Basics of Biology
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Lecture 27
Transcription (Part 1)

Hello everybody. This is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering, IIT, Guwahati. And what we were discussing? We were discussing about the, the different aspects related to living organisms. And in this context, in this particular module, we were discussing about the central dogma of life or the central dogma of molecular biology.

So if you recall in our previous lecture, we were discussing about the, the DNA dependent DNA polymerase and the process which is responsible for the DNA synthesis in the nucleus so that it can actually be duplicated. And once the DNA is duplicated and it makes the two copies then these two copies can be distributed among the, the, among the sister cells .

So when the cell divides and it requires the double amount of DNA, that process is going to be accomplished by the process of replications. And then in the previous lecture we have also discussed about how the, this basic process of DNA replication can be, can be performed under the in vitro chemical, under the in vitro conditions, and that was the, this, that was responsible for the discovery of the polymerase chain reactions.

And we have discussed about the different technical as well as the us, technical as well as the operational aspects related to PCR, and we have seen how we how we can be able to use the PCR for the different types of applications. So we have seen that the PCR can be used for even diagnostics of the different types of diseases, it can use for diagnosis of the different types of pathogenic organisms.

And then we, it can also be used for the many types of the criminal investigation or the paternal testings also. So in today's lecture we are going to discuss some more aspects related to central dogma of life. So what we have discussed so far? We have discussed

about the DNA dependent DNA synthesis, and the DNA dependent DNA synthesis is a process where you can be able to synthesize the new copy of DNA.

And once you have the cop, synthesize the new copy of DNA, that new copy of the DNA is responsible for the synthesis of the RNA molecule, and this process is called as the Transcription. So the RNA is going to be synthesized exactly the same sequence as the DNA molecule.

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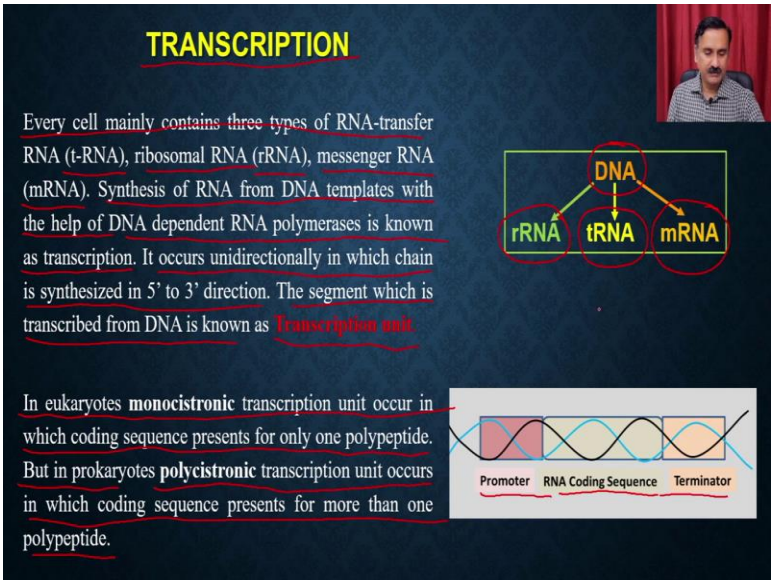

So in today's lecture, we are going to discuss about the transcriptions. So as the name suggests, the Transcription means deriving the information from the other molecules. So in this case you are actually taking the information from the DNA molecule and utilizing this information or utilizing the nucleotide sequences which are present on the DNA.

You are synthesizing the different types of RNA molecules, you are synthesizing the ribosomal RNA molecule, you are synthesizing the transfer RNA, and you are also synthesizing the messenger RNA molecule. And this process of synthesizing the different types of RNA molecule from the DNA is called as the transcription.

So every cell contains the three different types of RNA molecules, transfer RNA molecule or the t-RNA molecules, ribosomal RNA molecule or the rRNA molecule, and the messenger RNA molecule or the mRNA molecule. Synthesis of the RNA from the

DNA template with the help of an enzyme which is called as the DNA dependent RNA polymerase is known as the Transcriptions.

It occurs unidirectionally in which chain is synthesized is 5 prime to 3 prime and the segment which is transcribed from the DNA is known as the Transcription unit. So unlike the replications, if you recall the on, the replications, the replication is a bi-directional process, which means it can actually be, it can run from the five, it can be run from the left side to side or side to left side.

But in the case of the transcription, it is unidirectional and it, it runs only in the direction of 5 prime to 3 prime, and the segment, the one particular individual segment which is going to be synthesized or which is going to be transcribed from the DNA is known as the Transcriptional unit. In eukaryotic you have the monocistronic transcriptional unit which occurs, in which the coding sequence presents for only one polypeptide, whereas in the case of prokaryotes the polycistronic transcriptional unit occurs in which the coding sequence present for more than polypeptide sequences.

So what you see here is, this is actually a one individual transcriptional unit where you have, when you, when you transcribed the DNA, you are going to get the one transcriptional unit where you are going to have the promoters, you are going to have the RNA coding sequence and you are going to have the terminators. So, and during the transcription you are going to synthesize the three different types of RNA molecules.

You are going to synthesize the ribosomal RNA molecule, t-RNA molecule or to the messenger RNA molecules. Transcription as the, as you have seen when in, when we were discussing about the replication also, that replication also has the discrete steps like initiation, elongation and termination.

Similarly in the transcription also we have the discrete steps when the transcription is going to start and when the transcription is going to stop, so that it is actually going to give you a synthesized RNA, and that synthesized RNA is going to be called as the transcriptional unit.

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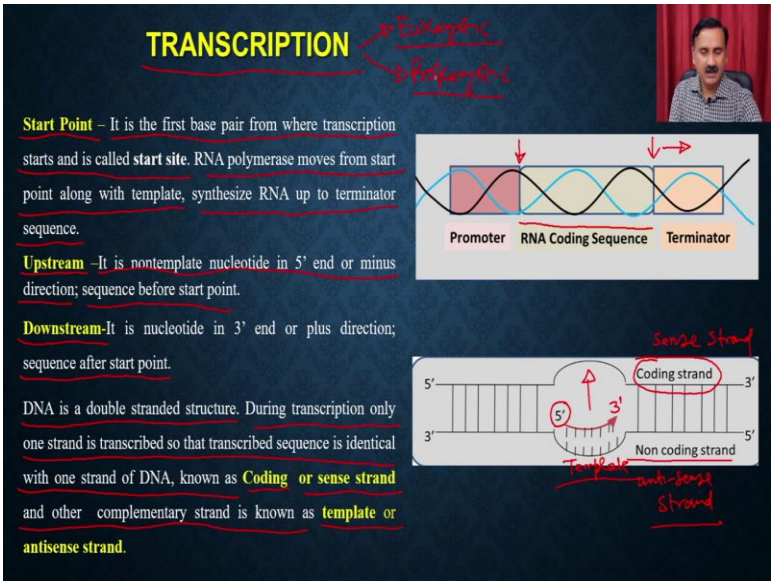

So let us see how do different steps which are there in the transcription. So transcription, you are going to have, or the transcriptional units. This is the, one of the transcriptional unit what you are going to have. So in this, you are going to have a start point, you are going to have the upstream sequences, you are going to have downstream sequences. So what is the start point?

