Eukaryotic Gene Expression

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Lecture No. # 14

Eukaryotic gene regulation: Regulation of tRNA and 5s rRNA synthesis by RNA polymerase III

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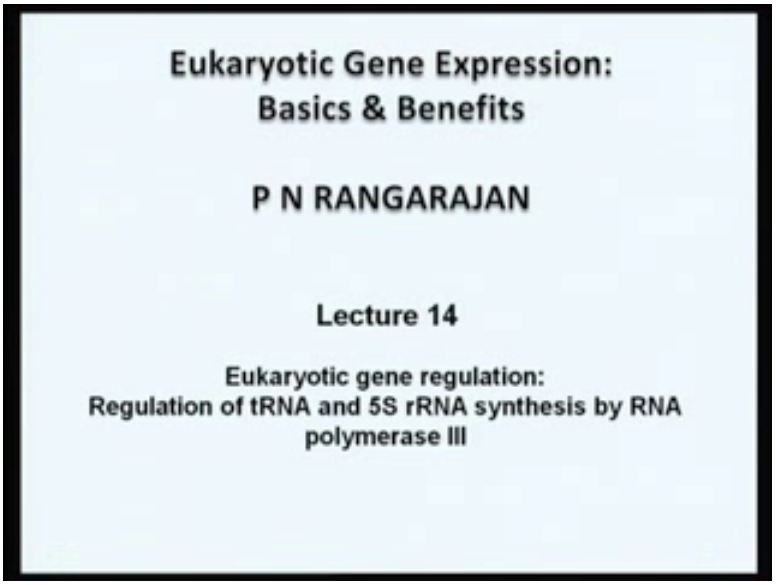

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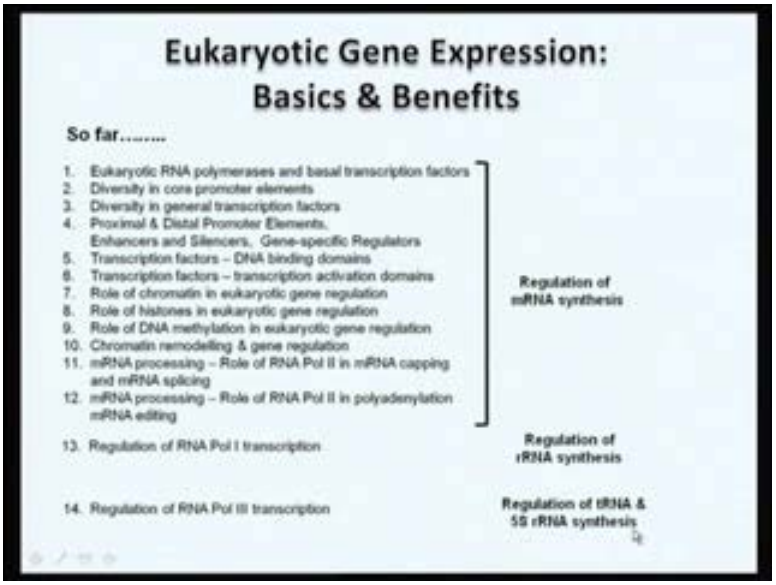

Welcome to this fourteenth lecture in this course on eukaryotic gene expression. Today, we are going to discuss about the regulation of bio synthesis of tRNA and 5S ribosomal RNA by RNA polymerase III. Now, the last twelve lectures or last thirteen lectures, our focus has been primarily on the regulation of gene expression by RNA polymerase II, which we took about twelve lectures, where I described clearly how RNA polymerase II along with general transcription factors and a variety of transcription factors, which bind to upstream promoter elements, enhancer sequence etcetera, go and bind to their corresponding response elements in the promoters and activate transcription. How differential gene expression takes place with the combination of a core promoter elements, combination of variety of general transcription factors as well as sequence specific DNA binding proteins binding to upstream enhancer sequences and silencers and so on and so forth.

We also discussed extensively about the role of chromatin in the regulation of gene expression. How crystal modification, DNA methylation etcetera affect gene regulation and then we discussed considerable amount of time discussing after transcription initiation, how RNA processing events takes place such as mRNA capping, mRNA splicing, poly relation so and so forth and very importantly we discussed about the role of RNA polymerase C-terminal domain in all these RNA processing events.

After discussing all these processes with relation to RNA polymerase II transcription, we spent the last lecture, we discussed primarily about the regulation of ribosomal RNA synthesis by RNA polymerase one. Today, we are going to discuss about the regulation of RNA polymerase III transcription, especially the regulation of tRNA and fiber ribosomal RNA synthesis by RNA polymerase III. So, this is going to be the focus of today's lecture.

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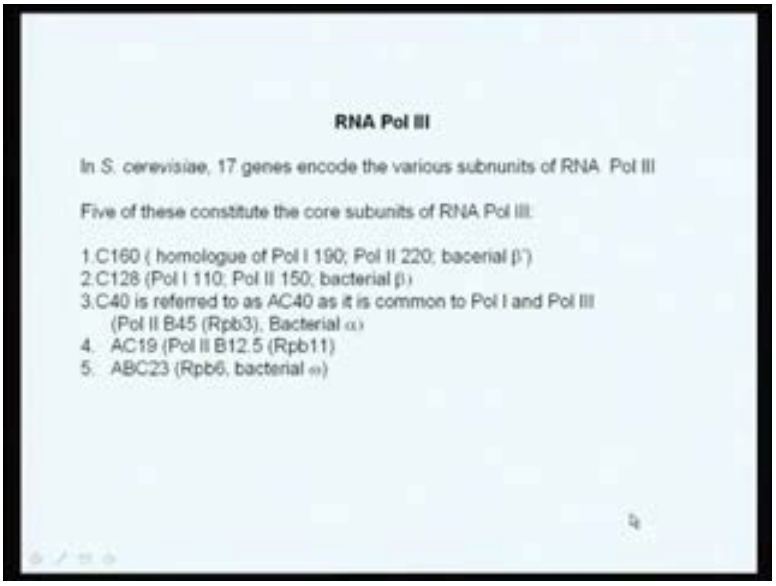

RNA polymerase III, as we have seen in the case of pol I or pol II, is also a multi subunit complex and in fact, there are about 17 genes which code the various subunits of RNA polymerase III. So, it is a multi-subunit complex. It is a huge protein complex of at least 17 different subunits. Of these 17 subunits, 5 of the subunits constitute the core subunit of RNA polymerase III and these core subunits most of the core subunits have homologues in both pol I and pol II; that means, these subunits are kind of shared by pol I and pol II, but they have homologues.

For example, the C160 subunit of RNA polymerase III is a homologue of pol I 190 subunit as well as pol II 220, which is the largest subunit of RNA polymerase II and is kind of homologue of bacterial beta prime subunit of E.coli RNA polymerase one RNA polymerase.

Similarly, the C128 subunit of RNA polymerase III is homologue of pol I 110 pol II 150 and bacterial beta subunit of E.coli polymerase. The C40 subunit of pol III is also referred to as AC40 because the same subunit is shared by both polymerase I which is referred as pol A and polymerase III which is often referred as polymerase III. So, it is called AC40 because this 40 care of subunit is shared by both polymerase I and polymerase III and is common to both pol I and pol III. In the case of pol II, its homologue is B45. Pol II B45 is also known as Rpb3 and it is also the bacterial E.coli or E.coli polymerase alpha is the homologue, in the case of prokaryotes. Similarly, the

AC19 - so called because this subunit is shared by both pol I and pol III and the homologue in the case of pol II is the pol II B12.5 or also known as Rpb11 subunit in the case of pol II.

Similarly ,the ABC23 is shared by all the 3 RNA polymerases namely pol I, pol II and pol III and it is known as Rpb6, in the case of RNA polymerase II and is the equivalent of bacterial omega subunit. So, this is in very brief, the subunit structure of RNA polymerase. We will not go into extensive details of how RNA polymerase II subunit is organized the structure and so and so forth, but suffice to know that these 5 subunits actually constitute the core RNA polymerase III and they have homologues, in case of pol I and pol III.

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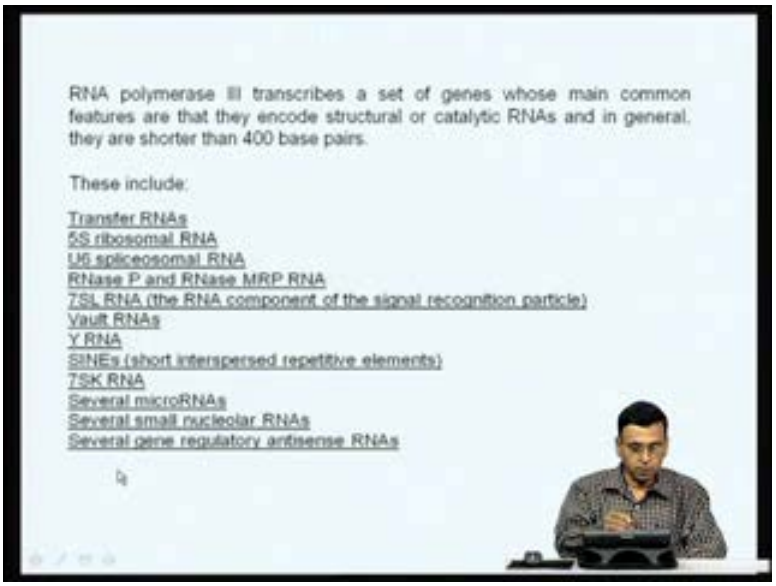

Now, RNA polymerase III transcribes a set of genes, whose main common features of that they encode structural or catalytic RNAs and in general, they are shorter than 400 base pairs. So, RNA polymerase III gives rise to RNA molecules, which are approximately 400 base pairs or shorter than that. And what are these kinds of RNA molecules which are made by RNA polymerase III? These include transfer RNAs, 5S ribosomal RNA, U6 spliceosomal RNA, RNase P and RNase MRP RNA. We will discuss each one of them in detail as we go along in this lecture series.

So, I am just making the list here. We will discuss each one of them. I will discuss each one of them at the end of the lecture, but in today's topic, primarily, we are going to

discuss about synthesis of 3 RNA species by RNA polymerase III namely, regulation of transfer RNA synthesis, mechanism and synthesis of 5S ribosomal RNA and U6 spliceosomal RNA.

How are these three major RNA species synthesized by RNA polymerase III? What is their promoter organization? What kind of transacting factors are involved or which coordinate RNA polymerase III leading to synthesis of these three image types? The others, I will just only make a passing note on that because it is not possible to cover everything.

