available for all these kinds of activities. So, histone dissociation, associations, and all these events have to be reversed once your replication is done.

Otherwise, this free DNA, which is not covered with the protein, would be vulnerable to the different types of DNAs and other kinds of enzymes that are present inside the nucleus. So, DNA replication is sandwiched between the two additional steps in eukaryotes: the dissociation of the histones and the synthesis of histones. So, methylation at the fifth position of the cysteine residue by the DNA methyltransferase appears to function by loosening up the chromatin structures. This allows the DNA access to the proteins and enzymes needed for the DNA applications. Remember that the DNA and the histones are attached to each other by a positive-negative interaction.

So it is because of these electrostatic interactions. So when the cytosine in the DNA is going to be methylated by the transferases, it is actually 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 proteins and enzymes needed for the DNA applications. Then it occurs simultaneously, so the synthesis of the histone occurs simultaneously with the DNA applications. So, these are the sequential steps in DNA replication: the first step is the formation of the V initiation 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 complete the telomeric regions.

So, the first step is to start with the pre-initiation. The pre-initiation step is the crucial step that prepares the DNA for the actual replication process. These steps primarily occur at the origin of replication, which is a specific DNA sequence where replication begins. The process of identifying these sequences is known as the replicator selection, which occurs in the G1 phase. This process leads to the assembly of multiprotein complexes at each replicator in the genome, and the origin activator only occurs after the cell enters the S phase and triggers the replicator-associated protein complex to initiate DNA unwinding and DNA polymerase recruitment.

So, the pre-initiation complex is what we are informed about regarding the origin of the applications, right? The combination of the ORC MCM 2 to 7 and CD66 and CDT1, along with the other regulatory protein factors, forms the pre-initiation complex at the origin. This complex serves as a platform for the initiation of replication. So, imagine that if this is the original replication, then the ORG will go and bind, and once the ORC goes and binds. Then the CDC 6th and CDT 11 are actually going to bind to this particular ORC, and once they bind, you will have the binding of the MCM 227. And these are going to bind; it is actually going to make the pre-initiation complex, and the job of this pre-initiation complex is to allow the recruitment of the DNA polymerase so that it will actually start the DNA synthesis.

These are some of the crucial steps in the pre-initiation complex formation. Now, you are going to have the initiation. So, it involves the coordinated action of various protein complexes and enzymes to ensure the accurate and faithful duplication of genetic material. This process ensures that 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 one contains approximately 400 automatic replicating sequences. So, these automatically replicated sequences are the independent sequences. They are

actually going to have their own origin of replication. So, they can start the replications, and remember that compared to the prokaryotic system, in the eukaryotic system, you are going to have multiple original applications. That is how you are actually going to complete the duplication of the genomic DNA at multiple points.

So it is not like DNA replication will start from one end of the DNA and then it will start over from there and finish at the end of the DNA. No, it is not like that. In the case of eukaryotic systems, DNA replication is going to start at multiple points, and you know that the replication rate is very low compared to the prokaryotic system. So, it is actually required that multiple points at which DNA replication is going to start. The second point is that because the DNA size is very big, it needs multiple machinery to replicate.

So, a specific site for the initiation of DNA replication is the 80 reach sequences, which are highly conserved 11 base pair sequences. So, mostly the 80 reach sequences are actually the original replication sequences or the site where the pre-initiation complex is going to assemble, and then the initiation is going to start. The multiple origins of replication are spaced from 300 to 300 KB apart, which means, for example, if you have DNA, then you are going to have multiple origins of replication. So, all these original replications would be somewhere around 300 KB, which means from this particular original replication, one fork will run in one direction and another fork will run in the other direction. And that is how it is actually going to complete the replication or duplication of this amount of DNA.