So it is the first base pair from where the transcription starts and it is called as the transcription start site. RNA polymerase moves from the start point along with the template and synthesize the RNA up to the terminator sequence, which means if this is the, this is the gene which is responsible for or which is going to be utilized for transcription then you are going to have a promoter sequence and then you are going to have the coding sequence and you are going to have termination sequence.

And in this, you are going to have the starting point which is going to be somewhere here and from here actually the first base pair of the DNA is going to transcribe and that is how you are going to have the RNA which is responsible for the coding sequence. And then the RNA will go up to the termination site and that is how it is actually going to give you a synthesis of the RNA which is responsible for this particular sequence.

Then in, along with this start, start, start site as well as the end site, you are actually going to have the upstream as well as the downstream sequences. So in the upstream it is a non-template nucleotide in the 5 prime end or the minus directions and sequence, these are the sequence before the starting point. So these are the upstream sequences where you are going to have the, which are actually going to facilitate the binding of the RNA polymerase.

And that is how they are going to help in terms of transcription of the, this particular sequence. Then you are going to have the downstream sequences. So downstream sequences are actually going to be present onto the plus directions, and these sequences are after the, a start point. So and since the DNA is double stranded, so you can actually have the two different templates which can be utilized for the transcription side.

You can have the, this, see in this one you have a blue colored DNA and you have a black colored DNA. So we used to, ideally, either of these DNA can be utilized for the transcriptions. So when you say about the transcription, that you see that the DNA is double stranded structures, so during transcription only one strand is transcribed, so that the transcript sequence is identical with one of the strand of the DNA.

And this strand which is going to be utilized for the transcription is called as the Coding or the sense strand, whereas the other complementary strand is known as the template or the antisense strand. So which means, if this is the double stranded DNA, you can have the two strands, you can have a strand which is running from the 5 prime to 3 prime.

And the other strand which is going to be run from the 3 prime to 5 prime, and what you see here is that the only one of the strand, as I said, transcription is unidirectional, it can be run for the both the templates, both the DNA strands, but it only will be in a single direction. And transcription is always in a direction of 5 prime to 3 primes.

Which means it is actually going to utilize the 3 prime to 5 prime sequence as a non-coding strands or it is actually also called as the template strands, whereas it is going to synthesize the RNA which is going to match with the DNA sequence which is present on to the 5 prime to 3 prime. And that is why this strand is called as the coding strand which

means this strand is going to called as the, also called as the sense trend, and the other strand is also called as the antisense strand.

So these are the different name for the different types of different strands. So, for example, the strand what is going to use for synthesizing the RNA is called as the non-coding strand or the template strands. So this is also called as template strand, so this is going to be serve as a template. And the RNA what is going to synthesize, that is going to be called as the coding strand.

So this is going to be, because the sequence, the nucleotide sequence of this RNA is going to match with the sequence what is present on to the DNA. And that is why this strand is going to called as coding strand or to the sense strand. Now, the transcription can happen in the, is the universal, so transcription can happen into the eukaryotic cell or transcription can happen into the prokaryotic cell.

So it can happen in prokaryotic cell. So in both the cells the transcription is, the steps are almost identical. You can have the initiation, you can have the elongation and you can have terminations. But the different types of factors are responsible for the eukaryotic or the prokaryotic transcriptions are different, the places where the eukaryotic or the prokaryotic transcription is taking place, that is also different, and the machinery what is participating into the eukaryotic or the prokaryotic transcription is also different.

So let us, before going into the discussion about the transcription in prokaryote or transcription in eukaryote, let us see the differences between the transcription of the eukaryotic as well as the prokaryotic organisms.

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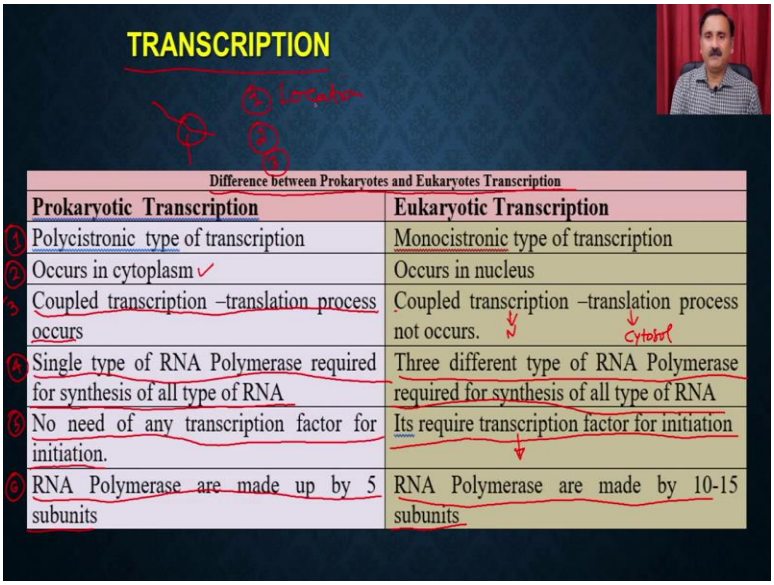

So transcription is different in terms of the machinery what is involved, in terms of the places where it is going to take place and the different types of steps. So what is the difference between the transcription, between transcription in eukaryotic as well as the prokaryotic cells. So first difference is that the, for the prokaryotic transcription it is polycisstronic which means the single RNA is going to transcribe for the different types of polypeptide chains.

So, and whereas in the case of eukaryotic it is going to be monocistronic. Then the second is the, as you know that the bacteria do not have the nucleus, so everything what is happening is inside the cell, is within the same cell or within the cytosol, so that is why the site of transcription in the case of prokaryote is cytosol, whereas the site of transcription in the case of eukaryotic is nucleus because the DNA is present inside the nucleus, so transcription is going to occur for the eukaryotic cell inside the nucleus.

And once the RNA is being synthesized then it is transported outside the nucleus and then it is going to participate into the protein synthesis. Since the, there is no nucleus then the, we can actually have the coupled transcription and the translation process, which means as soon as this RNA is going to be synthesized, it is going to be utilized for the protein production machinery.