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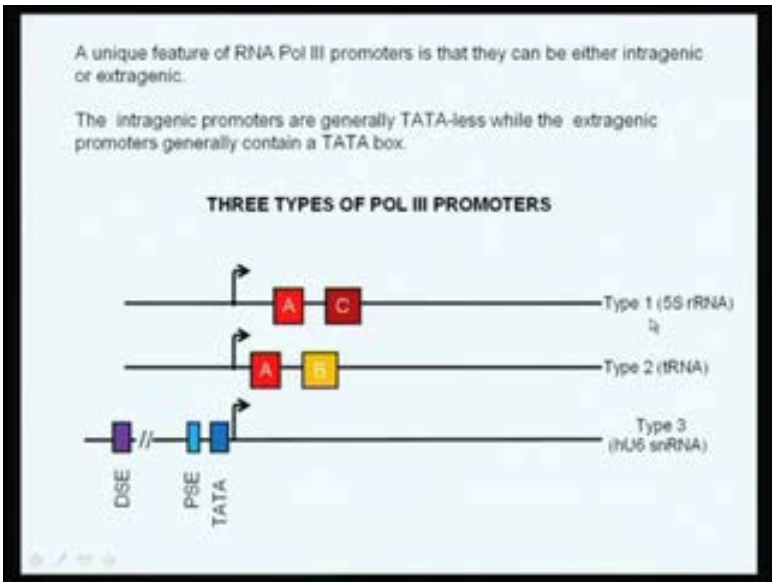

So, let us now try to understand how RNA polymerase III synthesizes transfer RNA, 5S ribosomal RNA and the U6 spliceosomal RNA. One of the very unique features about RNA polymerase III, which we have not found in case of polymerase II or polymerase I promoters is that the RNA polymerase III promoters can be either extragenic or intragenic, whereas so far, all our discussion in the last thirteen lectures which centered around RNA pol II transcription, pol I transcription, the promoter always lies towards the 5 prime end from the transcription start site.

So, they are almost referred to as the extragenic promoters. They do not The promoter lies 5 prime to the transcription start site and not within the coding region. It is only in the case of pol III transcription, some of these pol III transcription promoters or pol III transcription promoters, the promoter can be intragenic, that means, within the

transcribed region; it can also be extragenic. So, this is one of the fundamental differences between pol III transcription or promoters of pol III compared to pol I and pol II promoters.

The intragenic promoters are generally TATA-less, that means, they do not containing TATA box, while the extragenic promoters generally contain a TATA box. So, it is another important difference between the extragenic and intragenic promoters. So, let us just take bird's eye view of what are these three different pol III promoters? What are the major elements and then we will take each one of them and discuss in greater detail.

So, basically, depending upon whether the promoters are intragenic or extragenic, the pol III promoters are of three major types: they are called type 1, type 2 and type 3. The best examples for type 1 promoters are the promoters of genes encoding 5S ribosomal RNA. Remember when we discussed about pol I transcription regulation in the last class, we read we had clearly discussed that RNA component of the ribosomes consists of 28S, 18S, 5.8S and 5S and of which, the 5S rRNA is actually synthesized by pol III. So, that is what I am going to discuss in detail now. The other three are synthesized by RNA polymerase I.

And we have discussed very clearly, to get a functional ribosome, you require the coordinate function of all the 3 RNA polymerases namely, pol I which makes 28S, 18S and 5.8S RNA and 5S rRNA, which is made by pol III and all the ribosomal proteins are made by messenger RNAs, which are transcribed by the RNA polymerase II. So, to get a functional ribosome, all 3 RNA polymerase have to work together. Today, let us now try to understand how the 5S rRNA gene is transcribed by RNA polymerase III. How is the promoter organized?

So, it turns out, this 5S ribosomal RNA promoter consists of, it is an internal promoter like just previously, I had discussed that pol III promoters can be either extragenic or intragenic or it can be external or internal with reference to transcription start site, the type 1 promoters, which is an example we are going to discuss, the 5S ribosomal RNA promoter is actually intragenic promoter, where the promoter lies after the transcription start site and there are 2 key promoter elements, which are very, very important for this transcription of 5S rRNA gene; they are called as box A and box C.

In the same way, when you come to type 2, which all majority of the transfer RNA genes, majority of the genes encoding various types of transfer RNA fall under the category of again type 2 promoters. Again, this is an intragenic promoter because the two major promoter elements namely, the box A and box B is within the transcribed region and lies 3 prime of the transcription start site.

The type 3, we are going to discuss as an example, the human U6 snRNA. Remember the U6 snRNA are components of splicing machinery. The splicing machinery contain consists of number of fiber nuclear protein particles, which are RNA plus protein components and many of this RNA components are encoded by the pol III RNA polymerase and the U6 snRNA is one such promoter, we are going to discuss here and this comes under type 3 because here the promoter is extragenic because it is not within the transcribed region compared to other two; it lies in the untranscribed region over the 5 prime end or 5 prime to the transcription start site.

And there are three major promoter elements in the case of this U6 snRNA or type 3 promoters, which consists of TATA box, which consists of then a near upstream element called proximal sequential element or PSE and then further away you have what is called is DSE or a distal sequence element. So, remember, in the pol III transcription, the pol III promoters are of 3 distinct types type 1, type 2 and type 3; type 1 and type 2, the examples are 5S rRNA transfer RNA promoters; they are intragenic, whereas the type 3 promoters as exemplified by the human U6 snRNA is extragenic, since the promoter lies outside the transcribed region.

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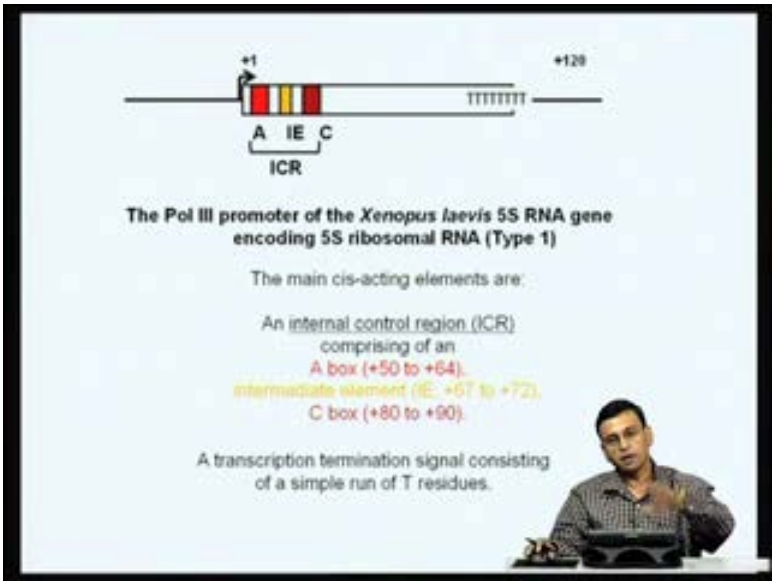

Let us now discuss little bit in greater detail, how is the pol III promoter of 5S rRNA gene organized. What are the key promoter elements? This belongs to type 1 and it is an intragenic promoter. It turns out the intragenic promoter in the case of 5S rRNA gene consists of three major elements, which together constitute what is called as a internal control region, often referred to as ICR.

Now, the internal control region comprises of three distinct elements. It consists of what is called as A box, which I have shown in red colour here, which spans from mark plus 50 to plus 64. So, the transcription start site is plus 1; so, the promoter lies after the transcript start site and that is why we call it as intragenic. So, the A box spans from plus 50 to plus 64. this Immediately after that, you have what is called as say the intermediate element, which consists spans from plus 67 to plus 72 with respect to transcription start site and then there is what is known as EC box, which spans from plus 80 to plus 90.

And it turns out in the case of all the 3 RNA pol promoters namely, the type 1, type 2 or type 3, whether it is extragenic or intragenic, the termination mechanism is more or less similar and it is attributed by a stretch of T's. We will discuss in more detail about transcription termination by RNA polymerase III later in this talk, but remember, a simple stretch of Ts, even 10 to 20 residues of T is good enough to terminate RNA pol III transcription.

And this transcription internal signal is common, whether it is type 1 or type 2 or type 3 promoters. So, transcription segment termination signal in the case of pol III promoters primarily consists of a stretch of T residues, ranging from 10 to 20 residues. So, this is basically how a pol III promoter of Xenopus laevis 5S rRNA gene is organized. Remember, there are three major elements box A, box C and in between is what is called as internal control a intermediate element; together they constitute what is called as a internal control region. So, these are the major cis-acting elements of a 5S rRNA promoter transcribed by RNA polymerase III.

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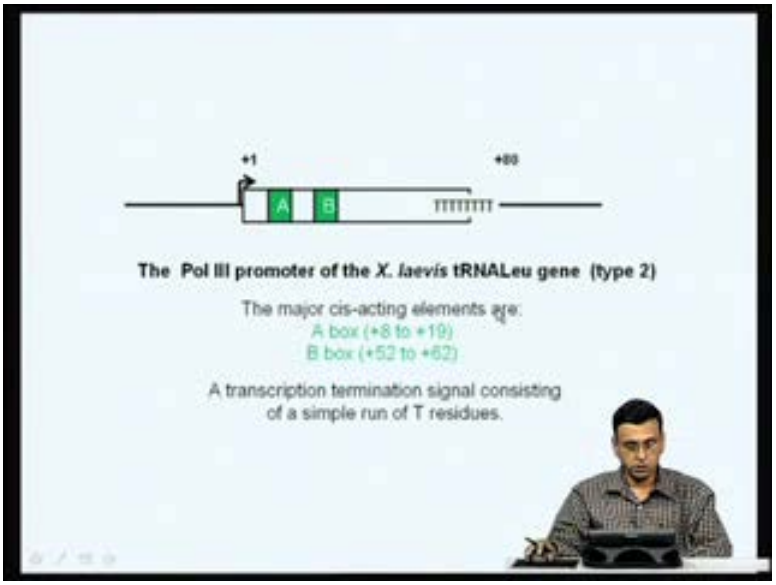

Now, let us look at the tRNA promoters. Here, they have taken an example of Xenopus laevis tRNA coding for leusin - the tRNA leusin gene, which belongs to the type 2 category. Unlike the 5S rRNA sub promoters, where we have discussed the major promoter elements of box A and box B box C, here the key promoter elements are known as box A and box B. Again, they are internal because they box A spans from plus 8 to plus 19, whereas box B spans from plus 52 to plus 62.

