Cell Biology: Cellular Organization, Division and Processes

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Lecture 10

DNA replication-Part I

Hello everyone! I am Shikha Laloraya, Professor of Biochemistry at IISc. Today's lecture is on DNA replication. In the first part of this lecture, we will discuss about the chemical basis for DNA replication and in the second part we will discuss the mechanism of DNA replication.

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This figure shows specific base pairing scheme in the double helix as was described by Watson and Crick. Note that the two strands are running anti parallel that is 5 prime to 3 prime on the left strand and 5 prime to 3 prime this way on the right side. So, they are running in opposite directions. The bases in the two anti-parallel strands are complementary to each other following the model of specific base pairing suggested by Watson and Crick.

That is A only pairs with T, and G only pair with C. So, this model actually suggested a mode for DNA replication or for the copying of the genetic material as mentioned in the famous statement by Watson and Crick in their paper, proposing the model of DNA structure. They had suggested that the specific base pairing proposed by them immediately suggested the possible copying mechanism for the genetic material.

This intrigued many scientists at that time and many possible models for DNA replication were debated.

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One possible model was the semiconservative model for DNA replication, which is illustrated in this slide. The two parallel strand separate, each serves as a template for the synthesis of a new daughter strand by complementary base pairing. In contrast with the conservative model which would predict that the entire new DNA duplex would be formed with both newly synthesized strands. Or, also in contrast with the dispersive model, where new DNA synthesis would start on both the strands at

short distances, forming regions of new DNA, which are dispersed among the old DNA, on both the strands.

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In order to test the models for DNA replication, 2 scientists, Meselson and Stahl did an elegant experiment using the heavy N^{15} isotope of Nitrogen to differentially label the parental strand of DNA to distinguish it from the daughter strands having N^{14} incorporated after shift to the N^{14} based medium. This experiment has also been referred to by some as the most beautiful experiment in Biology. And indeed, it is a very elegant, classic experiment that all of you should read about. This experiment made very clear predictions about the results that would support semi-conservative mode of replication versus conservative or dispersive mode of replication. So in semi-conservative mode of replication, the strands separate and each parental strand, templates the synthesis of one full new DNA strand.

So, there is still one parental strand in each daughter molecule. In case of conservative, both the parental strands remain together and form an entirely new DNA molecule that has both new strands. Whereas in case of dispersive each parental strand is broken at intervals and used to prime synthesis of short new strands that would be joined later on to form completely new strands. All the DNA strands produced would have both old as well as new DNA, which is non-conservative and a fully light would be formed after many many generations on N^{14} in the case of dispersive mode of replication. So here in this figure, bacterial cells were grown originally in N^{15} based medium, where ammonium chloride was the sole nitrogen source. Now if it was conservative, the heavy light (HL) intermediate would never have been seen. After one cycle you would see heavy heavy (HH) and light light (LL).

So basically, let us review the experiment, the cells were shifted to N^{14} medium and at each generation on doubling the DNA was isolated. And it was mixed with a caesium chloride solution, 6 molar with the density of 1.7 gram per ml, and this was then centrifuged in an ultracentrifuge at 140,000 X g for 48 hours to form a density gradient. In this the higher density would be at the bottom of the tube and the lower density would be near the top of the tube.

So looking at the results the DNA would sediment as per its density and what was observed was that after one generation, the DNA migrated at a region where there is intermediate density consistent with heavy light DNA and after two generations, from the same, one observed heavy light as well as light light DNA. And after an additional generation again heavy light and light light was found; this time there was more of the light light DNA being formed. And of course, heavy heavy was never recovered again. So, these results were consistent with the semiconservative mode of DNA replication. Because as we already discussed, if it was dispersive, then, although in the first cycle, it may look similar to semi-conservative, in the second cycle, the light light would not appear immediately, but we would observe and intermediate band between the heavy light and the light light, and likewise in the third, and so on. And fully light light in that case would be formed only after many generations. So these results certainly ruled out, both the dispersive as well as the conservative model. As I have already explained, in case of the conservative model, after one generation you would get heavy heavy and light light and you would not get intermediate one, and

that was of course, not observed. So these results were consistent with a semi-conservative mode of DNA replication.