And that is how it is actually going to start producing the protein. So this is called as the coupled transcription and the translational process, which means it is actually going to be called as co-translational process, which means that as soon as the transcription starts it is also allowing the protein, protein production machinery to attach to these RNA molecules and that is how it is actually going to start the translation.

Whereas in the eukaryotic cells, that is not the case, transcription is occurring inside the nucleus, so once the transcription is over, then the nucleus is going to transport the synthesized messenger RNA and as well as all other RNA species and then these RNA species are going to go into the cytosol, and that is how it is actually going to do the translation. So transcription is going to occur in the nucleus whereas the translation is going to occur inside the cytosol.

So that is why these two processes are not linked to each other, they are not going to occur simultaneously. In the case of prokaryotes, you require a single type of RNA polymerase for all type of RNA molecules whereas in the case of eukaryotes you require the three different types of RNA molecules or RNA polymerase for the synthesis of the all types of RNA molecules.

Then in the case of the prokaryotes you, you do not, no need to have any transcription factor for the initiation, which means the RNA polymerase what is present in the prokaryotic system is fully independent in terms of initiating the transcriptions whereas in the case of prokaryote, in the case of eukaryotes it requires the transcriptional factors for the initiation. So this is a very important step because it require, it actually gives the more control over the process of transcriptions.

In the case of prokaryotes, whenever the RNA polymerase wants, it actually can go and start to, start the transcriptions, whereas in the case of eukaryotes, it is not the case. Even if the RNA polymerase is present but the required transcription factor is not present, it cannot be start a transcription. So that is why the eukaryotic transcription is going to be more tightly controlled compared to the eukaryotic, prokaryotic transcriptions.

Then the, as far as the structure is of the prokaryo, polymerase is concerned, the polymerase in the case of the eukaryotic, prokaryotic system is made up of the 5 different subunits, whereas in the case of the RNA polymerase, RNA polymerase are made up of 10 to 15 subunits in the eukaryotic cells. So these are the few of the classical differences between, of the transcriptional process between the prokaryotic as well as the eukaryotic system.

So if you take home the message, what are the three important points, what are the differences between the transcription in the prokaryotic versus eukaryotic. The number one is the location, so location of the transcription, so location of the transcription in the case of prokaryote is cytosol whereas the location is going to be nucleus in the case of eukaryotes.

Then number two is the transcription and the translation are going to work together or simultaneously in the case of prokaryotes whereas it is going to be separate in the case of the eukaryotes. Number three is the, it is going to be more controlled in the case of eukaryotes, so transcription, because transcription depends on to the transcriptional factors so that is why it is going to be finer controlled.

And, in the case of eukaryotes whereas it is less controlled because it is going, not does not require the transcription factors. So we are going to start discussing about the transcription in the prokaryotes and then we are going to going to discuss about the transcription in the eukaryotes.

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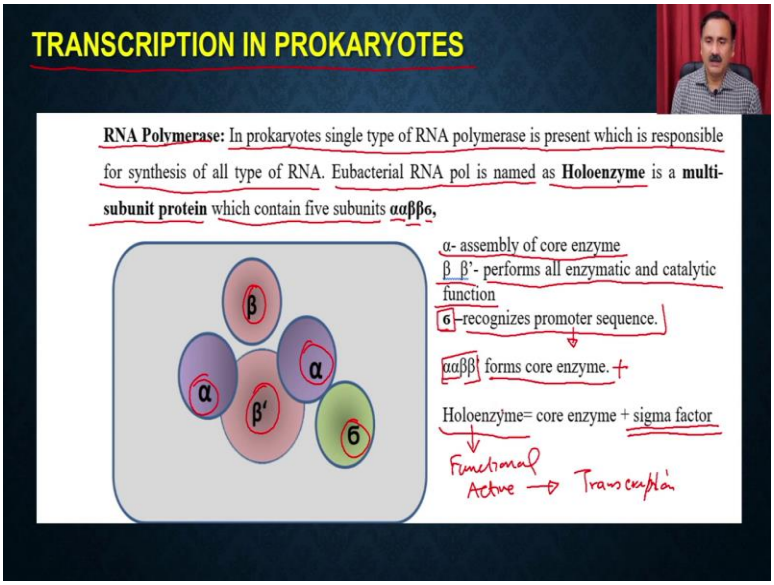

So transcription in prokaryotes. So when we want to start the discussion about the transcription in prokaryotes, first we have to discuss about the machinery. So in the case of transcription, in the case of prokaryotes, the only machinery what we require is the RNA polymerase. So in prokaryotes, a single type of RNA polymerase is present which is responsible for the synthesis of all type of RNA.

Eubacterial bacterial RNA polymerase is termed as the Holoenzyme and it is a multi subunit protein which contains the 5 subunits. You have the two alpha subunits, two beta subunits and two, one sigma subunit, which means you have the two-beta subunit, beta and beta prime, you have the two alpha subunit alpha and alpha, and you also have a sigma factor. So alpha is required for the assembly of the core enzyme.

The beta and beta prime are going to perform the all enzymatic and catalytic functions, and sigma is actually been required or sigma is a factor which is responsible for the recognition of the promoter sequence. So promoter is an upstream sequence which is going to say that okay this is the sequence where you have to go and bind, and then it is actually going to start the synthesis of the RNA polymerase.

So, it actually going to initiate the RNA, transcriptions, two alpha and two beta are together is going to be form and that is how going to form as core enzyme. When the core

enzyme is actually going to mix with the sigma factor it is actually going to form the Holoenzyme.

Which means the RNA polymerase in the prokaryotes is actually going to be present always in as a core enzyme, which means it is going to have the four subunits alpha and alpha and beta and beta prime, but as soon as the sigma factor will come and join, it is actually going to form the Holoenzyme, and this Holoenzyme is going to be functionally active, so it is going to start the, it will it is going to start in the transcription.

So it is going to do the, start the transcription. So as soon as, until the sigma factor is not going to combine with the core enzyme, the Holoenzyme is not going to form and that is why it is not going to participate into the transcriptional activities.