So, they come after the transcription start site. Therefore, they are known as intrinsic and also observe, in the case of the type 1 promoter of the 5S RNA promoter, even here the transcription termination is dictated by a series of T residues, which act as a transcription termination signal, even in the case of the type 2 promoters.

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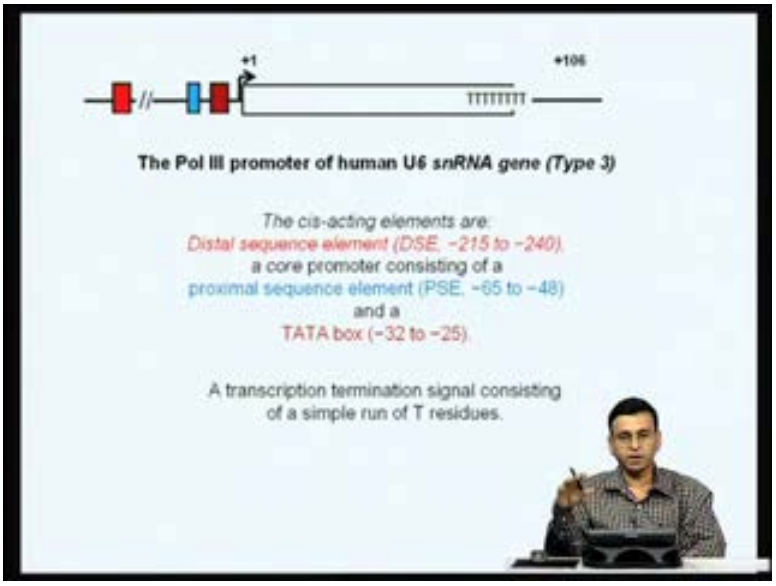

When you come to the pol III promoter of human U6 snRNA gene, remember, it is a type 3 promoter and it is an extragenic promoter, where all the cis-acting elements are present towards the 5 prime end before the transcription start site, whereas in the other two promoters, which we discussed so far, the promoters are internal.

So, the major cis-acting elements are what is called as a distal sequence elements, which usually lies about 200 base pairs away from the transcription start site and is often known as the DSE and it also consists of a core promoter. There are two major promoter elements in the core promoter element namely, the proximal sequence element or PSE which spans between minus 65 to minus 45; I have shown in blue colour here and a TATA box, which is minus 32 to minus 25.

So, the two intragenic promoters, 5S rRNA gene promoter and then tRNA gene promoter do not contain any TATA element and the promoter are internal, whereas in the case of human U6 snRNA gene, the promoter is extragenic and it contains a TATA element and two other promoter elements referred to as PSE and DSE and as seen in the case of the intragenic promoters, even here, a simple stretch of T residues towards the 3 prime end of the gene act as a transcription termination signal. So, irrespective of the promoters are intragenic or extragenic, the transcription termination mechanism is more or less same.

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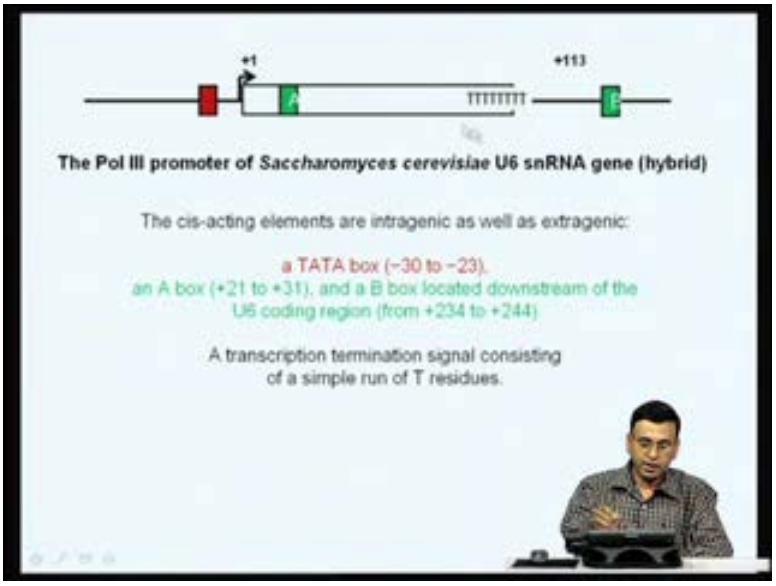

So, although I have described these three basic types of pol III promoters, there are always variations, there are always exceptions to this rule. For example, in the case of saccharomyces cerevisiae the U6 snRNA gene, promoter consists of cis-acting elements which are both extragenic as well as intragenic. So, although basically, there are 3 types of pol III promoters, there are variations, there are exceptions to this rule.

And one such exception is what I have shown here, where the pol III promoter of saccharomyces cerevisiae U6 snRNA gene is a kind of a hybrid promoter because it contains a TATA box, so, which is extragenic, 5 prime to the transcription site and it also contains a box A within the transcribed region and it contains a box B at the 3 prime flanking region of the U6 snRNA gene. So, the TATA box is located from minus 30 to minus 23, whereas the A box is located from plus 21 to plus 31 within the coding region, whereas the B box is located for downstream of the U6 coding region from plus 234 to plus 244. So, there are variations from the three basic types such as the saccharomyces cerevisiae U6, where it can be both extragenic as well as intragenic. Again, the transcription termination is brought about by a stretch of T residues, as seen in the case of the other pol III promoters.

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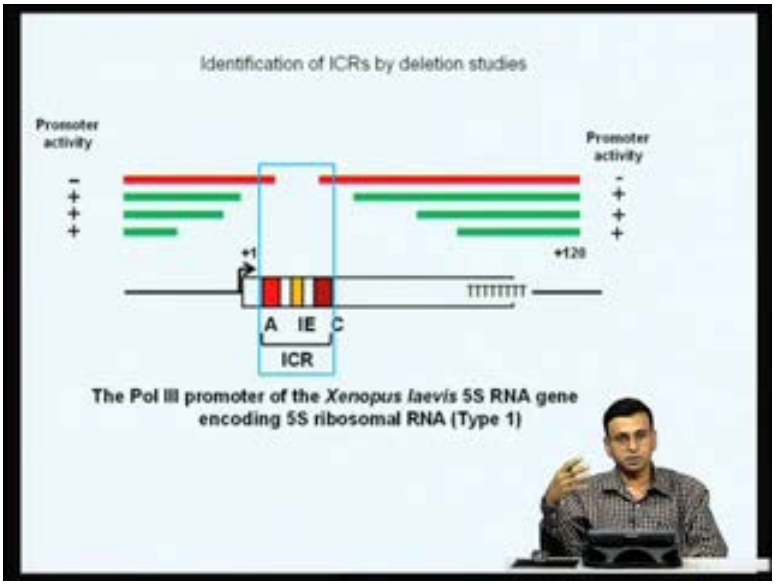

Now, how do people find out that the promoter in the case of the pol III transcription unit is internal? As we have studied in the case of pol II promoters, primarily studies involved in mutations, (( )) heamatogenesis and deletions have helped people to demark what are the major cis-acting elements involved in the pol III promoters.

For example, I have just shown a cartoon here to illustrating one experiment which actually demonstrated that the promoter in the case of the 5S rRNA gene is actually internal. So, basically, the strategy is similar to what you have studied in the case of the pol III transcriptions, where when people started making deletions towards the 5 prime end of the transcription start site, people found out when you delete the 5 prime regions before the transcription start site, it had no effect on the promoter activity.

The promoter activity was not affected when you make deletions 5 prime to the start site clearly indicating that the promote there is no cis-acting elements or there is no major promoter elements 5 prime to the transcription site, but the moment they made a deletion which spans after the transcription start site, there are some dramatic decrease in the promoter activity clearly indicating that the promoter region actually is after the transcription start site and these kinds of study gave a hint that yes, in the case of tRNA gene promoter, unlike what people have seen in the case of pol II and pol I, here the promoter may be actually internal.

Similarly, people have start making deletion from pre prime side and again, there was no effect as long as you delete different regions of the pre prime region, but the moment you start making deletions spanning the internal control region, there was a dramatic decrease in the promoter activity clearly indicating that these kinds of studies, when by doing deletion studies from both sides, people have been able to identify an internal control region, which spans after the transcription start site. This is how internal control regions of the internal promoters of pol II transcription genes were identified.

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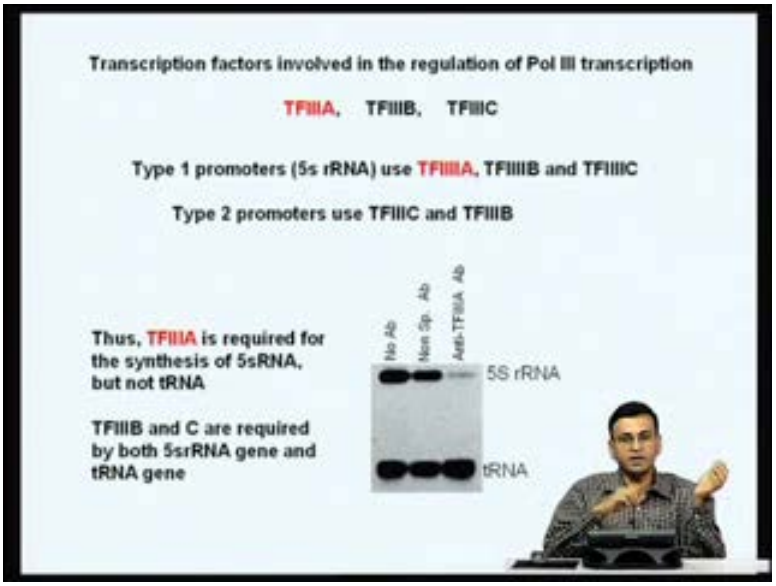

Now, so far, we have discussed about the major cis-acting elements which control RNA polymerase III transcription. As we discussed, we have type 1 promoter, we have type 2 and type 3 promoters and a typical example for type 1 promoter is the 5S ribosomal RNA gene, which contains box A and box B and we have the type 2 promoters, most of the tRNA genes fall under these category; they are the box A and box C and then we discussed about the extragenic promoters, in the case, of human U6 RNA, where the promoter is external or extragenic, which contains a TATA box, a proximal sequence element and a distal sequence element and there are variations as seen in the case of the saccharomyces U6 RNA gene.

