Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

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Week 01

Lecture 02: DNA Replication & Repair Mechanism

Namaskar. Welcome back to the very first week of the NPTEL lecture series, Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis. So, we have started the very basics and fundamentals of molecular biology and molecular diagnostics. Today we are going to discuss DNA replication and repair mechanism. Now, in this class the concepts which we are going to cover are the cross process of replication both in prokaryote and eukaryotes. Then the very important enzyme DNA polymerase which is required for replication.

Another very important aspects which should be discussed in replication that is telomere and an enzyme telomerase. Then different inhibitors of DNA replication and finally, different types of DNA damages and their respective repair mechanisms. So, let us go on to the very process of DNA replication. So, what is DNA replication? This is basically the synthesis of an identical copy of the parent DNA.

Now, DNA replication is basically synthesizing new DNA strands and that synthesis is semi conservative in nature and this proposal is given by Matthew Meselson and Franklin Stahl in 1957. So, what is this semi conservative nature or process of DNA replication? See here you can see the 2 strands of DNA these are the parental DNA from which the new daughter DNA strands will be synthesized. Now, here you can see both the strands both the strands can act as template over which the complementary strand complementary because the bases are complementary as per the base pairing rule. Now, both of these strands you can see acts as they can act as template over which new daughter strand is synthesized. So, each parent strand is having each daughter strand and finally, these are our target double strand DNA where one old parent strand and another new daughter strand is present.

So, this is a semi conservative nature of the DNA replication where a hybrid strand one

from parent and one new strand are synthesized. Now, this new strand synthesis is always from 5 prime to 3 prime direction. That means, the nucleotides are attached if the strand parent strand is like these the nucleotides are synthesized basically in this direction means the 5 prime end is here and in the 3 prime direction new nucleotides are synthesized and attached. Now, DNA synthesis is also semi discontinuous method why? A new strand of DNA is always synthesized in 5 prime to 3 prime direction because the free 3 prime hydroxyl group acts as a nucleophile where a new nucleotide is attached and elongated. Now, if you remember 2 of the strands in a double stranded DNA are anti parallel and both the strands are acting as template and template strand is always read from 3 prime to 5 prime direction because the daughter strand is synthesized from 5 templates are read from 3 prime to 5 prime direction because the daughter strand is synthesized from 5 to 3 prime end.

Now, before going to the semi discontinuous nature you need to know that there is formation of replication for what happens because the 2 parent strand are acting as templates they should be unowned. So, unwinding of the 2 DNA strands occurs that means, the hydrolysis of all the hydrogen bonds present over this segment has happened you can see this double stranded DNA has been opened up. So, that is the unounding and here you can see the formation is just like a fork. So, this is the replication for over which replication occurs or the new nucleotides are attached. Now, in one strand you can see if we start the replication here it can be read from 3 prime to 5 prime direction.

So, the synthesis can occur in this way. Now, what will happen in case of this strand because this strand has to be read from 3 prime to 5 prime direction which is here. So, the whole DNA remember the whole DNA is not unowned a part of the DNA is unowned forming the replication fork and when the new DNA strands are synthesized in this region again another region is opened up or unowned. So, what will happen in this region because a new strand has to be synthesized in this direction again when this part will be opened up nucleotide segment has to be synthesized in this direction. So, in one strand there is a continuous synthesis of parent DNA whereas, in the other strand there is segmental DNA synthesis a part of DNA is replicated after that when this part is completed this part is completed again another part is opened up and another new segment is synthesized.

So, the strand which has continuous replication or continuous addition of nucleotides this is our leading strand whereas, other strand which has segmental DNA synthesis that is known as lagging strand and this segments short segments or short fragments of DNA or short pieces of DNA which are synthesized over lagging strand they are known as Okazaki fragments. Now, remember Okazaki fragments though they are synthesized in segments they are joined or spliced together by the enzyme DNA ligase. So, DNA ligase is basically the enzyme which can seals the break in a double stranded DNA and also it

needs cofactor which is ATP in eukaryotes whereas, in prokaryotes this is mostly NAD plus. So, the concept of the semi discontinuous replication along with leading strand lagging strand and Okazaki fragments are now clear I hope. Now, let us move on to the very important enzyme that is DNA polymerase.