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So let us discuss the chemical basis and the requirements for DNA replication. Deoxyribose nucleotide triphosphates are utilised as substrates for addition of new nucleotides to the chain. Also something known as a primer template junction is required for DNA replication; certainly you need a primer to prime the synthesis or addition of the next base. And complementary base pairing with the template helps in inserting the correct base in the new DNA strand.

DNA synthesis occurs by extending the 3 Prime end of the primer. And the incoming nucleotide gets linked via phosphodiester bond to the C5 Prime of the deoxyribose part of the incoming nucleotide. The DNA polymerase enzyme is the one that catalyzes the formation of the phosphodiester bond joining the 3 Prime hydroxyl end of the primer to the alpha phosphate at the five prime carbon of the NTP and in this the process a pyrophosphate is released.

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All known DNA polymerases use a deoxyribonucleoside 5 prime triphosphate as a substrate and add the monophosphate building block to the three prime hydroxyl group of a growing DNA chain or the end of the primer strand. Shown here is the structure of a nucleotide that forms a building block of DNA, dAMP. The basic building block or repeating unit is a nucleotide composed of a base, a sugar and a phosphate.

The nucleotide AMP with one phosphate is shown here. It has a phosphate, a 2 prime deoxyribose sugar and the base adenine. The phosphate is attached at C5 prime. The base is attached via an N-O glycosidic bond at the C1 Prime of the sugar. In a DNA chain, another nucleotide can be added by the formation of a phosphodiester bond at the C3 Prime of the sugar of the first connected to the C5 Prime of the incoming nucleotide.

Thus, the polynucleotides have got a free 5 prime phosphate and 3 prime hydroxyl end. For the growth of the chain the hydroxyl at the 3 Prime position, on the terminal nucleotide, attacks the linkage between the alpha and beta phosphate or phosphoryl groups of the incoming precursor dNTP. One nucleotide gets added and the pyrophosphate ion composed of the beta and the gamma phosphates of the substrate is released. This reaction is catalyzed by the enzyme DNA polymerase.

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In the cell both the DNA strands are replicated simultaneously. So, the two strands of the DNA double helix, they separate to form the two template strands on which the new DNA will be built. And this Y shaped junction, which is there between the two unreplicated DNA and the newly

separated template strands is referred to as a replication fork. A Multi-enzyme complex that contains the DNA polymerase synthesizes DNA at the replication fork.

Now, recall that these two DNA strands are anti parallel, but at the same time DNA synthesis by DNA polymerase occurs only in the 5 prime to 3 prime direction.

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Hence, as a result of this, the replication fork is asymmetrical, due to mainly two reasons. One is the anti parallel strands and also that the polymerase synthesizes only in the 5 Prime to 3 Prime direction. So, the DNA polymerase, at this replication fork synthesizes only in one direction, from 5 prime to 3 prime, going this way. And the DNA polymerase initiates DNA synthesis by adding new nucleotides to the 3 Prime end of a primer. And the primer used is actually an RNA primer, the reason being that the DNA polymerase itself, it cannot put together or join two nucleotides by itself. It needs a primer; it needs a primer hydroxyl in a duplex. Since the two DNA strands are antiparallel the DNA, on one strand, which is referred to as the lagging strength is shown here, has to be synthesized in short segments.

And these segments are known as Okazaki fragments, which range from 100 to 2000 nucleotides based on the organism that you are working with. And these fragments later, after the end of the replication, are joined by DNA ligase. So, this was actually found by an interesting experiment done by the scientist Okazaki after whom these fragments are named. Tritiated thymidine was added to dividing bacteria for a few seconds to label only the most recently synthesized DNA fragments. And DNA fragments that were for about 1000 to 2000 nucleotides long were formed transiently. And these were made in the 5 prime to 3 prime direction and they were joined after synthesis and later on incorporated into longer DNA chain. So, these short fragments were referred to as Okazaki fragments that we will be referring to a lot. The direction of polymerase movement on the leading strand is the same as the overall direction of DNA replication. The direction of polymerase movement on the lagging strand is opposite to the overall direction of DNA replication.