Let us spend some time to understand what kind of transacting factors go and interact with the cis-acting elements. It turns out there are three major transacting factors, which govern the regulation of transcription by RNA polymerase III. These are known as

TFIIIA, TFIIIB and TFIIIC. Remember, whenever we talked about pol II transcription the designation has been TFII; all the factors are written as TFIIA, TFIIB, TFIIC and so on and so forth, whereas when it came to pol I, they are designated as TFI, whereas when it comes to pol III, you use the terminology TFIII. So, TFIII means the factor is associated the pol III transcription; TFII means the factor is associated with the pol II transcription; TFI means, this transcription factor is associated with pol I transcription. It turns out that type 1 promoters as discussed in the case of the 5S ribosomal RNA, it requires all these transcription factors namely, TFIIIA TFIIIB and TFIII in addition to RNA polymerase III, whereas the type 2 promoters which primarily consists of a majority of tRNA gene promoters, they require only TFIIIC and TFIIIB; they do not require TFIIIA. So, the TFIIIA is very specific for the transcription of 5S ribosomal RNA gene and is not required for the transcription of the transfer RNA genes.

Now, what kind of experiments, people have done to actually demonstrate that TFIIIA is required only for the 5S ribosomal RNA promoter, but not for the transcription of tRNA genes? As we have seen, in the case of pol II transcription, people have again made use of self-transcription studies. In fact, the pol III transcription studies and pol III cis-acting elements and transacting factors were identified much before the pol II transcription studies were initiated.

In fact, the TFIIIA was the first, eukaryotic transcription factor purified to homogeneity and studied very extensively. In fact, it was one of the first zinc finger proteins, which were identified. So, let us see for example, I have shown you an experimental simple transcription study, where you put all the transcription factors namely, RNA polymerase III nucleotides and you put a 5S RNA promoter as well as tRNA promoter link to the downstream gene and then see what kind of factors are required for the transcription.

And it turns out when you do not have TFIIIA, How do you deplete the extracts of TFIIIA? You simply add anti bodies raised against TFIIIA and these anti bodies raised against TFIIIA will prevent the TFIIIA from interacting with cis-acting element and therefore, makes unavailable for the cell-free transcription. So, when you make the TFIII in a cell-free extract nonfunctional by the addition of an anti-body, you can see the 5S RNA gene transcription is totally gone.

But in the extracts depleted of TFIIIA, tRNA transcription still proceeds. These two are controls where you have not had any anti body, you have added a non-specific anti body, but here you added a specific antibody raised for the which are specific for TFIIIA. So, when TFIIIA function is inactivated, only 5S ribosomal gene transcription gets affected, but not transfer RNA clearly indicating that the TFIIIA is a highly specific transcription factor required specifically for the transcription of 5S ribosomal RNA genes. So, these kinds of experiments clearly demonstrated which of these three transcription factors are required for 5S rRNA gene transcription as well as tRNA gene transcription.

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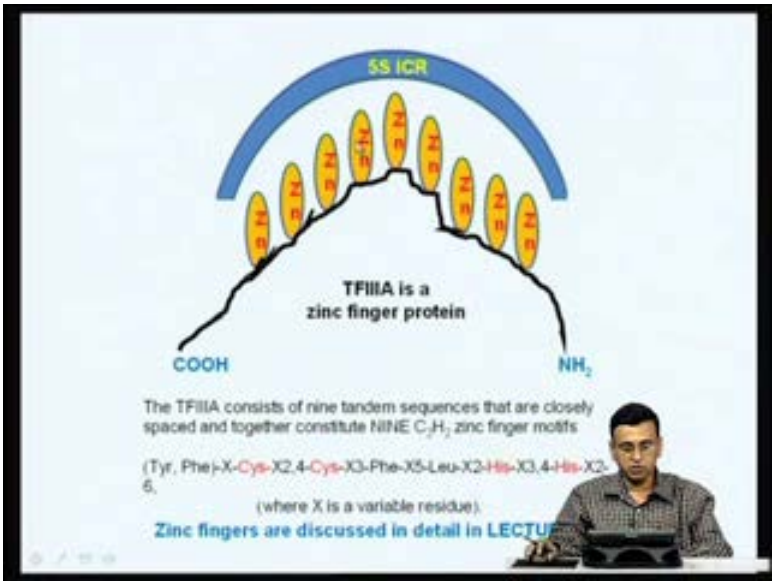

I have just described a cartoon which actually depicts the structure of the TFIIIA zinc finger protein. The TFIIIA consists of 9 zinc fingers and again I will not go in greater very detailed about zinc finger proteins because we have studied extensively in the RNA polymerase II lectures. In fact, if you want to know more about the zinc fingers, you must go to the lecture number five, where we have discussed in detail about the C2H2 kind of zinc fingers and other kind of zinc fingers in great detail and giving examples of pol II transcription factors.

So, the TFIIIA consists of 9 zinc fingers, whereas zinc atom is coordinate between 2 cysteines and 2 histidines and so, it is a C2H2 kind of a zinc finger module and consists of 9 tandem sequences that are very closely spaced, and together constitutes the 9 zinc finger motifs which go and interact with the 5S internal control region of the 5S rRNA

promoters and the kind of the consists amino acids sequence is shown here and as I said, more discussion about zinc fingers, we need to go back to lecture five and then come back here and then try to understand how the zinc atom is coordinated and what are the major structures of the major features of the zinc finger proteins.

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As I mentioned, there is extensive literature on the TFIIIA. In fact, TFIIIA is one of the first eukaryotic transcription factors which was purified to homogeneity. and there are a number of I have listed here, a number of review articles in the early and late 1990s, which discuss about the discovery and various structural, functional aspects of TFIIIA. So, you have to refer to some of these articles, if you want to learnt more about TFIIIA and it is very, very interesting protein and how it binds internal control and allows RNA polymerase to go through, there are very, very interesting mechanics studies, which have been studied to understand how TFIIIA binds and how RNA polymerase III transcribes the tRNA, 5S rRNA genes. We will not going to the details now.

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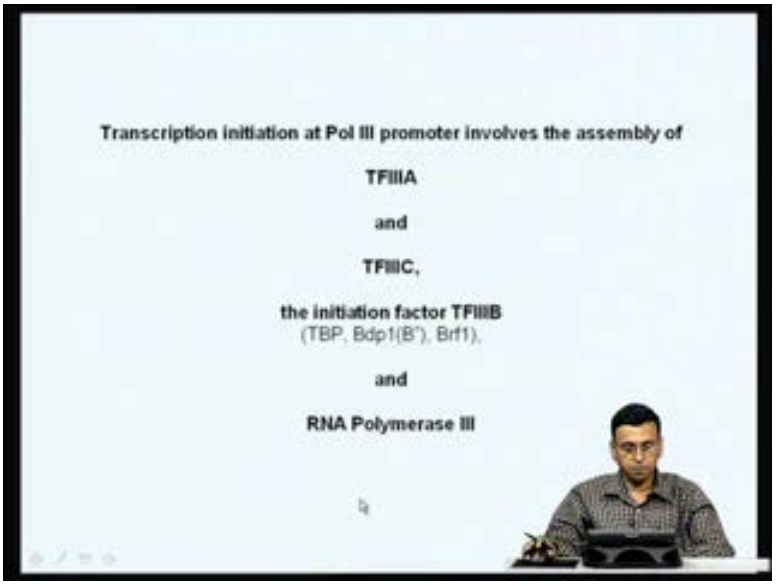

So, transcription initiation of the pol III promoter involves the assembly of TFIIIA and TFIIIC. and the TFIIIB As I said, TFIIIA is a zinc finger protein and in addition to TFIIIA and TFIIIC, it requires the initiation factor, TFIIIB. All these are multi-subunit proteins. So, remember they are not simple proteins. For sake of simplicity, to simply mention, they say single protein, but these are all multi-subunit proteins. For example, the TFIIIB consists of TATA binding protein Bdp1 as well as Brf1.

Again, we will not go into the details of subunit structure of all these transcription factors and their interaction so and so forth. Suffice to remember that three major transcription factors are required for the transcription of 5S ribosomal RNA gene namely, TFIIIA TFIIIB and TFIIIC, but in the case of tRNA gene transcription, you require only two factors namely, TFIIIB and TFIIIC. Of course, RNA polymerase is required in addition to all these transcription factors and assembly of all these 3 factors along with RNA polymerase completes the pre initiation formation in the case of pol III transcription.

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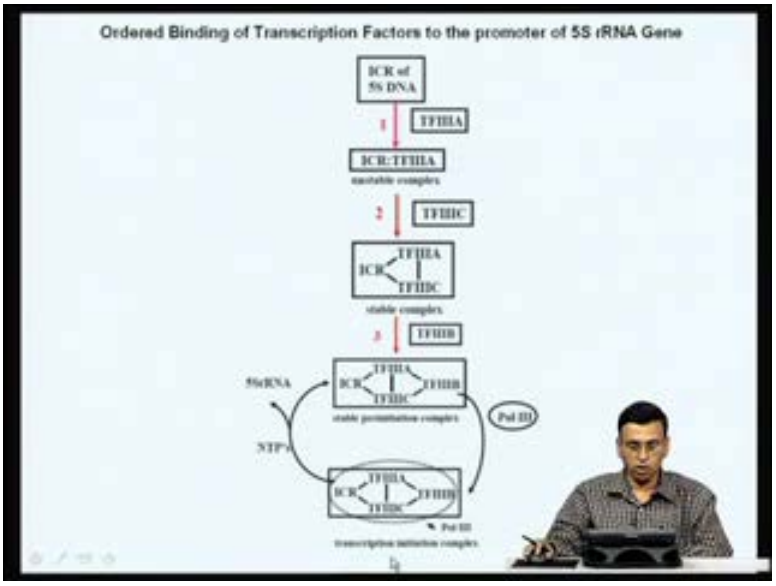

So, again as we seen in the case of the polymerase two transcription, where the TFIID and the TFIIA come and occupy first and then the RNA polymerase is brought in and other factors come and join and pre-initiation complex is formed, the same way, ordered sequential binding of transcription factors to the 5S RNA gene results in the formation of a pre initiation, in the case of pol III promoters. For example, the internal control division of the 5S rRNA gene is first bound to TFIIIA. So, TFIIIA say TFIIIA is the first transcription factor to go and bind the internal control region.

But this results in the formation of very unstable internal control region, TFIIIA complex. This DNA protein complex is stabilized by the joining of the TFIIIC. So, we get very stable DNA protein complex involving internal control region TFIIIA, TFIIIC and once the stable complex is formed, then the TFIIIB is recruited and you get a very stable pre-initiation complex and then the pol III is recruited and in presence of nucleotides, it makes the 5S ribosomal RNA gene and RNA transcription termination takes place and then the cycle continues. So, TFIIIA recruited first, TFIIIC is recruited next, followed by TFIIIB and RNA polymerase and all the all of them assemble together to synthesize the pol III transcription.