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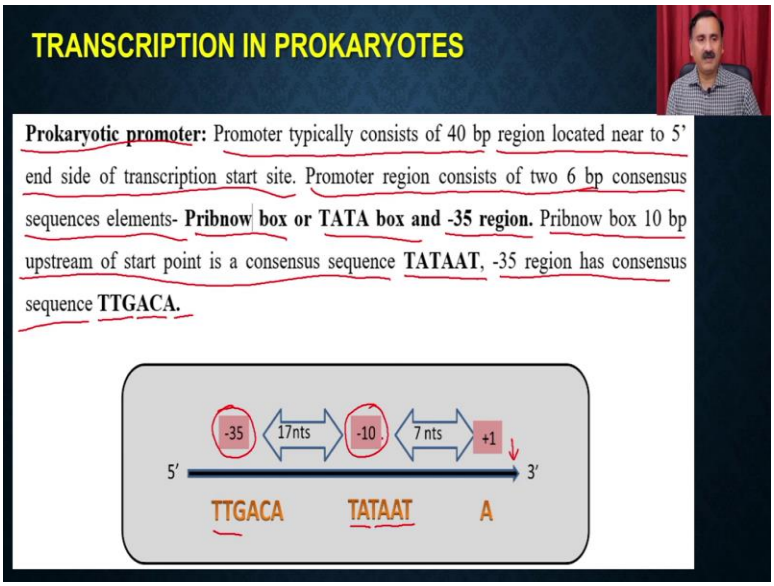

Now the second thing is about the promoter, so prokaryotic promoters, promoters typically consist of 40 base pair region located near to the 5 prime end of the transcriptional start site. So remember that when we were talking, when we, when we are showing you the typical transcriptional unit, we were having a, the coding sequence and then upstream to the coding sequence that is the region of the promoter.

Promoter region consists of the 6, two 6 base pair consensus sequence element which are called as the Pribnow box or to the TATA box, and the minus 35 regions. Then Pribnow

box is a 10 base pair upstream of a start point, and it is a consensus sequence of TATAAT, whereas -35 region has a consensus sequence which is called as the TTGACA So this is what is you are going to have, you have, you are going to have a minus 10 region and then you are going to have the minus 35 region.

In the minus 10 region you are going to have the Pribnow box, which is going to be having a consensus sequence, which is called as like TATAAT, whereas, so this is the, this is going to be the start point, so, and this is going to be plus one site, and below, above upstream to this, like the minus 10 region, you are going to have the Pribnow box, and the minus 35 region you are going to have the, these sequences.

So these are the optimal sequences what is present into the, as a, in the promoter of the prokaryotic genes, and they are actually going to be responsible for facilitating the transcription.

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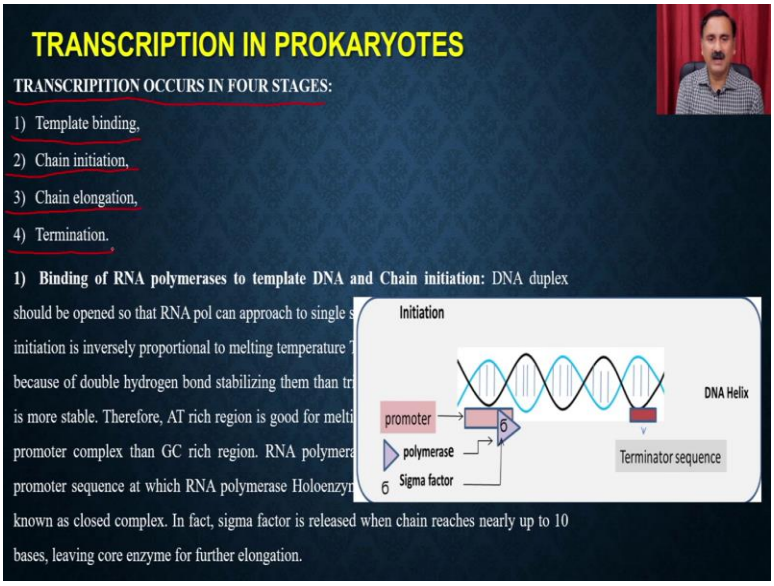

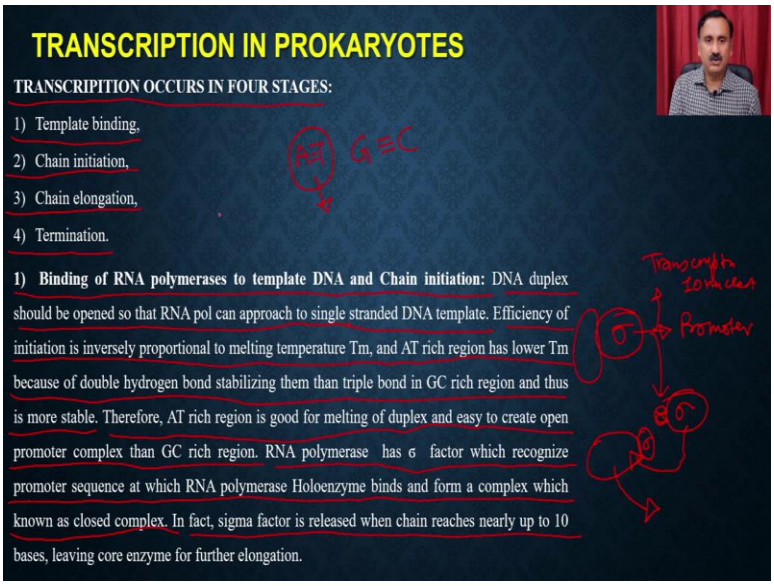

Now once we are talking about the process, so transcription occurs in the four steps in the in the case of transcription in prokaryotes. You are going to have the template binding, then you are going to have the chain initiation, then you are going to have elongation, and then you are going to have the terminations.

So the step one, you are going to have the binding of RNA polymerase to the template DNA and the chain initiations. So the DNA duplex should be opened so that the RNA polymerase can approach to the single stranded DNA templates. Efficiency of the initiation is inversely proportional to the melting temperature Tm, and the AT rich has a lower Tm because of the double hydrogen bonding stabilizing than the triple bond in the GC rich region.

So the place where it is actually going to be able to melt the DNA more nicely or more easily is that where you are going to have the AT rich sequences because AT is going to have the two DNA strands, two hydrogen bonding, whereas GC is going to have the three bonds, so three hydrogen bonding.

So because of that, the, if it, if the initiation is, if the DNA is going to have, is going to be opened up by the RNA polymerase very easily to, so that they can be able to utilize the DNA as a template if the DNA is going to have the more of the A or T sequences. The efficiency of initiation is inversely proportional to the melting temperature and the AT

rich region is going to have the lower Tm because of the double hydrogen bonding stabilization, than the GC rich region.

Therefore, the AT rich region is good for the melting of duplex and easy to create the open promoter complex than the GC rich region. RNA polymerase has sigma factor which recognize the promoter sequence at which the RNA polymerase Holoenzyme binds and forms a complex which is known as a closed complex.

