**Introduction to Cell Biology**
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**Central Dogma: Replication of DNA Part 2**

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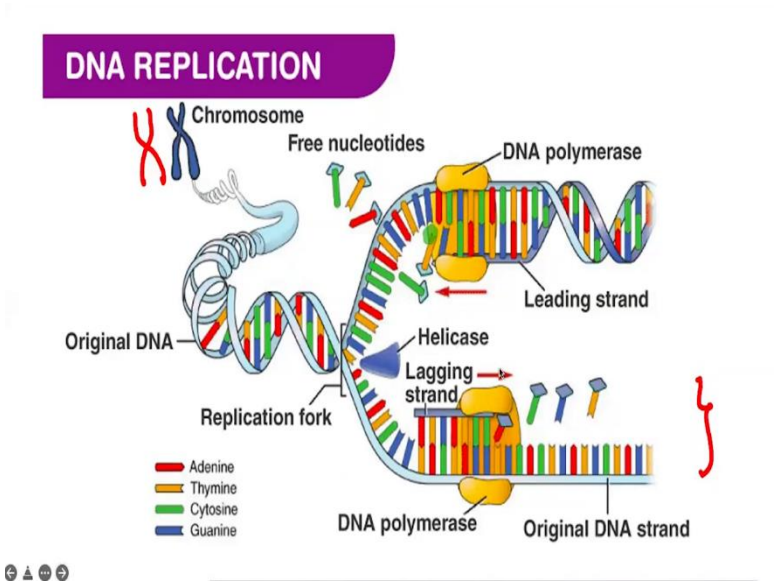


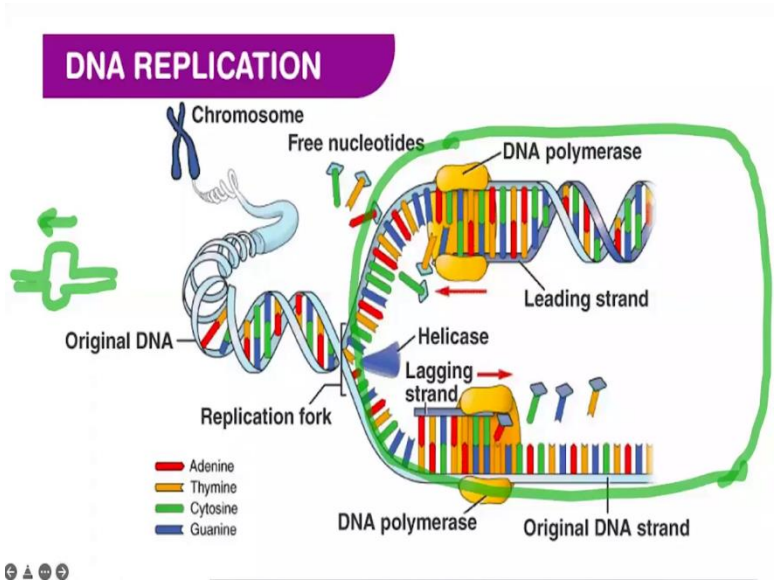


Professor: I will take questions now.

Student: Sir, in like in the lagging strand, is it always necessary that only one polymerase will be present or there can be five, six polymerases can present in the lagging strand.

Professor: So, in the lagging strand, you can have five, six polymerases, there is no reason why you should not have. And if you are going to ask me what dictates that it is one of the

open questions. I think the word open questions is going to be a favorite statement of mine. But yes, in theory, you can have multiple polymerases. And, in theory, and in practice, also a single DNA polymerase.

Once it sits, you will notice over here in this picture, DNA polymerase is sitting over here like a clamp. And I will tell you a little bit more about that. But at least when they are when they when the leading strand is being copied a single DNA polymerase, once it clamps onto DNA, it can keep on processing and keep on adding continuously, as long as helicase keeps on opening up the DNA and gives DNA polymerase access.

Because in the helical state of DNA, DNA polymerase does not have that necessarily have the ability to enter and start polymerization. Now, this terminology further terminology or here, this is called as a replication fork. And you will see why it is called a fork because this is where the two strands are forced apart and form a fork like structure. And you can see the helicase sitting over here. Questions?