So, for example, if it happens up to this point. So, from this original replication, it is only going to give you DNA until this. But then you are going to have another original replication that is also going to run in this direction and that direction, and that is also going to synthesize this amount of DNA. So, in the same amount of time, this original replication will give you DNA number 1, this will actually give you DNA number 2, and this again will give you DNA number 3, and so on. And that is how these are actually going to be assembled later on, and it will give you a complete synthesis of this particular whole stretch. So that is what the adaptation is, or that is what the ST is 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 two original replications is known as the replicons. So, this is actually a replicon; this is the one replicon that is actually going to participate in the replication. This is another replicon; this is another replicon. So, these are the multiple replicons that are going to be formed in the eukaryotic system. The AT-rich, also known as the ARS or the automatic replicating sequences, is similar to the AT-rich 13-mer present in the E.

coli OREC. It is also called the ORE or the origin replicating elements. The flanking sequences consist of the overlapping sequences that include the variant of the core sequences. ORE or ORC is called origin replicating elements, and ORC is called origin recognition complexes. ORE, which is a 111-base pair sequence in the core sequence, binds to a set of proteins; for example, DNA pol alpha, helicases, DNA pol delta, RFC, PCNA, SSB, and RFA. And that all are going to assemble onto the origin recognition complexes, and all these are going to make the origin recognition complexes, which are multimeric proteins.

The initiation of replication in all eukaryotes requires this multimeric protein, which binds to several sequences. So, ORE is located adjacent to the approximately 80 base pair rich sequence that is very easy to unwind. The binding of ORC to ORE causes the unwinding at the DU, which is the DNA unwinding element. Now, events in the replication fork initiate DNA synthesis by the ORC and ORE. The replication forks move bidirectionally, and replication proceeds simultaneously at as many as 200 forks, which means you are going to have 200 origins of replication, or replicons, working together simultaneously.

Then the formation of the replication fogs. The replication fog in the eukaryotes consists of four components that form in the following sequences. Sequence number one, the DNA helicase and DNA pol alpha unwind the short segment of the parental DNA at 80 base pairs, reaching sequences called the DU or the DNA unwinding elements. Then the DNA pol alpha initiates the synthesis of the RNA primer, which is going to be a 10-base pair RNA primer. Then the daughter strand synthesis is initiated by DNA pol epsilon and DNA pol delta in the leading strand, respectively. So this DNA pol epsilon and DNA pol hitha are going to have the initiation of the DNA in the leading strands.

SSB and RFA bind to the single-stranded DNA and prevent its reannealing. So DNAPOL epsilon and DNAPOL delta are going to have the initiation strand in DNA synthesis initiate into the leading as well as the lagging strands. Then the two additional factors that play an important role in the replication of eukaryotes are the PCNA and the RFC. So, PCNA is actually going to be polyphenolating cell nuclear antigens, and it acts as a clamp to prevent DNAPol delta from dissociating from the leading strand, thus increasing the processivity of DNAPol epsilon. Whereas the RFC is going to work as a clamp loader or matchmaker, its function is to assist the DNAPOL delta in forming the clamp between the DNA and the PCNA, and it helps in setting up a link between the DNAPOL delta and DNAPOL epsilon so that the leading and lagging strands can take place simultaneously.

So, this is one of the examples where the fork is running in both directions, and this is the situation in how the replication fork is going to be formed. So, the replication initiation complex is going to be assembled on both sides. What you see here is one side, one focus; one pre-initiation complex is going to be assembled on this side, and another pre-initiation complex is going to assemble on this side. And that is how it actually keeps you removing the association of the DNA from the nucleosome, and that is how this will run in this direction, and this will run in this direction.

Rate of the replication fork movement. So, the rate of the replication fork movement in eukaryotes is approximately 50 nucleotides per second, which is only one tenth of the E. coli replication rate. Replication of the human chromosome proceeds bidirectionally from multiple origins spaced 30 to 300 kb apart and is completed within an hour. An average chromosome contains nearly 100 replicates, and thus the replication proceeds simultaneously with as many as 200 replicates.

So this is all about the pre-initiation complex and initiation. Once the initiation is done, it is actually going to enter the next phase, which is called elongation. During elongation, an enzyme called DNA polymerase adds the DNA nucleotide to the 3 prime end of the newly synthesized polynucleotide strands. The template stands specify which of the four nucleotides that are ATGC is going to be added at the position along the new chain. So, you know that wherever you are in the template, if 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 strand. For example, when the DNA polymerase meets an adenosine nucleotide on the template strand, it adds thymine to the 3 prime end of the newly synthesized strand and then moves to the next nucleotide on the template strand. The above process will continue until the DNA polymerase reaches the end of the template strands. So these are some of the events that are going to happen in the elongation of the eukaryotic DNA applications.