So initially it will go and bind to the region where it, the, it is easy to melt the DNA and within the promoter region, and then it is actually going to unwind the DNA and that is how it is actually going to form the closed complex. And the sigma factor what is present in the Holoenzyme is responsible for recognizing the promoter sequence or it is actually recognizing the sequence where it is easy to melt.

So in fact, the sigma factor is released when the chain reaches nearly up to 10 bases leaving the core enzyme for the elongation. So as soon as the chain will going to enter or as soon as the RNA polymerase will enter into the initiation step and it will go for the 10 nucleotides, then it, the sigma factor is going to be removed, and that has a very good advantage because, you can have the sigma factor which is going to recognize the promoter. So it is going to recognize the promoter.

So as soon as the sigma factor will recognize which is a part of the RNA polymerase, it is going to recognize the promoter, and when the transcription is going to start, so, so you can imagine that transcription is going to start and it will go for the 10 nucleotides. Once the 10 nucleotides are over then the sigma factor is going to be released. So sigma factor is going to be released.

And now this sigma factor is free, this sigma factor can assemble with another RNA polymerase, so it can actually assemble with another RNA polymerase and that is how it can actually be able to help the another RNA polymerase molecule to recognize the another gene or another promoter, and that is how it actually can channelize the, the machinery for maximum outcome.

So you do not have to synthesize the many types of sigma factor. You can actually work with the one or two sigma factors to recognize the different types of promoters and that is how you can be able to distribute or you can be able to utilize the factors more optimally. Now once the binding of the RNA polymerase and the chain initiation is been done then it is actually going to enter into the next phase.

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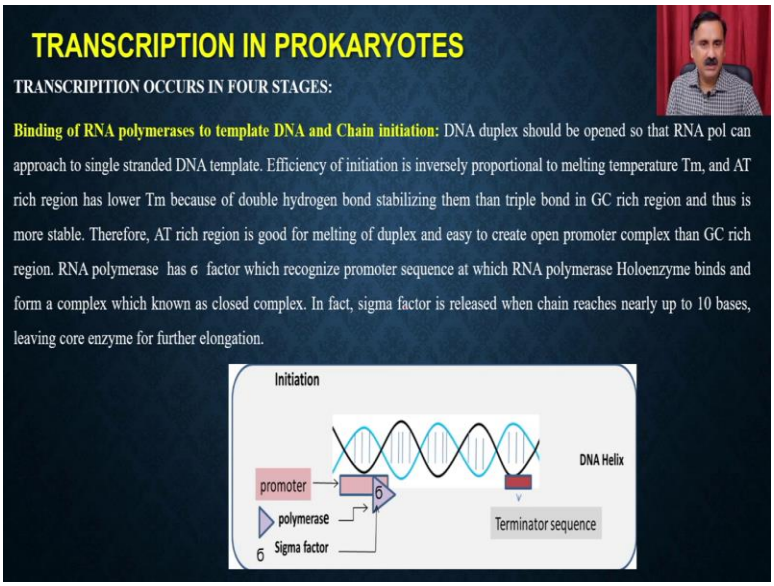

So in the next phase, it is actually going to have the binding of RNA polymerase to the chain initiation, that is already we have discussed.

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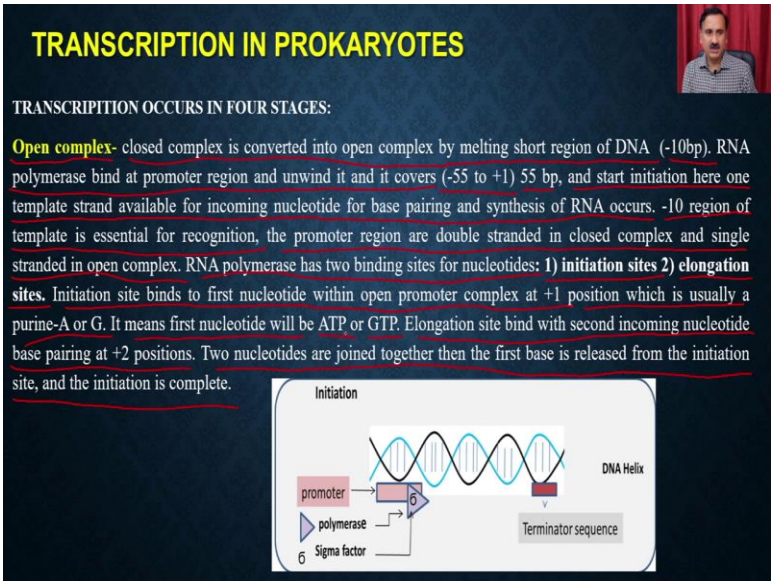

Then we can, actually going to form the open complex. So within the, that step, once the closed complex is converted into open complex by melting the short region of DNA like the 10, minus 10 base pair regions, the RNA polymerase binds at the promoter region and unwind it to cover the minus 55 to plus 1, which means the 55 minus, 5 to 50, to 55 base pairs, and it start the initiation here one template strand available for incoming nucleotide for the base pairing and the synthesis of RNA occurs.

So what will happen is that the RNA polymerase will assemble with the sigma factor and then it is actually going to bind to the template, and it is actually going to start melting the minus 10 regions. And once it start melting the minus 10 regions, and then it will bind the promoter region. So when it will going to bind the promoter region, it will going to start the initiation steps.

The minus 10 region of the template is essential for the recognitions, the promoter regions are doubly stranded in the closed complex and the single stranded in the open complex. So once it melts the DNA, then it is actually going to form the open complex, which means in the, in that open complex the, one of the template of the DNA is going to be available as a template.

The other strand, and to, so that the RNA polymerase will take that information, the nucleotide information from that template strand and then it start synthesizing the RNA. RNA polymerase has the two binding sites for the nucleotides. It has the initiation sites, and it has the elongation sites. So it has the two different types of activities for the nucleotide one. It actually can recognize the nucleotides and that is how it actually can participate into the initiation sites, or it can also have the elongations.

So initiation site bind the first nucleotide within the open promoter complex as the plus 1 site, which is usually a purine rich it A or G sequences. It means the first nucleotide can be ATP or the GTP. The elongation can, site binds with the second incoming nucleotide base pairing at the plus 2 positions. The two nucleotides are joined together and the first base is released from the initiation site and the initiation is complete, which means the RNA polymerase has the two site for the nucleotides.

One is initiation site, which is going to recognize the first nucleotides which is present at the plus one site, and that nucleotide could be either the A or the G. The second is the elongation site. That elongation site is going to recognize the second incoming nucleotide, and that second incoming nucleotide, so once it binds to the first nucleotide, and then it will enter, and start synthesizing the second nucleotide by utilizing the elongation sites. And once it enters into the elongation site then the, the initiation steps are over, then it will enter into the elongation phase.