Polymerase means it it is causing polymerization of the new nucleotides over the daughter strand. Now, the fundamental reaction in DNA polymerase is basically phosphoryl group transfer. So, as I told the new DNA strand is synthesized from 5 prime to 3 prime direction basically what is required is a free 3 prime hydroxyl group of the nucleotide and this acts as nucleophile. This nucleophilic attack over the alpha phosphorus of the incoming deoxynucleotide which is supposed to be joined incoming deoxynucleotide 5 prime 5 prime phosphate anything GTP, ATP or CTP whatever they attack this 3 prime hydroxyl group attack over this alpha phosphorus of the nucleotide triphosphate. What happens? A pyrophosphate is released.

So, here you can see the pyrophosphate is released along with that here is addition of a new nucleotide. For the action of DNA polymerase what are the requirements? Obviously, one template is required over which the complementary nucleotides will be attached a primer is required. Now, what is a primer? This is a RNA segment a complementary RNA segment whose base sequences are complementary to one end of the DNA and which has a free 3 prime hydroxyl group which act as a nucleophile. Also another very important thing about DNA polymerases are processivity. Now, processivity we are going to discuss later.

So, let us begin with prokaryotic DNA replication. Now, DNA replication begins at a specific start site replication occurs in 5 prime to 3 prime direction which what we need is one primer one are short RNA fragment the free prime the 3 3 prime hydroxyl group of that primer helps in nucleophilic attack to add nucleotides. Now, apart from the DNA polymerase itself there are multiple other proteins or enzymes which are required for DNA replication. One very important enzyme is helicase which causes opening of the double helix then single strand binding protein or SSBP which is required for unwinding of the DNA strand or the maintenance of the unwinding of the DNA strand because it binds with the single strand and prevent rejoining or reannealing. Then primase another important enzyme which is required to synthesize RNA primer.

Now, replication is a process of high fidelity and in prokaryote this high fidelity process is maintained by the property of DNA polymerase itself and this is known as proofreading. That means, no wrong nucleotide should be attached to its in the nucleotide or new DNA strand. Now, how this proofreading activity is maintained by the DNA polymerase? Now, the active side of DNA polymerase can accommodate only the base pairs as per the base pairing rule and that is prescribed by the very basic geometry

of the incoming nucleotides. What happens if an incorrect nucleotide is incorporated or has formed hydrogen bond with the with the template strand? The remedy is that it will not fit in the active side because there is dissimilarity in the geometry and thus the incorporate base can be rejected even before the phosphodiester bond with the previous nucleotide can be formed. Now, this is done by a very specific new enzyme activity or nucleus activity that is 3 prime to 5 prime exonuclease activity which is present in DNA polymerase and it is basically required for removal of the mis-pair nucleotide as it causes a double check over each nucleotide addition.

What happens? There is a kinetic pause. So, if any wrong nucleotide is added there is a kinetic pause because the translocation of the enzyme from one nucleotide to another is inhibited and in these pause this exonuclease removes the mis-pair nucleotide. So, this is how 3 prime to 5 prime exonuclease activity which is present in DNA polymerase can help in proofreading function. So, you can see there are 2 different sides that is one active side in the DNA polymerase another is a 3 prime to 5 prime proofreading or exonuclease activity it happens in another side. Now, there are different types of DNA polymerases present in prokaryotes.

The most studied one are the DNA polymerase in E. coli. Now, you can see there are 3 different types of DNA polymerase which are very much discussed in E. coli type 1, type 2 and type 3. Now, type 3 is the main enzyme which is required for the replication it causes the main polymerization function why because it has number 1 high processivity and number 2 high polymerization rate.

So, the number of nucleotides per seconds which is added over the template strand is highest in case of DNA polymerase. So, around 252000 nucleotides per second can be added and number 2 is processivity. Now, DNA polymerases cannot add the nucleotides at a stretch over the whole strand what happens after adding certain numbers of nucleotide it is detached from the template strand and then again reattached over it. Now, based on that how many nucleotides are added before the polymerase dissociates is the processivity of the enzyme. Now, if at a stretch mean in one single attachment more nucleotides are attached that will definitely help in replication and that is highest in case of DNA polymerase.