You are going to have the assembly of DNA pol delta and epsilon onto the leading and lagging strands, and that is why you are going to have the synthesis of the leading and lagging strands. So you are going to have the recruitment of the polymerase and primases onto both strands of the DNA, right? So this is going to be the leading strand, this is going to be the lagging strand, and then this clamp is actually going to keep sliding in this direction, and that is how it is actually going to keep unwinding the DNA, and the same is true for this one also, right? And in this one, you are going to have the synthesis of the

lagging strand. On 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, one of the important components of this whole reaction is the synthesis of the primer, which is being done by the enzyme called primase. So primase, all newly synthesized nucleotide strands must be initiated by the specialized RNA polymerase called primase.

It initiates the polynucleotide synthesis by creating a short RNA nucleotide strand complementary to the template DNA strands. The short stretch of RNA nucleotides is known as the primers. Once the RNA has been extended on the template strand, the primer exists and the DNA polymerase extends the new strand with the nucleotide complementary to the template strand. RNA nucleotides in the primers are removed by the DNA nucleotides with the help of DNA polymerase. Once the DNA replication is finished, the daughter molecules are made entirely of continuous DNA strands with no RNA portions.

The leading and lagging strands, the DNA polymerase can only synthesize a new strand in the 5' to 3' direction so that the two newly synthesized strands grow in opposite directions because the template strands at each replication fork are anti-parallel. Leading strand is synthesized continuously toward the replication fork as helicase unwinds the template on double-stranded DNA. Whereas for the lagging strand, it is synthesized in the direction away from the replication fork and away from the DNA helicase unwinding. This strand is synthesized in pieces known as the Okazaki fragments, and each fragment begins with its own RNA primer.

This is all we discussed when we were discussing the prokaryotic system. Then we have the synthesis of the leading strand. So, in the leading strand synthesis, it is initiated by the RNA primer synthesized by the primase unit of the DNA pol alpha. Then the DNA polymerase alpha adds a stretch of DNA to the primers. At this point, the RFC carried out a process known as polymerase switching, and RFC removed DNA pol alpha and assembled the PCNA in the region of the primer strand terminus. Then, the DNAPol epsilon binds to the PSNA and carries out leading strand synthesis due to the 5'-3' polymerase activity.

After the addition of several nucleotides, the total strand is removed by DNAPol epsilon due to its 3'-5' exonuclease activity, and the gap is also filled by the same polymerase again. Then, the NIC is sealed by the DNA ligase, and finally, the fidelity of the replication is compromised by the DNAPol delta due to its proofreading activity. Then we have the synthesis of the lagging strand. So, lagging strand synthesis of the Okazaki fragments is initiated in the same way as the leading strand synthesis.

RNA is primarily synthesized by DNA pol alpha due to its primase activity. The primer is then extended by DNA pol delta due to its 5' to 3' polymerase activity using the dNTPs. All but one of the ribonucleotides in RNA primers is removed by RNase H1. Then the exonuclease activity of FEN and the RTH1 complex removes the remaining nucleotide. The gap is filled by the DNA epsilon through the 5 prime to 3 prime activity, and the DNA ligase joins the Okazaki fragments of the growing DNA strands. So, this is all about what we are going to show regarding the lagging strand synthesis.

In the lagging strand synthesis, you will have the synthesis of RNA, the unwinding of DNA, and so on. Combined activity of DNAPol delta and DNAPol epsilon. So, looping of the lagging strand allows a combined DNAPol delta and DNAPol epsilon asymmetric dimer to assemble and elongate both the leading and lagging strands in the same overall direction of the fork movement, and then the last portion or the last step is the termination. So, when the replication forks meet each other, the termination occurs.

It will result in the formation of two duplex DNA molecules. Even though the replication is terminated, the 5-5 end of the telomeric part of the new cellulose DNA molecule is found to have a shorter DNA strand than the template strand. This shortage is corrected by the actions of an enzyme. The only actual replication is completed. The enzyme is called telomerase. So, when replication is happening, it is going to start from the center; one of the folks will go in this direction, and the other folk will go in that direction, and that is how, when they meet each other or meet the folk of the other, replication is going to stop.