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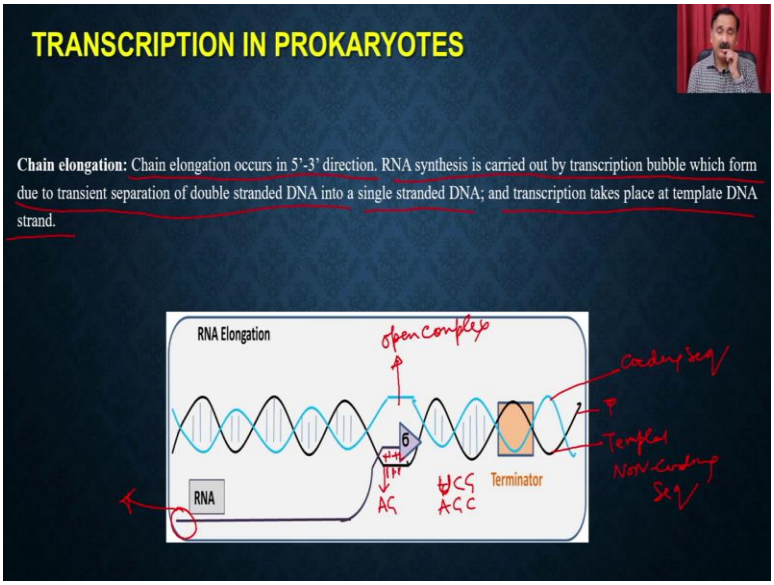

What will happen in the elongation phase? The elongation, the chain elongation occurs in the 5 prime to 3 prime directions. The RNA synthesis is carried out by the transcriptional bubble which forms due to the transient separation of double stranded DNA into a signal stranded DNA, and the transcription takes place at the template strand.

So what you have is, see, this is the site where the, the DNA is binding. So this is the site where you have the promoter. So one, initially the sigma factor will come and start binding, then it is going to form the open complex. So this is the, actually the open complex. Now within the open complex, you have, this is the template strand, like, this is the template strand, so this black one is a template strand.

And this blue one is the, the coding sequence, or this is called as non-coding sequence. And what will happen is the RNA at the Site 1, so plus 1 site, you are going to have plus 2 site. So on plus 1 site, the RNA polymerase will go and sit and then it will actually be looking for the nucleotide like A or G, and then it is going to start the transcription.

As soon as it will start transcription, so it is actually going to keep utilizing this information. So if you have the A, it is going to put the T into the into the template, and so on. So if it is A, it is actually going, not T, so synthesize the u, and so on. So if you have G, it is going to synthesize the C, and if you have a C it is going to, so it is going to

read these nucleotides, the incoming nucleotides utilizing the elongation site within the RNA polymerase and that is how it is actually going to start synthesizing the RNA.

And you see the RNA is going to come out from the, from this transcriptional unit and that is how this RNA is going to be utilized by the protein synthesis machinery, that we are going to discuss when we are going to discuss in our subsequent lecture, that how the, the, the protein synthesis machinery is going to bind this nascent RNA, and that is how it is actually going to participate into the protein synthesis.

So the chain elongation is going to occur in the 5 prime to 3 prime direction, and it is going to be unidirectional.

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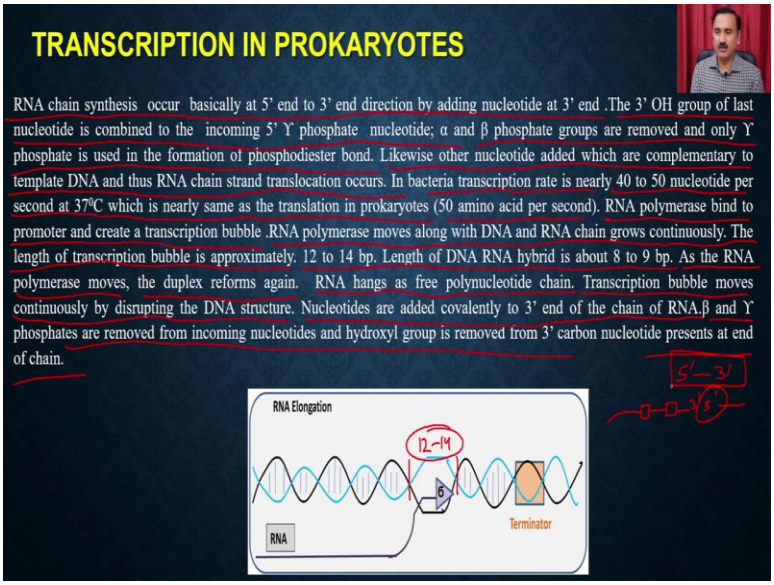

Then it is going to enter, so chain, RNA chain synthesis occurs basically at 5 prime to 3 prime end direction by adding the nucleotide at the 3 prime end. The 3 prime OH of the last nucleotide is combined to the incoming 5 prime nucleotide and the alpha and beta phosphate groups are removed and only the gamma phosphate is used in the formation of phosphodiester bonds.

Likewise, the other nucleotide added which are complementary to T template strand, and thus the RNA chain strands translocation occurs. In bacteria, the transcription rate is nearly the 40 to 50 nucleotides per second at 37 degree Celsius, which is nearly same as

the translation in the prokaryotes which means the 50 amino acid per second. RNA polymerase binds to the promoter and create a transcriptional bubble.

See RNA polymerase moves along with the DNA and the RNA chains grows continuously. The length of the transcriptional bubble is approximately 12 to 14 base pair. So this trans, 12 to 14 base pair transcriptional bubble is going to be open for allowing the RNA polymerase to read the template strands and then it actually going to, based on the nucleotide present onto the template strand, it is going to start putting the complementary strands into the RNA molecules.

So the 12 to 14 base pair length of the DNA-RNA hybrid is about to 8 to 9 base pair. So within this, 8 to 9 base pair is going to form the DNA-RNA hybrids. As the RNA polymerase moves, the duplex reforms again. So as soon as the RNA polymerase moves on, the, the remaining our DNA strand is actually going to coil back because see that there is a big difference between the replication and the transcription.

In the replications, you are supposed to remove the helicases and topoisomerases, so and then you also have to remove the single stranded DNA binding protein so that you can be able to unbind the DNA whereas in this case there is no such requirement. The RNA polymerase will able to melt the DNA and will be able to start the transcription. So it is actually going to, so as soon as the RNA polymerase will move on, it, there will be no factor responsible for the DNA to remain the single stranded.