So, while others are like DNA polymerase 3 to 200 in one single addition whereas, 1500 nucleotides in DNA polymerase 2 in case of DNA polymerase 3 the processivity is more than 5 lakhs. So, this is why these DNA polymerase 3 is basically taking part in main replication process. Apart from that there are different structural differences like DNA polymerase 3 has more than 10 subunits here you can see there are different subunits like beta, delta, gamma, tau, delta dash, beta clamp like that they have more than 10 subunits. Also 3 prime to 5 prime proofreading exonuclease activity which is

present in all the DNA polymerases, but another exonuclease activity which is required for a nick translation process that is 5 prime to 3 prime exonuclease activity is only present in DNA polymerase 1. Now, what is the function of this 5 prime to 3 prime exonuclease activity? It is required for removal of the primers remember the primers which has been attached that is an RNA segment.

So, we need to remove that and that part of. So, here you can see these are our okazaki fragments and here is our RNA primers. So, what we need we need to remove this part. So, what will happen there will be a gap creation and these gaps needs to be filled up by addition of deoxynucleotides and they should be joined. So, this filling up is done after removing the primers this filling up is done by nick translation process which is the property of 5 prime to 3 prime exonuclease activity which is present in DNA polymerase 1.

Now, DNA polymerase apart from this 5 prime to 3 prime exonuclease activity has the polymerization function as well as the proofreading that is 3 prime to 5 prime exonuclease activity. Now, in DNA polymerase 1 this 5 prime to 3 prime exonuclease segment is attached loosely which if detached the fragment which remains is known as Kleenau segment which contains this polymerization and proofreading function. So, as I told that the replication begins at a very specific site in you in prokaryotes that specific site is known as origin of replication that is a single AT rich region and this in this region a complex proteins along with enzymes bind. So, here you can see this is the whole segment is basically the or C segment where different types of sequences are consensus sequences are present. In case of eukaryotes there is multiple site of origin of replication in case of prokaryote that is only one which is known as or you see.

Now, before origin of the replication the 2 DNA strands needs to be separated which causes a replication bubble and at the end of each replication bubble there is formation of replication for how? Remember in case of prokaryote we are having circular DNA. So, if their DNA is like these double stranded DNA is like these what happen due to unwinding consider this part is announced. So, here see a bubble is formed. So, this is our replication bubble and at the end of each replication bubble there is our replication fork and this is how replication can proceed in both the direction this way as well as this way. Now, as I discussed the or you see locus in case of E.

coli are basically a 245 base pair region which contains different types of sequences tandem arrays of 13 base pair sequences which are rich in AT base pairs and over which multiple types of proteins or enzymes are attached. One such protein is DNA A protein it identifies the oracy sites attached it is attached over with over this region and then DNA B protein which has the helicase function binds over this also it helps in the binding of multiple proteins helps in relieving the positive super coils by topoisomerase I. And

finally, in this region along with all this DNA A, DNA B and topoisomerase and other proteins that is formation of a pre priming complex. So, in this pre priming complex basically the single strand which are announced thus those single strands are accessible for other enzymes to bind and beginning of the replication. So, here are these proteins DNA A, B, C, DNA helicase that is DNA B they are bound SSBP single strand binding protein stabilizes the single strand and finally, the pre priming complex is there.

Now, what happens we need primer that primer is synthesized by primase in prokaryote that primase is DNA G. So, primase is basically nothing, but a specialized RNA polymerase which synthesizes short stretch of RNA which is known as primer that is 10 to 200 nucleotide long and that is complementary to one of the template strand. This primase attaches over the pre priming complex and form a multi subunit assembly which is known as primosome and remember as I discussed the RNA primers are removed by 5 prime to 3 prime exonuclease activity and this exonuclease activity in case of E. coli it is present in DNA polymerase I. Now, DNA polymerase III is the main enzyme it takes over the whole process and causes the replication that is addition of new nucleotides over the daughter strands.

Now, this is the initiation of replication. Next we are coming to elongation. Now, in elongation the two strands replication that is leading strand and lagging strand replications are different. Now, remember both the replicate both the strands replication occurs via same replicosome complex that is one DNA polymerase enzyme which is loop through which is causing the looping of one DNA strand that is the lagging strand and another strand is straightforward attached over it. So, here you can see this is our leading strand and this is our lagging strand.