Student: Sir, how do we exactly know these details? Is it like through microscopes, or chemistry or is it like computer simulation that we know this in such a detailed way?

Professor: So the answer is, through all of these, but the real answer is, by using the human mind, by making mutations by doing experiments, which are at an atomic level where you cannot see making sure that the results of those experiments were using color changes, or using something that you could visualize using our own eyes.

So what I am trying to say is that you cannot see things at atomic level, but you can link it up with things we can see. And the output if it is easy to visualize, either through looking at the expression of a gene, or the gene converted to the, or looking at, let us say EM pictures, which were not always very clear. It is a combination of things. But human imagination paid a very large role. And mutagenesis played a very large role.

So, for example, if you have a organism with a single DNA polymerase, and you knock down all the 10,000 genes in the DNA in that animal, and one of those genes, when you knock it down, or 5 of those genes, when you knock it down, stops DNA replication, you know that those 5 genes are something related to replication, when you look at what proteins are made by those 5 genes, and by this in a stepwise manner, you can identify DNA polymerase.

And then of course, the tricky part of figuring out how it does things is a completely different part. An example is Okazaki fragments. So people who are doing working on DNA replication, were puzzled that you are getting these little bits and pieces of DNA. And the person who figured it out was called Okazaki, Japanese scientist, and that was a mystery.

When it was solved, we knew that there was a lagging strand, and things were made discontinuously in a lagging strand. So biology moves forward stepwise one step at a time. And after 30 years, which is pretty much what we are looking at, we have a more complete picture. And we are still learning things as we go along. Questions.

Student: DNA is basically wound on histone octomers and then it is made into chromosomes, so how does it completely unwind like this?

Professor: So in eukaryotes, it is true in prokaryotes, they are DNA binding proteins, but they are not architecturally things equal to nucleosomes. But good you are using your imagination. Part of opening up strands is to unwind it from DNA. So you will literally have to imagine that this piece of DNA which is on a chromosome, and remember, chromosomes are nothing but compact pieces of DNA wrapped around many orders of magnitude of nucleosomes.

So you have to bring them off the nucleosome. Temporarily, you have to unwind it, you have to polymerize it. And each of these strands will have its own set of nucleosomes which have to be ready for it to wind around so that from one chromosome after the end of the entire cycle of replication for that chromosome, you will end up with basically two chromosomes.

So now your question is how exactly is this happening? It is a manufacturing process. And we are learning steps of the manufacturing process. And it is simple manufacturing process in the sense that a DNA architecture is just ATGC on a phosphodiester backbone, and you are just making a copy of it. So it sounds simple, but it is not so simple to do all of this. I' will show you a few animations in a few minutes. Maybe that will help you.

Student: So what happens when there are errors in replication?

Professor: So the polymerase itself, most polymerases, have the ability to detect errors and repair them, repair it themselves. So let us take this one as an example. This is a DNA polymerase. And I will draw and draw here. So it is, it is putting in strands. It is copying,

doing its copying function as well as it can, let us say it makes a mistake somewhere over here.

It will go ahead. Once it puts in a nucleotide, it has a checking function. It is like seeing have I done the good or done the right thing. And it by the time it is checked, it is already moved ahead of that base, and it is putting the next base. But the moment it notices, that as an error, it will come back to slide back and it will remove the nucleotide and put the correct nucleotide. So error checking is part of the process of DNA polymerases. In general, there will be DNA polymerases, which cannot do error checking.

Student: Now what are the nucleosomes, you used the word in the previous explanation?

Professor: I actually have shown it in the last class, can you go back to that lecture because I do not have pictures right now to explain nucleosomes right now. And I had rather not go back and open that slide again. So back and look. And if you still are not clear, I will explain it as in the question answer sessions.

Student: Sir, why do not the cut fragments of DNA gets joined again, before DNA polymerase approaches?

Professor: Which cut fragments.

Student: Helicase unwinds; they can join together after helicase unwinds them?