So it will again unwind. And the RNA hangs as the free polypeptide chained, polynucleotide chains. So transcriptional bubble moves continuously by the disrupting the DNA structures. Nucleotides are added covalently to the 3 prime end of the chain of the RNA, beta and gamma phosphates are removed from the incoming nucleotide and hydroxyl group is removed and present at the end of the chain.

So as soon, as soon as the RNA polymerase is moving the, every five, every for, and then it starts adding the nucleotide. And this in this case it is actually going to utilize the 3 prime end of the chain. So 3 prime end of the chain it is going to have the 5 prime of the

nucleotide, incoming nucleotide, and that is how it is going to synthesize, and that is how it is going to have the transcription in the direction of the 5 prime to 3 prime.

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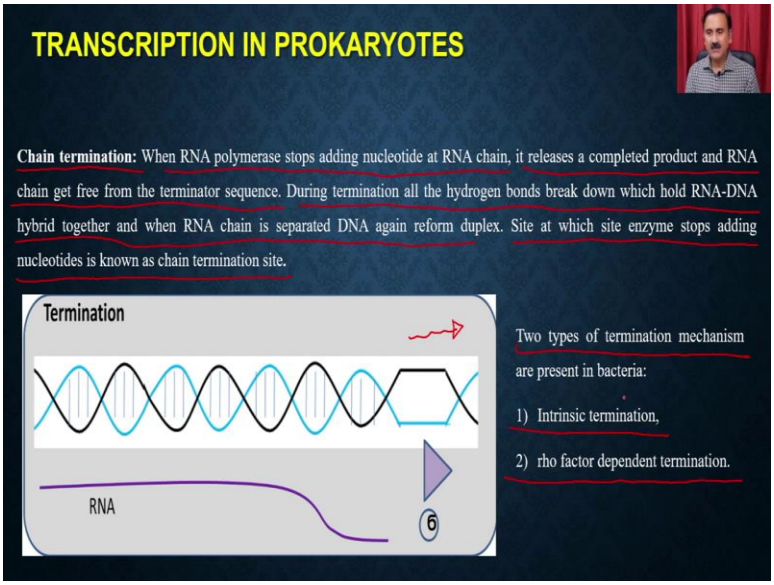

Now, we are going to have the next stage. So next stage is the chain termination. So when the RNA polymerase stop adding nucleotide at RNA chain, it releases a complete product and the RNA gets free from the, free from the termination sequences. During termination, all the hydrogen bonding breaks down which holds the RNA-DNA hybrid together and then the RNA chain is separated; DNA again will form the duplex, site at which the site, the enzyme stop adding nucleotide is known as the termination site.

So when this bubble, like transcriptional bubble is keep moving, keep moving and then it reaches to the transcriptional site, or transcriptional termination sites, and then it actually, at that site, since the, the there is no, there is no template strand, like the, so template strand does not provide any information to that, you add the nucleotides, then that is why the RNA polymerase stop adding the nucleotide.

As soon as the RNA polymerase stop adding the nucleotides, then the, the, DNA, DNA-RNA hybrid is actually getting destabilized and that is how it is actually going to be removed, and that is how it actually ends up in doing the termination of the transcriptions. In prokaryotes, we can have the two different types of terminations. One is

called as the intrinsic termination, the other one is called as a row factor dependent terminations.

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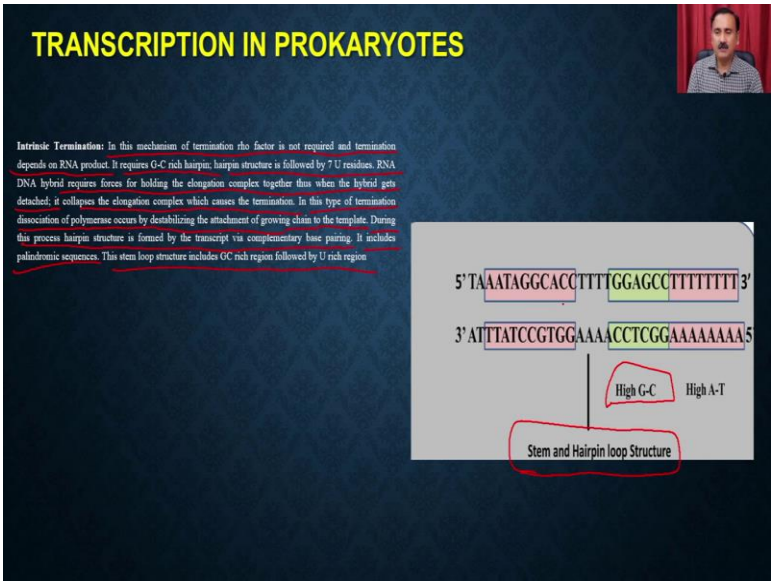

So let us see about the intrinsic terminations. In an intrinsic termination, in this mechanism of termination, the row factor is not required for the termination, and it depends on to the RNA products. It require a GC rich hairpin. So hairpin structure is followed by the seven new structure. So what you see here is that the, this is the termination sequence where you have a very high GC rich content.

So when you have a very high GC rich content, first of all the, this particular region is not going to be melted out by the RNA polymerase. And then it also has a hair leave, hairpin loop kind of structure. So you see it is going to flip, and that is how it is actually going to form a hairpin. So RNA-DNA hybrid requires the forces for holding the elongation factors, elongation complex together, then the hybrid gets detached.

It collapses the elongation complex which causes the termination. In this type of termination, the dissociation of the polymerization occurs by destabilizing the attachment of the growing chain to the template. During this process, the hairpin structure is formed by the transcription via the complementary base pair.

It includes palindromic sequences, this step-loop structure includes the GC rich region by the U region. So what you see here is that it has a GC rich hairpin. So it has a GC rich hairpin, and because of this and then it has followed by the seven new residues on both the site, and that is how it is actually going to form the hairpin loop.