So, in this way unwinding is occurring in case of lagging strand we are having one primer only which is attached here and then the replication or the addition of new nucleotides are occurring in this direction. Once this part is replicated again unwinding of this part this region will happen and this is how the strand will be extended, but in case of lagging strand in case of lagging strand there are multiple RNA primers. So, here you can see one RNA primer here is another RNA primer. So, there are short segments of DNA I form this is our Okazaki fragments. Now, with the help of the DNA polymerase 1 these primers will be removed and it will be filled up by the deoxynucleotides and then this Okazaki fragments will be joined by DNA ligase.

As I told there is looping of the one strand here you can see this is our lagging strand it is looped over the same replicosome complex. So, basically polymerase 3 dimer helps in the same direction new nucleotide synthesis. Next termination of the replication termination occurs via TASTER complex. So, what is TASTER complex? TUR is the termination sequence basically the specific sequence when encountered by the

replication fork here is the termination of replication. So, 2 replication forks of the circular chromosome meet at one terminal region containing multiple copies of 20 base pair sequences called TUR.

Over this TUR sequence one protein binds that is known as TUS or Terminus Utilization Substance and this TUR complex causes halt of the replication fork in one direction. The other when meets the first replication fork in that case replication in the other strand is stopped. This is how the termination of replication is termination of replication happened via forming the TUR complex which is a complex process I have simplified here. Then we are coming to eukaryotes. In eukaryotes the replication the mechanism is same, but the process is more complex why because of the size of DNA or chromosome which is larger in case of eukaryote.

The number of chromosome in case of prokaryote E. coli there is one single chromosome whereas, in case of human there are 23 pairs of chromosome. Also the prokaryotes are having circular DNA whereas, the eukaryotes are having linear chromosomes or linear DNA. So, basically the replication is the process is complex because of these 3 and how these are resolved because the larger size and more numbers of chromosome. So, at a stretch replication begins in multiple sites.

So, multiple origin of replication presence which are located between 30 to 300 kilo base pair of parts. In case of human it is around 30,000 origin of replication and that replication units are known as replicon. And this replication in case of human or eukaryote is linked with the eukaryotic cell cycle why because remember in cell cycle there is a phase or synthetic phase where DNA replication occurs synthesis of new DNA occurs after that there is mitosis. So, cell cycles are arranged in such a way that during the S phase the whole replication process is completed and it must not reach the mitotic phase and that is regulated by multiple checkpoints in our cell cycle. Now, in eukaryote the origin of replication or ORC site is not well defined always, but in case of yeast a segment is found that is known as autonomously replicating sequence or ARS which resembles the origin of replication and it acts as a docking site of ORC that is origin of replication complex and if the complex contains multiple types of enzymes and proteins which helps in replication.

Then DNA polymerase alpha is the initiating polymerase. So, it has the polymerase function as well as the primase function. So, it itself synthesizes the RNA primers, but it does not have any exonuclease property. What happens after this DNA polymerase alpha has synthesized around a few nucleotides over the template strand there is polymerase switching that is detachment of the polymerase alpha followed by attachment of another polymerase that is polymerase delta which has the proofreading function. So, basically it has the high processivity as well as the proofreading function.

Here also replication occurs continues in both direction till because I told you there are multiple origin of replication. So, replication continues in both the direction until adjacent replicant meets and fuse with each other. In case of eukaryotes RNA primers and removed and DNA fragments are ligated by ligase the RNA primers are removed by RNAs H and flap endonuclease 1 and polymerase beta is the enzyme which is taking part which plays the role of gap filling as well as DNA repair mechanism in case of eukaryotes. Next we are coming to one very important topic which is relevant in case of eukaryotes as I told eukaryotes are having linear chromosomes or linear DNA. So, the ends of this linear DNA are known as telomeres.

So, it is present in the tail end of the DNA and that is that consist of 1000 repeats of hexanucleotide sequence that is AGGTT. Now, in human there are 46 chromosome thus 92 telomeres one at each end. Now, the actual termini of the mammalian telomeres is not a blunted rather it consist of a single stranded 3 prime protrusion over a G strand known as 3 prime overhang. So, this is our 3 prime overhang and that single stranded region loops back to form a DNA duplex. So, here you can see there is a looping of the end which causes a DNA duplex and that is stabilized by telomerase binding protein.