Professor: Yes. So this is why I want you to use your imagination. You know, this process has to be done. And now you have all these questions? How is it happening? The answers to your questions are in the questions themselves. There are approximately 30 to 40 proteins whose job it is to make sure that this little this so I am going to draw a sort of.

So this whole thing over here, if you look at it under an EM microscope, which has a high resolution EM microscope, it looks like a bubble like this, and this bubble moves forward in the direction replication is taking place. And to maintain this bubble, you have a large number of proteins, and all of them have not been discovered yet.

So as I said, the answer is in your question you need it is like using your two arms to keep a wire mesh open, you have to apply force. Similarly, there are proteins whose job it is to make sure that the bubble remains open so that DNA polymerase can do its job. So I cannot tell you

the identities of all these proteins, it will complicate things, but in an animation which is coming up, you will see some of these proteins.

Student: So, why do we use the word 5 dash or 5 prime and 3 prime and 3 prime? Like why do we use the dash? Why not just 5 and 3?

Professor: That was the way it was? It was it is I am giving you an analogy, why do we call a dog a dog? Or when why do we not call it a cat? Because the first human being who used the English as a language, and pointed at a dog said that was a dog. So nomenclature is giving credit to the people who first defined it. Many of them were chemists. And in chemistry, the prime nomenclature is news used for the sugar quite routinely. So we are just following nomenclature.

Student: So what activates the unwinding of the DNA helix by helicase?

Professor: Very difficult question. It is an open question. It is you are basically asking me what triggers DNA replication. And once DNA replication is triggered, helicases told no who tells helicase it is not very clear. Go to a certain spot in DNA. It is called the origin of replication. It has a specific name and start opening up and create a bubble.

Once the bubble is created, like a swarm of minions, I am sure most of you have seen minions, the movie. Minions with different functions swarm on the bubble. And polymerase, which is the which is one of the major actors starts making both the lag make making copies of the lagging and leading straps.

So there are not any clear answers. We know the way biology works. Things are triggered. Machines do their work, events happen, but it is being very difficult to trace back to what started everything. Obviously, it is a synchronized process. And whenever a bacteria, for example decides to make two bacteria, and it will decide to make two bacteria when nutritional conditions are reasonable.

This process is happening in the DNA of the bacteria. And it has to happen very quickly, maybe in a few minutes. And remember, bacterial DNA is not small it is 10 raise to 4, 10 raise to 5 base pairs. So, these machines have to be not only efficient and accurate, they have to be very, very fast. And the numbers when you hear about them will astound you, they

work at the same rate your fan works when it is at full speed. And you will see this in a few slides.

Student: Sir, how the leading and lagging strand is decided?

Professor: It is based on I guess, where the replication fork opens. And at that point, any, so you have to remember that the leading strand is a strand where the polymerase is heading in the 3 prime direction, these are nomenclatures. And the lagging strand is the strand, which is the opposite strand to the opposite strand to the leading strand where the polymerase cannot continuously make.

Because the orientation of the DNA it is copying is not the correct orientation. You have to sit down draw this and think about it, which is why I am emphasizing so much on making simple models of DNA, which I showed you in the first movie.

Student: So just a small question, that green colored enzyme was ligase?

Professor: I guess so I will have to look at it a little bit more carefully. But yes, no Okazaki fragments have to be put together with the ligase.

Student: Can we say that are leading strand act as a template for future lagging strand and vice versa?

Professor: You see, finally DNA is double stranded. And it has to be replicated in both directions, you have to take two strands of DNA and end up with four strands. Now, the definition of leading strand and lagging strand is arbitrary. These are two anti-parallel strands. The leading strand is a strand where the polymerase can clamp and continuously work without any breaks.

Whereas in the lagging strand, and it is nomenclature, that polymerase unfortunately does not have access to the DNA. Because it is the direction it is moving. It can only do things in short bursts. So leading and lagging are nomenclatures. I am not saying they are not important, but they are arbitrary. You could have called leading strand alpha; you could have called the lagging strand beta. So if you had used those terminologies, we will be saying alpha strand and beta strand.

Student: The enzymes come from the opposite direction, then the leading strand becomes a lagging strand and the lagging strand becomes a leading strand?