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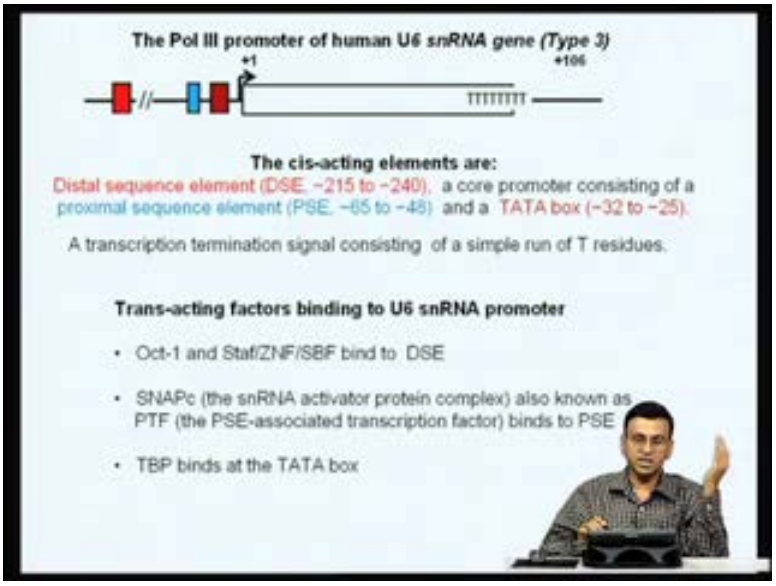

So far, we discussed about the transcription factors binding to the internal control intragenic promoters namely, the 5S rRNA, tRNA genes, where you have the TFIIIA in the case of the 5S ribosomal RNA gene and we have in addition to TFIIIA, also TFIIIC and TFIIIB in the case of the tRNA gene. So, let us very briefly discuss about the various transacting factors, which are involved in the regulation of the extragenic promoter namely, the human U6 snRNA gene, where we do not have either box A or box B or box C, but you actually have extragenic promoters in the form of a TATA box, a proximal sequence element and a distal sequence element.

So, what kinds of proteins factors go and bind to this? The transacting factors which binds to the U6 snRNA promoter in the case of humans, where the promoter is extragenic are primarily, an Oct 1 and a Staf, ZNF or SBF protein; these are variously named; they bind to this distal sequence element, whereas the protein called SNAPc or the snRNA activator protein complex, also known as the PTF or the proximal sequence element associated transcription factor binds to the proximal sequence element and of course, the TATA binding protein occupies the TATA box.

So, these are the major transacting factors, which interact to the cis-acting elements of the human U6 snRNA gene, where the promoter is extragenic, whereas in the case of the other two namely, the 5S rRNA gene, you have TFIIIA, TFIIIB and TFIIIC and in the

case of tRNA gene, it is TFIIIB and TFIIIC. So, these are the cis-acting elements and transacting factors involved in the regulation of pol III transcription.

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As I said, I have made a very, very simplistic view of pol III transcription. It is much more complicated than what I have discussed just now. The idea is not to confuse the viewers the learners, but give a very overall view of the mechanism by which the pol III transcription takes place. So, I have given a very bird's eye view of the major cis-acting elements, transacting factors involved in the pol III transcription.

You want to study more details about the pol III transcription, there is an excellent review, genes and development in 2002 by Schramm and Hernandez about the recruitment of RNA polymerase III target promoters, how all these transacting factors TFIIIA, TFIIIB and TFIIIC interact with each other to recruit the RNA polymerase together and then how the RNA polymerase actually transcribes and how the transcription termination takes place, so on and so forth. So, for more details, you should read review articles such as these. There are a number of other articles, but this one of the very, very interesting and very elaborative.

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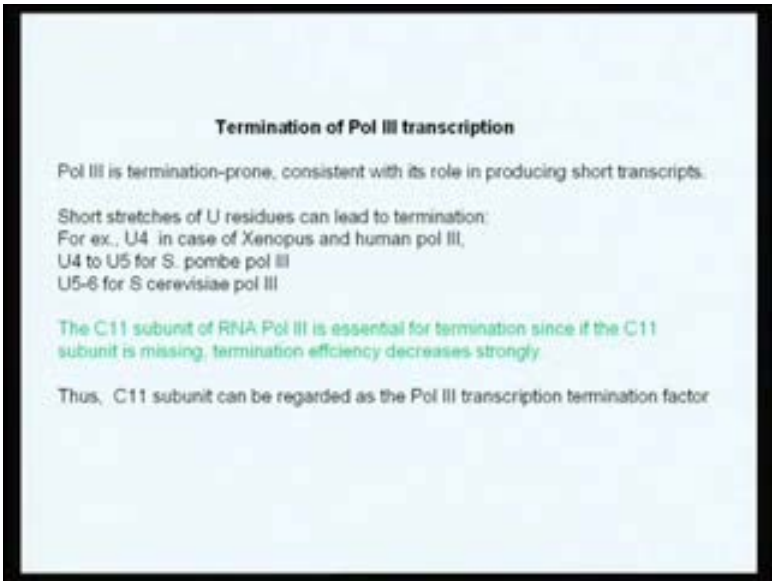

Now, as I said, termination of pol III transcription is very, very simple and it is actually determined by a short stretch of U residues, in the case of RNA or T stretches, which is corresponding to the T residues, in the case of the genes. So, a very short stretch of U residues can lead to transcription termination, in the case of the RNA polymerase III and this is one of the reasons, why polymerase III actually produces very short transcripts, less than 400 base pairs like tRNA, 5S RNA and so on and so forth.

For example, in the case of the U4 snRNA and the human pol III, the U4 to U5, just 4 residues of U is good enough to bring about transcription termination, in the case of the human and Xenopus polymerase III, whereas in the case of saccharomyces cerevisiae polymerase III, about 4 to 5 residues of Ts or Us, good enough to bring about transcription termination, whereas in the case of saccharomyces cerevisiae pol III, about 5 to 6 residues of T in the gene or U residues in the RNA is good enough to bring about transcriptions termination.

And it turns out of the various subunits of RNA polymerase III, which we described here, the C11 subunit of RNA polymerase III is actually essential for the termination, since if the C11 subunit is deleted or mutated, termination efficiency decreases strongly. So, of the various RNA polymerase II subunits, I think about 15 or so, the C11 subunit is actually responsible for transcription termination. So, the C11 subunit of pol III is actually viewed as the transcription termination factor. So, remember, transcription

termination in the case of pol III is very simple. It simply consists of a stretch of series of T residues, which can range anywhere from 4 to 6, depending upon the species, whether it is saccharomyces cerevisiae, human or a species.

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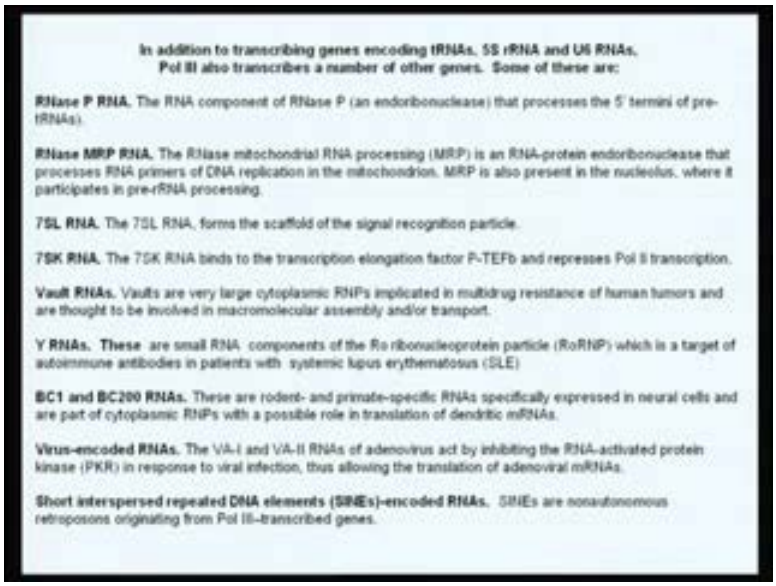

It looks very extensive, but the purpose is not to scare you, but just to tell you, in addition to the 5S ribosomal RNA tRNA and U6 RNA, the RNA polymerase III also (( )) number of other RNA (( )) number of other molecules, many a times you do not know. Whenever we ask you a question, usually students say pol III transcription primarily means transfer RNA genes, but it is not true. In addition to the transfer RNA genes, pol I RNA polymerase III also transcribes 5S ribosomal RNA, many of the RNAs involved in RNA splicing, let us say such as U6 and so on, so forth. It also makes a number RNAs which are (( )).

So, let us spend a few minutes to understand what are these various RNA molecules, what are their biological functions and why these RNAs are also being synthesized by RNA polymerase III. For example, in addition to the tRNAs and in addition to 5S ribosomal RNA and U6 snRNA, it also makes all these RNAs. The RNase P RNA is Actually, RNase P is endoribonuclease that processes the pre prime 5 prime terminal of the pre-tRNAs.

So, ribonucleases, which is ribonucleoprotein and RNase P is a ribonucleoprotein and RNA component of ribonucleoprotein, which is also required for the tRNA processing is

actually synthesized by RNA polymerase III. Similarly, there is an RNA called MRP RNA which actually refers for RNase involved in mitochondrial RNA processing, which is an RNA dependent endoribonuclease that processes RNA primers for DNA replication in mitochondria and is also present in nucleolus, where it is required in pre-rRNA processing. So, this MRP RNA is also made by RNA polymerase III.

And there is a very important RNA called 7SL RNA, which actually is a part of the signal recognition particle, which is very, very important for bringing the nascent poly peptide chains to the rough endoplasmic reticulum and channelizing proteins towards the secretory pathway. So, all the secretory proteins which contain a signal sequence, they are brought about and during translation, they are translated on rough endoplasmic reticulum and a key protein called as a signal recognition particle plays a very crucial role in this process and a signal recognition particle is a ribonucleoprotein and RNA component of signal recognition particle is actually made by RNA polymerase III.

Similarly, you have a 7SK RNA, which actually binds to transcription elongation factor P- TEFb and is involved in preparation of pol II transcription and this regulative RNA is also made by RNA polymerase III. Similarly, there are what are called as vault RNAs, which are large cytoplasmic ribonucleoproteins implicated in multidrug resistance of human tumors and are thought to be involved macro molecular assembly and transport.