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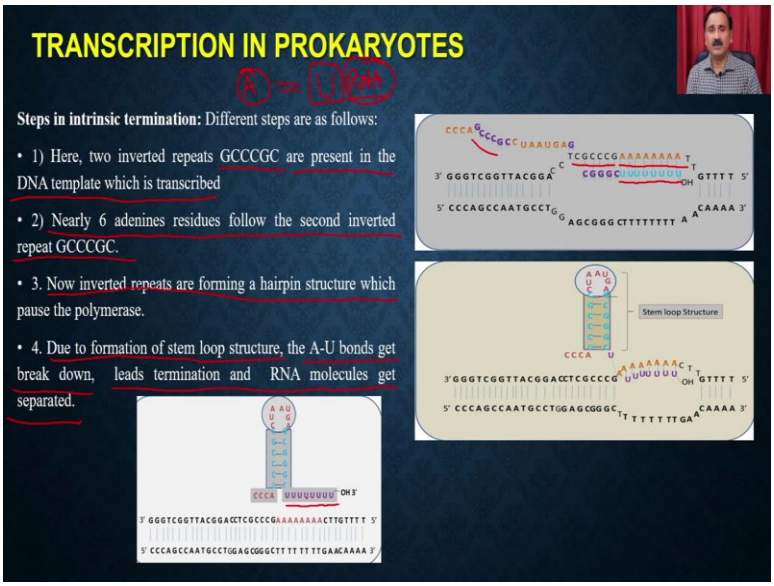

So what are the different steps in the intrinsic terminations? So here we have the two inverted GC rich regions. So you have the two inverted repeats like GCCCGC. So this is what you have here. So, and these are present in the DNA template which is transcribed. So, nearly 6 adenine residues follow the second inverted repeat. So you have the two repeats, one is on this side, the other one is on this side.

So as soon as you have this inverted repeat is being transcribed it is going to be followed by the seven A residues. So this is going to be seven A residue which are going to be formed. And you know that the A is actually going to have the, when the A is present in the DNA, it is actually going to give you the U in the RNA molecule, because that is the complementary.

So it is going to give you the A in the U in the RNA molecule, which means the, it is actually going to form a very less stable DNA-RNA hybrids. And now, at the same time, what will happen? The inverted repeats are forming a hairpin like structure. So you see

that all these inverted repeats what are present here is actually going to form a hairpin like structure.

So one repeat you have on this side, the other repeat you have on this side, and that is how, it, when they will come together, when they this, they will be form into the RNA, they will actually going to form a hairpin like structure like this, and this hairpin like structure is going to be attached or going to be connected onto the DNA by the UUU like sequences.

And then these UU like sequences or the DNA-RNA hybrids because of the interaction between the A to U is going to be weak, and because it is going to be weak, it is actually going to be detached from the template. So, due to the formation of the stem loop structure, the AU bond gets broken down, and it, that leads to the termination and the RNA molecule gets separated. So that is how you, that, this is going to happen.

Once the RNA polymerase is going to be reached to the termination site, at the termination side you are going to have this GC clamp, and GC clamp is followed by the A, seven A nucleotides. And this GC clamp is actually going to form a hairpin like structures, and once the hairpin like structure is formed, it actually going to stop the growth of the RNA polymerase, and that is how it is actually going to hold here.

But since the, it cannot be remain there on to the template for longer period of time because the stability of the A to U interaction is very low, and because of that the RNA polymerase, along with the, the RNA, synthesized RNA is going to be removed from the templates.

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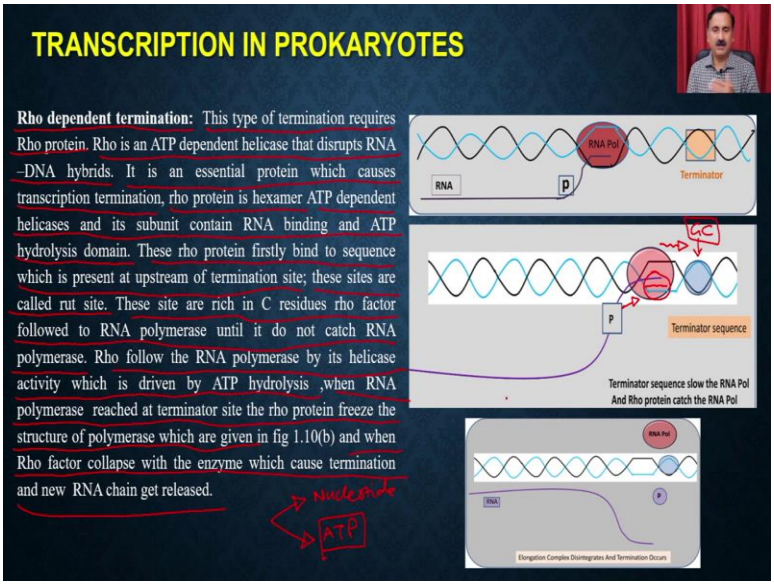

Now the second mechanism is the Rho dependent mechanism. So row dependent mechanism is depending on a protein which is called as the Rho protein. So this type of termination requires the rho proteins, and rho is a ATP dependent helicase that disrupts the RNA-DNA hybrid. It is a essential protein which causes the transcriptional terminations. And rho protein is a hexamer. So rho protein is actually a hexamer and that actually binds to the RNA.

So in a ATP dependent it is, and it is a ATP dependent helicase, and its subunit contain the RNA binding and the ATP hydrolysis domain. So in the rho protein you have the two activities. You have nucleotide binding activity or the RNA binding activity, and then you also have the, the ATP hydrolysis activity. So when, you have, so you utilize the ATP hydrolysis and then you actually bind the RNA.

So these rho protein firstly bind to the sequence which is present in the upstream of the termination sites. These sites are called as the rut sites, and these sites are rich in the C residues. So the rho factor followed to the RNA polymerase until it does not cache the RNA polymerases. So, rho follow the RNA polymerase by its helicase activity which is driven by the ATP hydrolysis.

When the RNA polymerase reached at the termination site, the rho protein freezes the structure of the polymerase. And when the rho factors collapse with the enzyme which causes the termination and the new RNA protein chain can released. So this is what exactly happened. So this is the RNA chain, the rho protein will go and bind to the this growing chain, and then it will keep reaching or keep progressing or keep moving towards the, the termination, transcription bubble.

But what will happen is that when it reaches the termination site, that at the termination side the RNA polymerase speed is going to be reduced because the transcription site is going to be free, rich of GC sequences, and because of that, it is difficult for the RNA polymerase to go there.

And once the RNA polymerase cannot move a very long time, then the row factor is going to come and it is actually going to utilize its helicase activity to break the interaction between the RNA and DNA hybrid. And because of that the, the whole complex, the RNA polymerase, the RNA is going to jump into the cytosol, and that is how it is actually going to cause the terminations.

So this is all about the transcription in the prokaryotes. And in our subsequent lecture, we are going to discuss more about the transcription in eukaryotes, and then we are also going to discuss about the post trans, post transcriptional modifications. So what we have discussed? We have discussed about the, the transcription in prokaryotes, we have also discussed about the differences of the transcription between the eukaryotic as well as the prokaryotic organisms.

And so with this, I would like to conclude my lecture here. In our subsequent lecture, we are going to discuss about the transcription in eukaryotes, and then we are also going to discuss about the post-transcriptional modifications. Thank you.