So, this is the general structure of telomere. Now, why I am discussing this telomere? So, in case of eukaryotes there is a phenomena which is known as telomeric end replication problem. So, what is the problem? Remember DNA polymerase cannot initiate the DNA synthesis DNA what it needs is a 3 prime end or 3 prime hydroxyl group free hydroxyl group which can act as a nucleophile and this is the stem of end replication problem because remember in case of. So, this is our 2 parent strand. Now, in case of leading strand there is no problem because there is one primer which is attached and then DNA is synthesized in this direction fine and it can reach the end. In case of lagging strand what will happen? The DNA will be synthesized in this direction and there is multiple such fragments.

This is our 5 prime end. Now, remember the opening of the double stranded DNA is this direction. So, at the end when the first primer will be removed when this primer will be removed these gap cannot be filled because it does not have any 3 prime overhang. So, the last primer over the lagging strand in a linear template is the terminal end and when it is removed cannot be replaced by a double strand DNA. So, what will happen? There will be a loss of terminal sequence and that loss of terminal sequence will happen at every round. So, in every round there will be progressive shortening of the chromosomal ends and this is dangerous or deleterious.

So, how this is solved by the enzyme telomerase. Now, telomerase is responsible for the end replication or terminal replication and thus it helps in maintaining the length of the

chromosome or the length of the telomere. Now telomerase is one unique polymerase polymerase which has its own RNA template. So, basically inside the telomerase there is an enzyme function that is known as telomerase reverse transcriptase enzyme as well as one RNA segment telomerase RNA or TAR. And this template has the complementary sequence or serve as the template for elongation of the G string or G rich strand. Thus the enzyme has you can see an RNA segment which acts as a template which elongates the telomeres in such a way that new nucleotides can be attached.

Now, telomeres function are mostly seen over gametes and stem cells very high in tumor cells, but in case of somatic cell proliferation senescence follows approximately 50 to 70 cell division. So, the shortening of cells somatic cells begins. Now how telomerase helps? Here you can see in the lagging strand this part is empty because there is no after the RNA primer there is no free 3 prime hydroxyl group. So, what telomerase does it extends this 3 prime overhang in such a way that new RNA primer can be attached and strands can be synthesized.

So, this is how telomerase solves the end replication problem. Now, we are coming to inhibitors of DNA replication. DNA replication can be inhibited at different phases in case of prokaryotes DNA gyrus which is a type of topomerase can be inhibited by quinolone group of antibiotic one such example is ciprofloxacin. Then the 2 strands of DNA can be intercalated to inhibit the replication by the anticancer group of drugs like actinomycin, adriamycin, doxorubicin. Topoisomerase in case of eukaryotes can be inhibited by etoposides which is a anticancer drug. Then DNA polymerase in case of eukaryotes can be inhibited by different nucleoside analogs like zidovudine, didanosine which are anti HIV drugs also 6 mercoptopurine which is a nucleotide analog.

Next we are moving on to different types of DNA damages and their sources. Now, DNA bases can be modified via different processes like oxidation, alkylation, hydrolysis. Different adducts can be formed like benzyl pyrondiol epoxide causing the DG adduct, guanosine adducts. Mismatching of bases can be there due to errors in general replication. And what are the sources for these types of DNA damages? Ultraviolet rays, UV rays, ionization radiance like gamma rays they are responsible UVB.

This causes direct damage it causes cross linking of adjacent cytosine and thymine forming pyrimidine dimers. Then UVA synthesizes free radical mediate and that free radical mediates indirect damage over the DNA. Then temperature causes depurination and single strand breaks also chemicals like aromatic hydrocarbon can form DNA adduct. So, these are the types of DNA damage which can be handled by cells own repair mechanisms. Now, different types of repair mechanisms are there one such is base excision repair.

Now, in case of base excision repair the damage nucleotide base is basically removed by excision of a short piece of the poly nucleotide and finally, the proper synthesis of proper complementary base is re synthesized. And base excision repair mainly acts in minor damages like alkylation or deamination. The two important enzyme is DNA glycosylase and then epi endonuclease also associated with exonuclease DNA polymerase 1 and DNA ligase. DNA glycosylase basically cleave the N-glycosine ting bond instead of the phosphodiester bond this is how the base is removed. And finally, an apurinic or apiremidinic site is formed that is known as epi site that epi site is now removed by chain breakage or chain cleavage that is cleaving the phosphodiester bond.