The Okazaki fragments are made in a sequential order; those which are nearest to the fork would be the ones which have been synthesized most recently and older ones are further away from the fork.

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An enzyme, DNA primase, synthesizes the short RNA primers, which could be 10 nucleotides long using ribo NTPs as the substrates. This process is shown here where the Primase is putting together the first 2 nucleotides to start the RNA primer. DNA polymerase as I mentioned cannot initiate polymerization de novo. It can only add to the 3 prime end of an existing nucleotide that is part of a nucleotide acid chain base paired to DNA. However, this enzyme DNA Primase can start a new polynucleotide chain by joining 2 nucleoside triphosphates together. And this is shown as the first step here. Now of course, remember that in RNA instead of thymine, you have Uracil. So that is what has been incorporated here. The primer is being denoted in yellow and this theme will be followed in many of the future slides and animation. So please bear it in mind that the primer would be labelled

yellow. Ok, so after starting this chain, it can synthesize a short chain also in the 5 Prime to 3 Prime direction and then it stops and then it makes the 3 prime end available to the DNA polymerase for further extension which is shown in the second panel. So the DNA polymerases can add to the new nucleotides to the 3 prime hydroxyl and then start DNA synthesis.

Now, primase enzyme adds nucleotides to a single stranded DNA template and its activity is enhanced when it is bound to another enzyme, the DNA helicase enzyme, at the replication fork. So these properties ensure that it is only active at the replication fork, and not anywhere else where there is single stranded DNA. The process of replication continues in this way; now the DNA polymerase can take over and start synthesizing the DNA chain.

In the end these RNA Primers have to be removed and there would be a gap created, which has to be filled by a DNA polymerase. And then, the neighbouring segments of DNA have to be ligated at that site.

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For DNA synthesis to occur, the two DNA strands that are held together by hydrogen bonding between the bases, they need to be separated. Local opening of the DNA helix happens. And this is first followed by synthesis of the RNA primer. DNA synthesis starts at this primer by the DNA polymerase, initiating the leading strand DNA synthesis. Next, additional primers are sequentially laid down for the lagging strand DNA synthesis, as is shown here.

And this results in the formation of Okazaki fragments. This structure in which both the strands have been separated from each other and both of them are now serving as templates for DNA synthesis is referred to as a replication bubble.

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So, shown here is generic diagram showing prokaryotic DNA replication. In prokaryotes, the chromosome is circular and replication initiates at an origin or an initiator sequence. The replication origin binds a sequence-specific DNA-binding protein referred to as the initiator protein. The origins also have an easily unwound region, which is AT rich in nature. So an AT rich segment is easily unwound relative to other random sequences, because as you know AT base pairs are only two hydrogen bonds as opposed to GC base pairs which have 3 hydrogen bonds.

Replication initiates with the help of initiator protein and a primosome. Primosome consists of a DNA helicase and a Primase. The DNA helicase unwinds the DNA and the primase of course as explained lays down the primer. The DNA polymerase synthesizes leading as well as lagging strand DNA and replication proceeds bi-directionally. So there are two forks moving away from each other.

And they keep on progressing and new DNA keeps on getting synthesized until at the other end they collide with each other and then here the primosome is then disassembled and DNA replication is completed resulting in the formation of two copies of the chromosome, that is 2 daughter chromosomes.

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So, some of the key requirements for DNA replication in prokaryotes are mentioned in this slide. One that is already mentioned is the initiator or the DnaA protein. It binds to the origin DNA and it melts the AT rich region. The helicase or the DnaB protein unwinds DNA at the replication fork to generate single strand DNA template strands. Primase or the DnaG protein synthesizes the RNA primer for DNA synthesis by DNA polymerase.