So, the RNA components of these ribonucleoproteins are also made by RNA polymerase III and there are what are called as Y RNAs, which are very small RNAs of the ribonucleoprotein, RoRNP, which is the target of autoimmune antibodies in patients with the auto human disorder called systemic lupus erythematosus. So, you can see pol III is not just involved in transcription of tRNA and 5S RNA, it also seems to be involved in number of other important, less known RNAs, but they are very, very important.

Similarly, in the case of nervous systems, we have what are called as BC1 and BC200 RNAs, which are RNAs very specifically expressed in neural cells and they form very important components of cytoplasmic ribonucleoproteins and they play very important role in the translation of dendritic messenger RNAs, in the case of nervous system – so, neural cells. Similarly, there are what are called as virus encoded RNAs, VA-I and VA-II, which act by inhibiting the RNA activated protein kinase in response to viral infection, thus allowing the translation of adenoviral mRNAs.

And there are what are called as SINEs - short interspersed repeated elements encode RNAs. These are nonautonomous retroposons originating from pol III transcribed genes. I have made these particulars just to tell you that tomorrow if somebody asks you what is the functional of RNA polymerase III in eukaryotic cells, do not say that it simply makes transfer RNA; you have to say it makes transfer RNA, primary transfer 5S ribosomal RNA and U6 RNAs splicing, but it is also makes a number of other less known RNAs some of which have listed here. So, pol III has very, very important functions in the cells.

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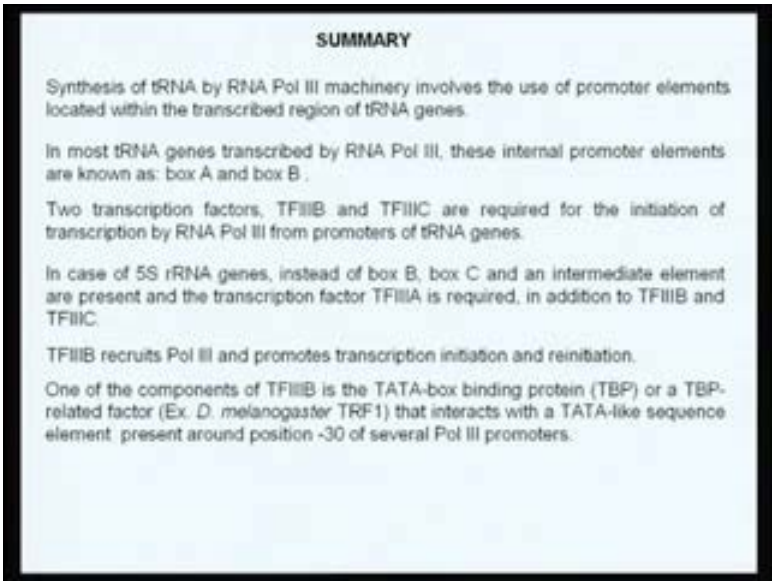

So just to recapitulate your (( )) what we have discussed so far, I have just summarized here. Synthesis of transfer RNA by RNA polymerase III machinery involves the use of promoter elements located within the transcribed region of tRNA genes. So, the tRNA gene promoter, the promoters are internal. In most tRNA genes transcribed by RNA polymerase through polymerase III, these internal promoter elements are known as: box A and box B. So, all tRNA gene promoters are internal; they contain two important elements called box A and box B. The transcription factor TFIIIB and TFIIIC are required for the initiation of transcription by pol III promoters of tRNA genes.

So, if somebody asks you to describe a tRNA gene promoter and the transacting factors, it is very simple. The tRNA gene promoters are internal. They consist of two major cis-acting elements box A and box B and two transcription factors called TFIIIB and TFIIIC

together with RNA polymerase III are involved in transcription initiation in the case of the tRNA gene promoters, whereas if you come to 5S rRNA, it is slightly more complicated than tRNA gene promoters where the cis-acting elements, instead of box A and B are designated as box B and box C and in addition to TFIIIB and TFIIIC, they also require TFIIIA. So, three major transacting factors are required for transcription of 5S rRNA transcription promoters, whereas only two transacting factors are required for the transcription of tRNA gene promoters; this is the major distinction between the two.

The TFIIIB basically recruits the RNA polymerase III and promotes transcription initiation as well as re-initiation and one of the components of TFIIIB is the TATA binding protein and also in the case of Drosophila Melanogaster, there is a protein called TBP related factor or TRF1 and these actually interact with the TATA-like sequence which is usually present around minus 30 regions from the transcription start site in many of the pol III promoters. So, when you have an extragenic promoter, you require a TATA binding protein, which binds to a TATA sequence.

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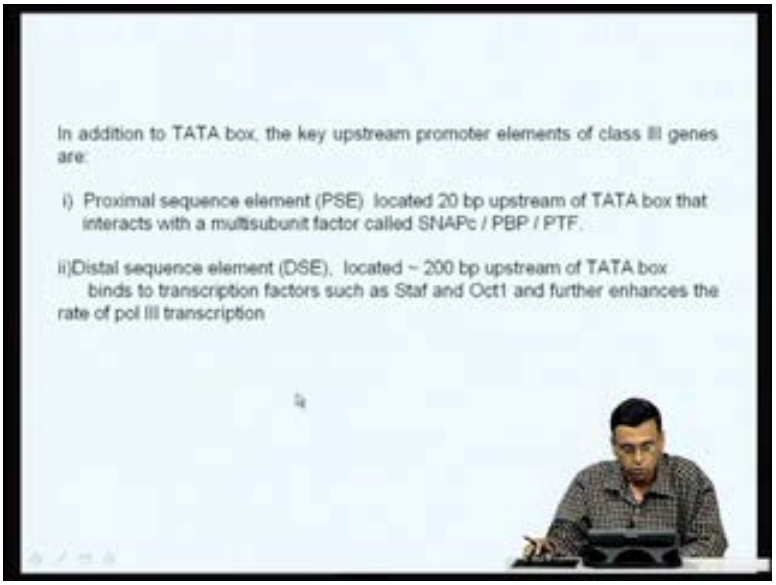

So, in addition to the TATA box, that is, in the case of extragenic promoters in addition of TATA box, you require two major elements namely, the proximal sequence elements located about 20 base pairs upstream of TATA box and a protein called SNAPc also known as PBP or PTF binds through this proximal sequence element, and another element is the DSE or the distal sequence element located about 20 base pairs upstream

of TATA box and it binds to transcription factors such as Staf and Oct1 and further enhances the rate of polymerase transcription.

So, I have type 1 promoters; they are represented by tRNA, which requires box A, box B and it requires two transcription factors TFIIIB and TFIIIC. We have 5S rRNA promoters, we have box A and box C, which are occupied by TFIIIB, TFIIIC and also, a unique transcription called TFIIIA, which is zinc finger protein, whereas when you come to extragenic promoters, there are three major cis-acting elements, the TATA box, proximal sequence element and distal sequence elements and these are occupied by distinct transcription factors. This is in a nutshell, the organization of polymerase III promoters and how RNA polymerase III transcribes pol III promoters tRNA, 5S RNA and U6 RNA, various other promoters using specific transcription factors.

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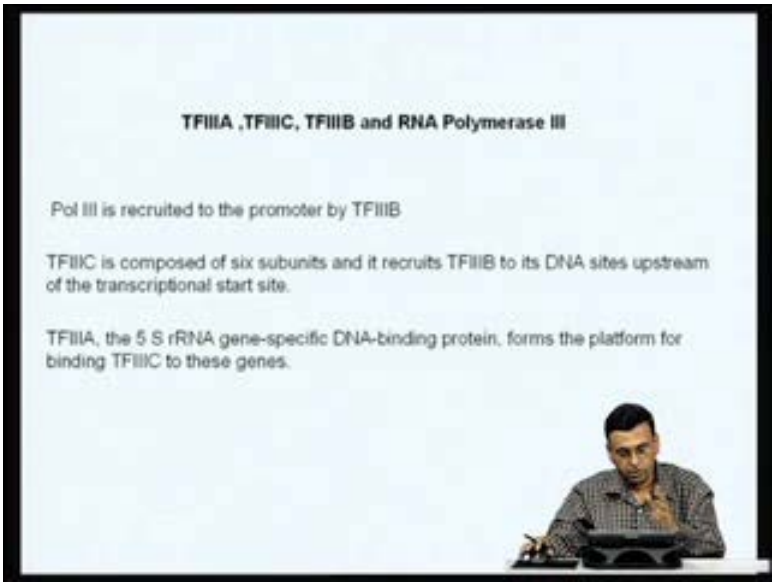

So, the polymerase III is recruited to the promoter by TFIIIB, and TFIIIC is composed of 6 subunits and it recruits TFIIIB to the DNA sites upstream of the transcription start site. So, as I said, each one of them is a multi-subunit complex; do not think it is as simple as what I mentioned here. It is much more complicated. TFIIIB, TFIII each one of them is a multi-subunit complex and each subunit has a very, very important regulatory role and TFIIIA, which is the 5S rRNA gene specific DNA binding protein, it forms the platform for binding of TFIIIC to these genes.

So, if somebody asks you, what is the function of TFIIIA, you have to mention TFIIIA is a very important transcription factor required for the transcription of 5S ribosomal RNA genes. So, that is the summary of what we have discussed so far.

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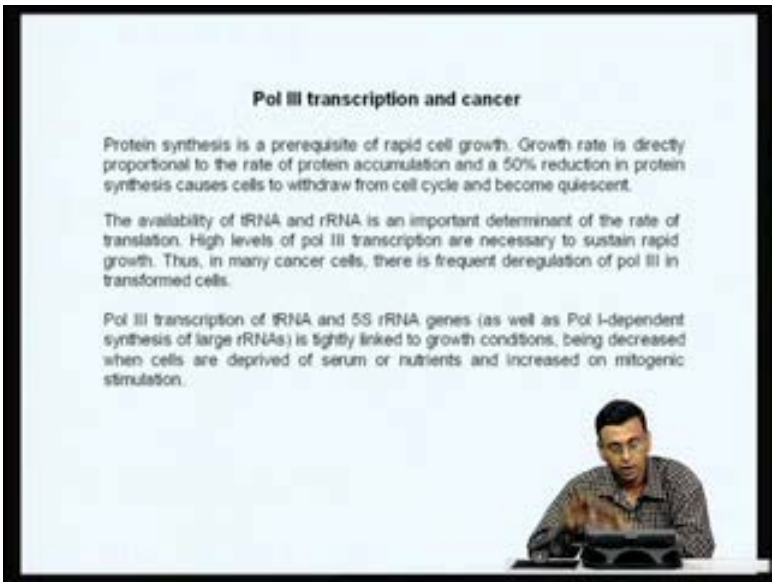

Now, we will spend about maybe, 10 minutes or so now, to discuss what is the physiological significance of pol III transcription. As I said, in general people tend to concentrate primarily on RNA polymerase II transcription. How protein coding genes are transcribed? How messenger RNA is synthesized? Most of the times the focus is primarily on the TFIID, TFIIB, RNA polymerase II, CTD, splicing, capping, polyadnelation so on, so forth; very rarely, people describe on pol I and pol III transcription.