But at these corners, what will happen is that the synthesis will not be complete. The last primer that you are going to use is not going to allow the synthesis of the last portion of the DNA, and this portion is actually going to be synthesized by an enzyme called telomerase. Until telomerase comes and synthesizes the telomeres, it is very difficult to say that DNA replication is complete because if that does not happen, then this portion is going to be lost, and if it keeps losing, then there will be a shortening of the genomic DNA. Telomerases are the enzymes that actually synthesize the telomeres or that are going to complete the synthesis of the telomeres. So, during eukaryotic replication, telomerase plays a crucial role in ensuring the accurate replication of the linear chromosome. Eukaryotic cells use a semi-conservative method to replicate their 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. A short stretch of the 5 to 8 base pairs and the tandem repeats, as well as the G-series nucleotide sequences, is actually going to be a problem for telomerase. So, telomerase is actually going to fill in these gaps. Now, what is the end-replication

problem? So, the linear genome, including those of several viruses as well as the chromosome of the hepatic cell, poses a special problem for the completion of replication of the lagging strand. Excision of the RNA primer from the 5' end of the linear molecule would leave a gap known as the primer gap, right? And this primer gap cannot be filled by the action of DNA polymerase because of the absence of the primer terminus to that 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 called telomerases. So, telomerase is also known as the RNA-dependent DNA polymerase, right? It is a ribonucleotide containing the RNA component with repeats of 9 to 30 nucleotides long. This RNA component serves as a template for the synthesis of the telomeric repeat at the parental DNA ends. So, telomerase is used 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 component as a template. and it has a 5' to 3' RNA-dependent DNA polymerase activity due to which it adds the successive telomeric repeats to the parental DNA strand at its 3' 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, add that repeat on multiple occasions, and fill the last gap of that particular DNA. So, the regeneration of the telomerases and telomeric DNA consists of a simple tandem repeat sequence at the 5 prime end; for example, in humans, you have AGGGTT, in higher plants you have AGGTTTT, in algae you have AGGGTTTT, in protozoans you have GGGG and TTTT, and in yeast you have GGG and T. So these are the repeat sequences that are present in 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, through its RNA-dependent DNA polymerase activity, it repeatedly adds the telomerase sequences to the 3' end of the parental DNA, and then it is released. At last, the RNA primers of telomerase are bound near the leading lagging strand, and they are extended by the DNA polymerase. Thus, the synthesis of the lagging strand is completed. So, this is exactly what is going to happen, right? So, for example, once you have this kind of situation where this portion needs to be synthesized, right? So, telomerase is actually going to bind to the 3 prime end of the telomere, 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 that has already been present inside the telomerase, and it is going to synthesize

this. So, this 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: keeping synthesizing this and utilizing this DNA polymerase will actually bind the complementary DNA lagging strand, and it is actually going to synthesize this strand.

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 the gaps with the help of telomerase known as modified reverse transcriptase or telomere transferase so that the genes are ended or conserved. In humans, the RNA template consists of AAUCCC repeats. Examples of repetitive sequences that arise among species. For example, Tetrahyna is going to have the AACCC, and Oxiridica is going to have the AAACCC.

So, this is the mechanism through which telomerase is going to fill the gaps. You are going to have 3 prime ends of the lagging strand base paired with the unique region of the telomerase-associated RNA. The telomerase catalytic site at the deoxy nucleotide uses the RNA molecule as a template, and the telomerase then translocates to the new 3 prime end by pairing with the RNA template, and it continues with the reverse transcription. DNA polymerase uses the newly made primer for the synthesis of DNA to fill in the remaining gap. The primer is removed, and the nick between the fragments is sealed by the DNA ligase.

So, this is all about the DNA applications in prokaryotic and eukaryotic systems. What have we discussed so far? We have discussed the DNA application in the eukaryotic system and how it is different from the prokaryotic system in terms of machinery, processivity, and processes. 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 DNA replication. Thank you.