Also, of course, most importantly, you need the DNA Poll II holoenzyme that synthesizes DNA. It consists of a clamp loader, which is a complex that loads another protein complex known as a sliding clamp on to a primer template duplex, the tau protein which connects the clamp loader to the DNA polymerase 3 core. And this also later on contacts and stimulates the helicase. The DNA Polymerase 3 core enzyme which is, of course required for DNA synthesis and in the holoenzyme, there are three copies of this and the sliding clamp which clamps the DNA polymerase 3 core onto the DNA and it enhances its processivity to ensure that it does not fall of and diffuse away. So this helps in maintaining efficiency and speed of progression of DNA synthesis. You also need single-strand DNA binding proteins-they are quite important as they bind to single stranded DNA near the replication fork and prevent the annealing of the single strand DNA template strands either with itself or with the other complementary strand.

One also needs enzymes, DNA Pol 1 and in some systems there is a different enzyme that does this job of primer removal and a ligase which is required for ligation of the Okazaki fragments in the end in the lagging strand. One also needs a class of enzymes known as topoisomerases; in *E. coli* they are known as Topo 1 and Gyrase. And the main function of these enzymes is that they prevent DNA tangling during replication and they remove super coils that are formed because of the progression of the replication fork.

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Although the preceding description I gave are quite simple, DNA replication is a highly complex process, which is brought about by coordinated action of the replisome. The replisome is a complex molecular machine that consists of various enzymes and other proteins, which are important for replication and these are assembled at the replication fork. You can see some of the proteins here doing the process of replication.

Replication in *E. coli* happens quite fast. The DNA polymerase at the fork can add nucleotides at the rate of up to 1000 per second. It is a very complex and dynamic process. This replisome machine has got many moving parts, as you can see. There are additional regulatory modules also involved in checkpoint control etcetera, which are not shown here. The helicase enzyme is shown in blue over here, which is unwinding the DNA duplex. You can see other continuous synthesis on the leading

strand below; I am playing it again to explain this complicated animation, which was provided to me by Drew Berry and Etsuko Uno, and so here you can see the helicase which was this blue enzyme over there, Yeah. It is unwinding the DNA into two single strands and this side on the right side is the leading strand and on the top is the lagging strand which is forming these loops and moving around a lot.

So you can see actually the primase, just now it arrived and it formed this yellow primer and very quickly the action happened where the clamp loader attached the clamp to it and the DNA polymerase, the lagging strand DNA polymerase became attached to it. So, just watch this a little more to make it clearer. I think we have covered that entire animation. So here you can see this loop which increases in size and that is seen on the lagging strand only. And this model of replication is referred to as a trombone model after a musical instrument where you see such an increasing size of the one of the parts of the instrument. So here is the helicase and there is the yellow primer and the clamp loader. This is indeed a fascinating process and in fact lot of problems can also happen when there is a complex process going on that may need to be corrected or monitored by the checkpoints.

There again, that is the lagging strand letting go of the polymerase. So, I think it would be clear.

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This is very complex process of course, the key enzyme here is the DNA polymerase enzyme. And I just want to review some of the main properties of the DNA polymerase, which is important for this synthesis reaction in cells. The DNA polymerases they synthesize DNA by adding a nucleotide to the 3 prime hydroxyl of a primer. The DNA polymerase enzyme, it has got a single active site that is capable of catalysing DNA synthesis. DNA polymerase only incorporates correctly paired basis as specified by the complementary sequence of the template strand. Incorrectly paired bases are not incorporated by this enzyme. The structure of DNA polymerase enzyme resembles a partially closed hand and the DNA fits in a large cleft in that molecule. Also, DNA polymerases are processive enzymes and the *E. coli* DNA polymerase, DNA Pol3, which is the replicative polymerase can add up to a thousand nucleotides per second.

And of course, as I already explained this processivity is enhanced by being attached to the sliding clamp, which keeps it in place attached to the DNA strand. Even if it were to get disturbed or fall off, the sliding clamp, puts it right back to do its job and prevents it from floating away, which it would do if it was not tethered to the clamp. DNA polymerases also have a 3 prime to 5 prime proofreading exonuclease activity that removes unpaired on mispaired bases.

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So, listed here a couple of references pertaining to the Meselson and Stahl experiment that had proved the semiconservative nature of DNA replication, it is worth looking into. Stay tuned for the next part of this lecture, which will be on the mechanism of DNA replication. Thank you.