So, the last class, when I discussed pol I transcription, I have mentioned very clearly why it is important to understand the rDNA transcription and how rDNA transcription plays a very important role in cell growth and in many of the cancers, the pol III transcription is enhanced and you do require both pol I transcription, pol III transcription because cell has to grow and divide; proteins have to be made and if proteins have to be made, you require ribosomes and you require transfer RNA and therefore, these two transcriptions are also very, very important from the point of cell physiology and function.

It turns out, just as we have seen in the case of the RNA polymerase I transcription, where it plays a very, very important role and in many cancers, the pol I transcription is

either enhanced. Many of the pol I transcription factors are over expressed. The same way the pol III transcription also plays a very, very important role in cancer. Let us now try to understand how it is.

We all know protein synthesis is a prerequisite for rapid cell growth. So, if cells are to grow and divide, proteins have to be constantly made. The growth rate is directly proportional to the rate of protein accumulation and it turns out, if the protein synthesis is reduced by 50 percent, the cells withdraw from the cell cycle and go and go into quiescent state. So, the very if the cells have to continuously divide, protein synthesis has take place continuously and (( )) take a place continuously, since ribosomes have to made, tRNAs have to made, pol I transcription and pol III transcription have to be regulated continuously.

And if protein synthesis falls 50 percent of the normal, the cells will exit the cycle and go into the quiescent state. This is the basic principle. Now, the availability of transfer RNA and ribosomal RNA therefore, is a very important determinant for the rate of translation. So, if protein synthesis is very, very important for the cells to grow and divide, the protein synthesis is controlled by pol I, pol III transcription.

High levels of pol III transcription are therefore, necessary to sustain rapid growth. Thus in many cancer cells, there is a frequent deregulation of pol III in transformed cells. So, if the cancer cells have to divide rapidly, protein synthesis has to take place in a very high amount. Therefore, more ribosomes are required, more transfer RNA is required and therefore, in many of these cancer cells, the pol III transcription is up regulated.

So, pol III transcription of transfer RNA and 5S ribosomal RNA genes as well as pol I dependent synthesis of large ribosomal RNAs, this aspect we discussed in the last class, is very tightly linked to growth conditions. It decreases when cells are deprived of serum or nutrients and it increases, when cells are simulated with mitogens. So, when there is a mitogenic simulation, when cells have to divide, when cells are mitotically active, both pol I and pol III transcriptions go up; when the cells have to differentiate or cells are going to quiescent state, the pol III and pol I transcription goes down. So, pol I and pol III transcription is very beautifully regulated, depending upon the need of the cells, whether to grow or divide or remain quiescent.

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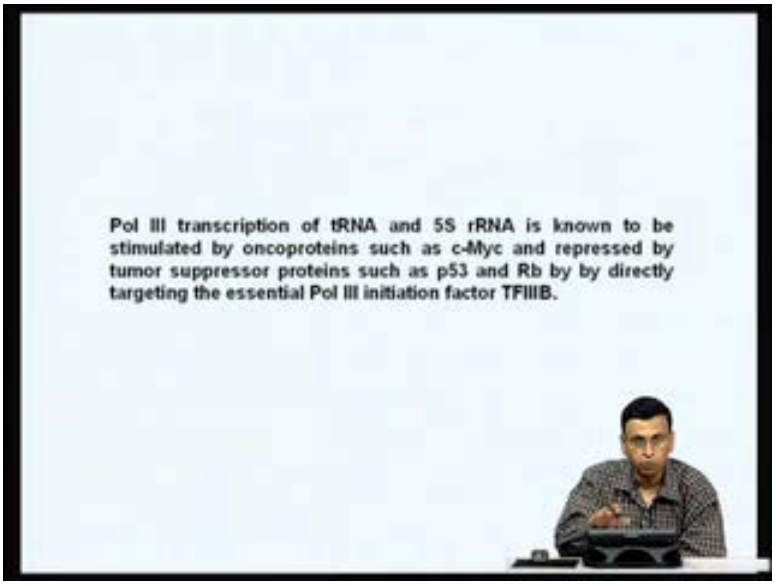

So, the pol III transcription of transfer RNA and 5S ribosomal RNA is known to be simulated by oncoproteins such as c-Myc and repressed by tumor suppressor protein such as p53 and Rb by directly targeting the essential pol III transcription factor TFIIIB. So, because if the cancer cell have to become tumorigenic, if the cancer cells have to keep on dividing, they want more and more transfer RNA to be made, they want more and more ribosomal pro ribosomal proteins or ribosomal RNAs to be made and therefore, the first thing that happens in cancer cells is to up regulate the transfer RNA and ribosomal RNA synthesis and therefore, the oncoproteins has a c-Myc as well as tumour suppress proteins such as p53 and Rb, play a very important role interacting with transcription factors, which are involved in pol III transcription, especially the TFIIIB. Let us spend some time to understand, how exactly these TFIIIB function is regulated by oncoproteins and tumor suppressor proteins involved in cancer.

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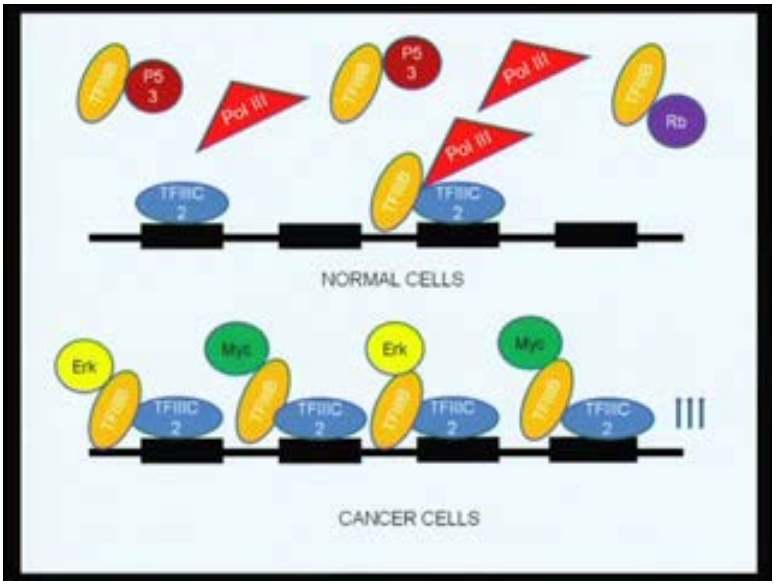

For example, as we all know, there are tRNA genes organized in tandem units; there are several copies of tRNA genes arranged in a head to tail fashion in the genome and normally, in normal cells, the amount of transacting factors such of polymerase III or TFIIIB or TFIIIC is limiting and therefore, not all tRNA gene promoters are occupied by these transacting factors and therefore, the level of transcription of the level of synthesis of tRNA is not maximum because all the transacting factors do not occupy all the tRNA promoters; only a certain level of tRNA as if transcription takes place and this signifies the normal transcription, normal situation.

So, previous session complex are actually assembled depending upon the availability of all the transcription factors and it turns out proteins like p53, which actually are very important molecules regulating cell cycle and very important mutations in p53 can lead to cancer; it turns out p52 goes and interacts with TFIIIB and makes it unavailable for formation of initiation complex.

So, in normal cells, tRNA synthesis is regulated because TFIIIB molecules interact with the p53 and as a result, TFIIIB is not available for the formation of the pre-initiation complex and TFIIIC transcription itself is rather regulated and therefore, TFIII is also not present in very high amount and therefore, you only get a few molecules of transfer RNA made just sufficient for the normal protein synthesis to take place.

Now, let us see what happens in cancer cells. In addition to p53, the other tumor suppression in Rb is also known to interact with TFIIIB. So, remember, in normal cells, the tRNA synthesis is regulated primarily by making TFIIIB unavailable for the formation of pre-initiation complex in case of pol III promoters because Rb and p53 sequest of the TFIIIB protein and depending upon the since these proteins are present in very levels are very finely regulated, depending upon the level of p53 and Rb, the tRNA level gene transcription is modulated.

Now, let us see what happens in the case of cancer cell. So, in the cancer cells, the first thing that happens is the one of the components of TFIIIC is called TFIIIC 2. As I said, I have not discussed in detail about the subunit structure of each one of these factors involved in pol III transcription. TFIIIC is a very complicated transcription factor and consist of a number of subunits and one of the TFIIIC subunits is called TFIIIC 2 and it turns out the TFIIIC subunit itself, which is DNA binding component itself, the levels itself is up regulated in many of the cancer cells. So, compared to normal cells, in cancer cells, there are very high levels of TFIIIC and therefore, the promoter the number of tRNA gene promoters which are occupied by TFIIIC is much higher compared to normal cells.

Finally, and Also, in many of the cancer cells, the cancer is because of mutations in tumor suppressor genes like p53 or Rb and when you have mutations of p53 or Rb, they cannot interact with TFIII B and therefore, they are not able to sequest that TFIIIB and make them unavailable. Therefore, now TFIIIB is freely available for go and binding to the promoter regions of tRNA gene and initiate transcription and in turn, the TFIIIB now associates with oncoproteins such as Erk, c-Myc and so on so forth, which are growth promoters.

So, you can see in the normal cells, when the growth has to be regulated tumor suppressor proteins like P53, Rb interact with TFIIIB and make them unavailable for tRNA gene transcription and therefore, tRNA gene transcription proceeds at a very slow pace. Whereas when cells become cancerous because of mutation p53 or Rb, these mutant proteins cannot interact with TFIIIB. On the contrary, the TFIIIB comes and interacts with the oncoproteins, which are growth promoting agents like Erk and p53; as a result of interaction, the tRNA gene transcription proceeds much faster and therefore, very high levels of tRNA are synthesized. So, you can see how cells have devised

strategies for increasing the rate of tRNA gene synthesis by using either oncoproteins or tumor suppressor proteins and this is how a normal cell will become cancer cell.