Professor: That is what I am saying it is nomenclature, it is arbitrary. What is important is you have to remember that DNA is an anti-parallel helix, the two strands are in opposite directions. And bases are complementary. So it is redundant information. If you take once, if you if you take a double stranded DNA helix, rip apart, the two strands throw off one strand into the garbage bin, the second strand has all the information you need.

And it is very easy to copy because you can now copy it in a single direction. So one way of replication could have been and this is, this does not happen. But I just want you to think about it is you open up the DNA, and you completely degrade one strand because it is redundant information anyway. And then you copy the second strand, that is it you have you end up with a double helix.

The problem is, when you are making two bacteria from one bacteria, you want exactly the same double stranded helix repeated in the new bacteria, which is why you take two strands and make a total of four strands. And the pairs of strands come together and they separate into two bacteria.

Student: You said that if we keep only one strand, and then as you explained something after that I did not get.

Professor: What I am trying to tell you is DNA polymerase does not work to make four strands as a machine. It is copying a single strand. The reason you are making four strands is basically one organism is going to become two organisms, one bacteria is going to become two bacteria and bacterial division under good conditions is let us say about 20 minutes.

So the bacteria notes that in 20 minutes it has to make a second copy of itself. And part of the process is that the DNA which is a single strand of DNA, circular DNA in the case of bacteria has to replicate itself in around 10 to 15 minutes. So you have to start with two strand and make four strands which is why this entire process is happening.

Very let me use the term imperfect process, which is not a good word to use. I guess for young students in biology where one strand is being made continuously and the second strand you are doing it bits and pieces and then you are pasting them together.

Student: The 3 base pair number that you mentioned that was also arbitrary?

Professor: It was arbitrary. Depends on how big the bubble is. The proteins which are opening up the bubble, if they can open up a 50 base pair bubble, then the lagging strand can do 50 base pairs at a time.

Student: Is it possible to see live replication going on through electron microscope?

Professor: No, not at all, which is why we are showing you these animations.

Student: Sir, the lagging strand are made in fragments. And the leading strand is made continuously. Is it because the specific nature of the enzyme DNA polymerase is specific for its reagent?

Professor: Yes, that is what I am trying been trying to tell. DNA polymerase can only extend in one direction. There is no DNA polymerase in existence that we know about which can extend in the 5 prime direction, it can only add nucleotides to the three prime direction.

Student: Sir, since DNA has existed, like in life, has it always replicated this way or has it evolved? And has it evolved to what it is today?

Professor: Impossible question to answer, because we only know life as it is today, the best we can do is we can argue that the Archaeal Kingdom is have maintaining the same kind of machinery for replication as it is maintaining today, a million years ago, and that may not be true.

And if we look at Archaea, and we see that Archaea is doing DNA replication exactly the same way, as let us say mammalian cell is doing, then we say that the replication process has not changed in a million years. But that is an extrapolation. We cannot go back in time and see how things were 2 billion years ago.

Student: Sir, maybe making 2 enzymes for DNA replication took time. So they evolved for making only 1 enzyme.

Professor: So your guess is as good as mine, nobody really knows why.

Student: Sir, every chromosome has only one origin of replication, or can it have multiple?

Professor: It can have multiple origins of replication, in fact, for very large chromosomes, you if you need to do the replication of the entire stretch of DNA, so it is almost like think of putting tar on roads on a city like Pune or your city, Delhi, Bangalore, there are it's a huge number of roads, you can say, we give one contractor money contractor starts from the Main Street, which is many times called Mahatma Gandhi road, and then it moves throughout the whole city.

But no matter how fast the contractor goes, it takes a long time. So if you are if you have to do the project before the rains come in this in this particular case, let us say in bacteria in 20 minutes, it makes sense to have multiple origins of replication. But that depends on the speed of making DNA. If you can make an entire copy of a DNA genome in 10 minutes.

Then you do not need multiple replicate replication fork because the government has given you 10 minutes to make a copy of DNA. But in the case of eukaryotic gene, if you have to do the work in 30 minutes, and it is impossible at the speed at which polymerase works, it is the rate of synthesis of doing it in, let us say, 20 minutes, then you open up multiple replication forks.