And that epi site is removed by the enzyme epi endonuclease then the process is very smooth the DNA polymerase 1 fills up the gap and ligase joins. So, that is that is the base excision repair. Now, in case of a large distortion where a large segment is having problem there is nucleotide excision repair. So, nucleotide excision repair with the help of the enzyme exonuclease it removes a long segment of DNA by breaking the phosphodiester bond at the both sides here you can see at the both sides there is breakage of phosphodiester bond and the strand is removed and that gap then is filled and repaired and ligated. So, in E coli the UVR system of ABC exonuclease is very popular in this regard.

Then coming to the mismatch repair system which is important in case of wrong or incorrect DNA replication. Now, mismatch base pair basically causes distortion of the geometry in the double helix and that is identified by the mismatch repair system. Now, how in a double stranded DNA which is newly synthesized how the system can identify which strand is daughter strand and which strand is the parent strand. So, that is done by the dam methylase which is present mostly in U carriers in E coli the dam methylase basically methylates the adenosine residues of the parental strand.

So, methylated residues are present over the parental strand. So, the non methylated DNA strand is the daughter strand where the correction is required, but you can in case of U carriers this discrimination process is not well explained. Now, there are multiple proteins or enzymes in case of E coli mutH mutL mutS those are responsible for mismatch repair system they are contemporary enzymes are present in U carriers. Then the other two type that is double strand DNA damage and direct repair system double strand DNA damage is mostly repaired by homologous recombination. So, basically in case of double strand the strand breakage is there.

So, there is a gap then strand adduct formation can be there. So, these type of problem can be solved by another pair of homologous chromosomes. Now, this homologous recombination we will discuss in our later lectures. Another mechanism that is translation error prone DNA synthesis or SOS response also helps in double strand DNA

damage. Here the repair mechanism is such that the gap is filled without any template proper template. So, basically there is just gap filling and that is why it is quite error prone, but the broken DNA strand is much more dangerous than the error prone DNA strand.

So, there is translation error prone DNA synthesis. Then direct repair system it acts directly on the damage nucleotides converts the damage nucleotides back to its original structure and that is done by photo reactivation is a light dependent direct system. Mostly responsible for repairing pyrimidine dimer and the enzyme which is responsible is a DNA photolyase when stimulated this enzyme stimulated by light it binds to the pyrimidine dimer and converts them back to the original monomeric nucleotides. Now, there are different diseases which are associated with this DNA repair mechanism or defective DNA repair mechanism. One such is xeroderma pigmentosa which is associated with defective nucleotide excision repair mechanism. Now, because of this UV radiation induced damage is very high in those patients and they are having skin cancers different malignancies of skin.

Ataxia telangiaceae is one such disease which has defective ATM gene. Now, ATM gene is basically responsible for identifying the DNA damages and that signal is delivered to the repair system because of there is ATM gene defect the DNA damage is not transmitted to the repair system. So, the repair damage remains it affects the nervous system as well as the immune system. Fanconi anemia where the defect is in DNA cross link repair there is DNA cross link which is not properly repaired and increased occurrence of cancer in the Fanconi's anemia. Bloom syndrome associated with abnormal DNA helicase formation which is responsible for lympho reticular malignancies.

Cokines syndrome again a nucleotide excision repair mechanism defect. So, basically the UV induced DNA damage after the UV radiation causes the DNA damage the transcription process is also damaged. So, this is how Cokines syndrome happens and finally, hereditary polyposis colonic cancer HNPCC or Lynch syndrome it is associated with defective mismatch repair. So, this is all about the class. Coming to the summary the replication starts at a very specific site which is or you see in case of prokaryotes in eukaryotes it is not well explained. What is required is a DNA primer because DNA polymerase cannot synthesize DNA segments de novo.

DNA synthesis is a semi continuous phenomena the leading state synthesize continuously whereas, the lagging strands are formed in fragments several fragments which is known as okazaki fragments. Different enzymes are required in replication DNA polymerase helicase topoisomerase primase proteins like single strand binding protein enzymes DNA ligase. Then the both strands are synthesized from 5 prime to 3

prime direction what happens the primers are attached by the primase then those primers are removed and that gap is filled up by DNA polymerase. In case of eukaryotes that is flap endonuclease and RNase H the pieces are ligated by DNA polymerase. Proofreading is done by DNA polymerase itself replication can be inhibited in multiple sites and different drugs are associated with that then we have discussed different types of DNA damage and associated cellular repair mechanisms.

So, these are the references from which I have taken the contents. Thank you all and see you in the next class. .