So the answer is many times in bacteria, small genomes, there can be a single replication fork but in larger genomes, we do not have a choice, you need multiple replication forks and multiple origins of replication. So remember, each chromosome.

Student: At every origin of replication of DNA just gets disconnected from the remaining strand?

Professor: Well, it opens up in a bubble temporarily. It is not opened up completely, it only opens up in the replication bubble.

Student: How to determine and how to I mean, when to stop, so that all those pieces can stick back together.

Professor: Which all those pieces?

Student: You said, that there would be multiple origins of replication. So, there would be lengths of pieces of DNA then there will be again.

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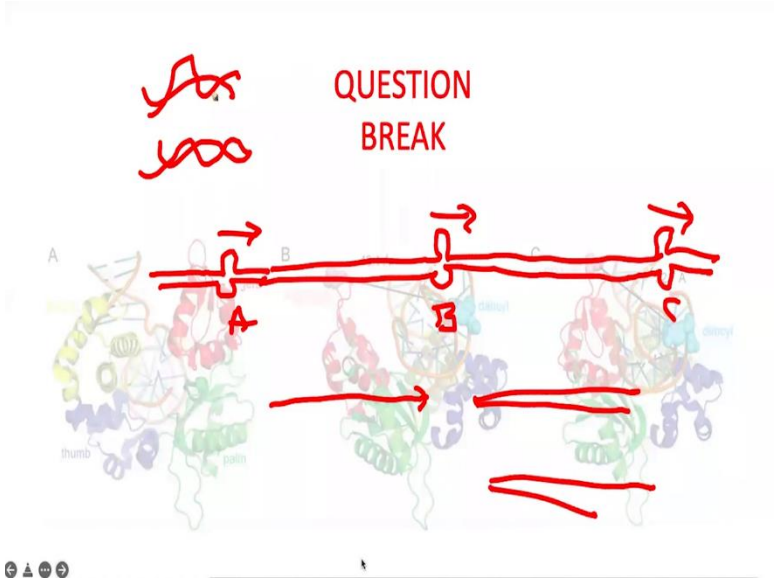


Professor: So I think, let us try and visualize this again. What you will end up basically having is if this is a log you will have a bubble here. You will have a bubble here and you will have a bubble here. The bubble is where the second strand is being made. Now, this bubble will move in this direction, this bubble will move in this direction, this bubble will move in this direction.

By the time bubble A comes to the side where bubble B is when it moves in this direction, B and B to C has already been replicated, you already have two strands of DNA being made. So what I am trying to tell you is that separation of the double stranded DNA happens transiently at the bubble.

And once the bubble does its job, you have two pairs of DNA instead of one pair of DNA. And both pairs are happily complementing each other. So you have to, you have to sit and visualize how this is happening inside the cell.

Student: It always takes a lot of energy to unwind the DNA?

Professor: Yes, it takes a lot of energy because you have to make enough nucleotides and the nucleotide biosynthetic pathway is an independent pathway, then you need to open up the bubble which takes energy polymerase, it is literally like paying laborers, laborers have a rate per hour.

The DNA polymerase has a rate per hour per nucleotide of how many ATP molecules it will consume. So all these processes are intense processes, energy, intense processes. And the currency of energy in the cell is ATP and similar energy rich triphosphate compounds, let us say, and this energy has to be used to do all these processes.

Student: So how long does it take for the replication to be complete? It cannot be done at a single stretch?

Professor: So what I can get, as you are asking me the time required.

Student: Yes.

Professor: The time required, is based on the processivity of the polymerase. And I will pull out the numbers for you. These are extremely efficient machines. They, for example, you heard in the movie that the ligase moves at 10,000 RPM. That is like the RPM in a jet engine, it moves extremely rapidly. So things are happening at a very fast pace inside the cell. But I have to give you the numbers.

I do not have the numbers on me right now. The way I tried to explain this to us to try and tell you that entire bacteria is going to divide into another bacteria in 10 minutes, which means that the whole process has to finish in 10 minutes.

Student: Yes, sir.

Professor: Okay.