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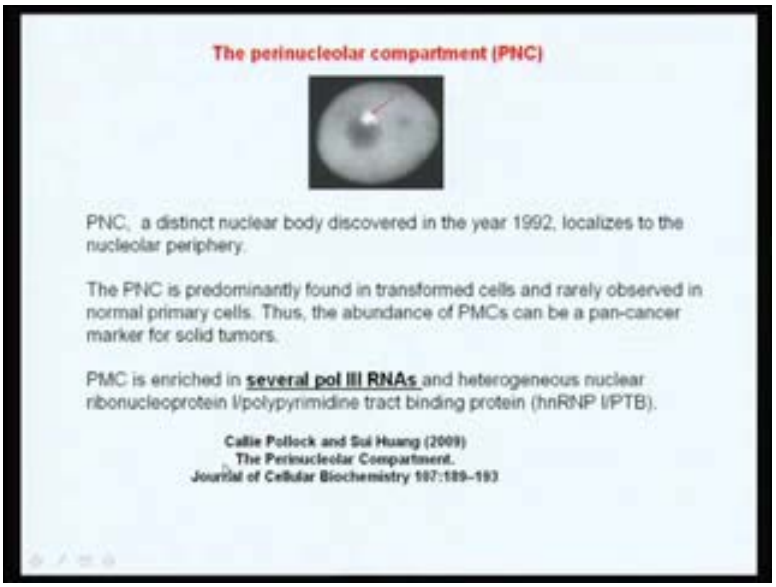

It also turns out there is a very, very distinct compartment called perinucleolar compartment which is just on the periphery of nucleolus which is the nucleolus and the dark body is a nucleolus and the white body shown by the red arrow is what is called as a perinucleolar compartment. This sub nuclear structure called perinucleolar compartment was discovered in the 1992 and is present on the periphery nucleolus and this PNC is predominantly found in transformed cells of the cancer cells and is rarely observed in the normal primary cells.

And in fact, the presence of PNC is often considered as a marker for distinguishing a cancer cell to normal cell. So, in normal cells, you rarely see a perinucleolar compartment, whereas in the case of cancer cells, this is very, very prominent and it turns out, this PNC is highly enriched in several pol III RNAs as well as heterogeneous nuclear RNA protein I, polypyrimidine track binding protein involved in hnRNA synthesis.

So, you can see, when a normal cell becomes a cancer cells, in addition to the regulation of TFIIIB and increase in tRNA synthesis, there is also accumulation of pol III transcripts and certain hnRNP particles in a perinucleolar compartment and therefore, this become highly visible and this PNC compartment is actually is a one of the markers

to distinguish a normal cell from a cancer cell. A very interesting review article on the perinucleolar compartment is available. In this review article, in general by bio chemistry just last year and those who are interested in understanding more about this perinucleolar compartment can read this article.

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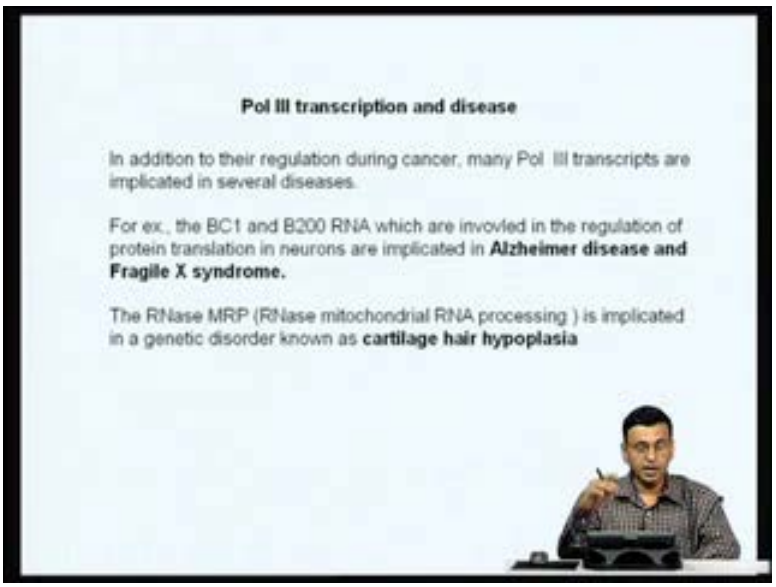

So, in addition to this regulation of pol III transcription in cancer, the pol III transcription is also implicated in a number of diseases. For example, in the case of, as I mentioned in that long list of tables about the various RNAs, which are transcribed by pol III, in the case of neural cells, there is what is called BC1 and BC200 RNA, which are involved in the regulation of protein translation and both BC1 and BC200 are transcribed by RNA polymerase III.

These are present only in the neural cells and when you have problems in BC1 and BC200 RNA synthesis, it leads to disease like Alzheimer disease or fragile x syndrome. So, pol III transcription is involved in neuronal disorder such as Alzheimer disease and fragile x syndrome. Similarly, the ribonucleus is MRP, which stands for the mitochondrial RNA processing RNA, which is RNA involved in mitochondrial RNA processing, it is implicated in genetic disorder called cartilage hair hypoplasia. So, you can see, if there are problems in pol III transcription or factors involved in pol III transcription, it can lead to cancer; it can lead to number of disorders, especially neural disorders such as Alzheimer and fragile x syndrome, so on and so forth.

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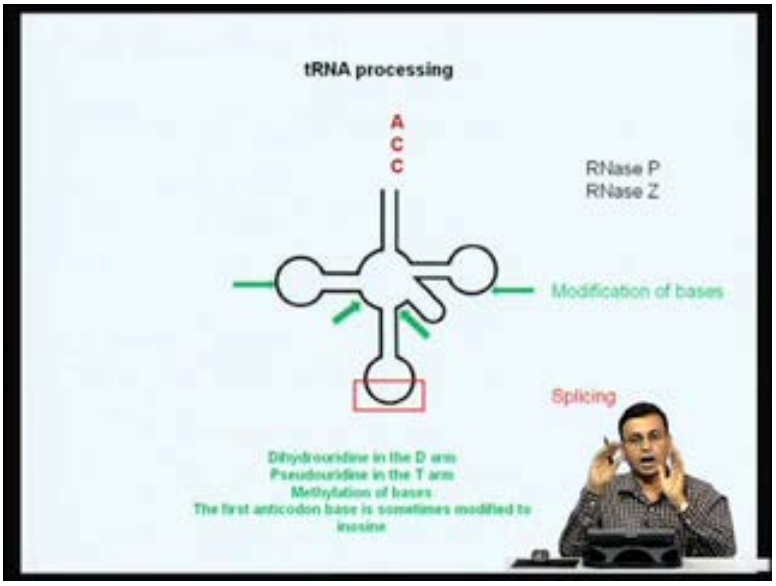

So, I think what I have described to you so far is about the general mechanisms or the major cis-acting elements involved in pol III transcription, various transacting factors involved in pol III transcription, what are the various types of pol III promoters - type 1, type 2 and type 3, and what is the organization of these promoters, what kind of transacting factors go and bind and how these factors associated with RNA polymerase III regulate pol III transcription, then we discussed very briefly how a pol III transcription plays a very, very important in the normal growth as well as in transformation.

And the synthesis of transfer RNA is very finely regulated. A number of oncoproteins and tumor suppressor proteins interact with the transacting factors involved in pol III transcription and as a result of these interactions, either the tRNA gene transcription is increased or decreased and when the tRNA gene transcription is increased, it can lead to cancer, whereas in normal cells, the tRNA transcription takes place at a very sub optimal level and not all the tRNA gene promoters are actually occupied by these transacting factors because they are sequested by tumor suppressor protein p53 and Rb.

Now, we will spend may be a few minutes to understand, once the tRNA is synthesized, what kind of processing takes place. Just like in the case of pol II transcripts, we have seen the pre-messenger RNA undergoes a number of RNA processing steps like capping, splicing and polyadnelation. Similarly, in the case of ribosomal RNA, we have shown it

is synthesized as a precursor ribosomal RNA, which then gets cleaved to 28S or 18S and 5.8S rRNA, whereas in the case of tRNA, let us see what kind of processing takes place. One of the first There are about four major steps in the case of tRNA processing. One of the first steps is removal by specific ribonucleases, the 5 prime and 3 prime regions of the tRNA.

You all know the tRNA forms a typical structure called the cloverleaf structure primarily because of a secondary structure. The RNA folds itself immediately after transcription and forms a typical cloverleaf structure and a number of processing mechanisms takes place and one of the first steps of the synthesis of tRNA, percussive tRNA is the removal of the 5 prime and 3 prime overhangs by 2 ribonucleous called the RNase P and RNase Z. So, this is the first step in RNA processing. And once this 5 prime and 3 primes overhangs are removed, there is also very short intron in the pre tRNA molecules in the anti-codon loop of the transfer RNA and this intron also gets spliced. So, the removal of the intron from the anti-codon loop is another important step in the tRNA processing.

So, the 5 prime and 3 prime overhangs are removed, a shot intron which is from the anti-codon is spliced out and then the amino acid acceptor called CCA, which is actually added to the 3 prime end of the eukaryotic messenger RNA, whereas in the case of prokaryotic tRNAs, it is part of the transcription; the CCA sequence is a part of the transcribed RNA, whereas in the case of eukaryotes, the CCA sequence is added post transcriptional.

Remember, the CCA which is very, very important for the tRNA function is added post transcriptionally in the case of eukaryotes. So, it is another important modification that takes place and known as the tRNA processing. Of course, we all know that just like in the case of ribosomal RNA, in the case of tRNA also, a number of unusual modified bases are present in the tRNA. So, this base modification also is a part of pre-RNA processing mechanism.

For example, the dihydrouridine is present in the D arm and a pseudouridine is present in the T arm and a number of bases like cytosine and guanine and adenine, they all get methylated and the first anticodon base sometimes is modified to inosine. So, all these base modifications also take place. So, the cleavage of 5 prime and 3 prime overhangs, the removal of an intron in anticodon loop, the addition of CCA in the 3 prime end of

tRNA as well as base modification, all these things happen, once the tRNA is synthesized. Ultimately, you get the matured transfer RNA, which is then used for protein synthesis and translation.

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So, I think I will stop here. What I have listed here is, in addition to the articles which have listed in between during the course of this lecture, I have also listed some very important papers, which are very important key contributions for the RNA polymerase III transcription studies, especially Bob Roeder's group, who made a very pioneering contribution and I have listed some of the articles, especially from Bob Roeder's lab.

There are also many other important articles, but these are some of the key factor papers that one should read, if you have to understood little bit more greater detail the various aspects of pol III transcription. I have also listed in couple of review articles, which very nicely discuss about the various aspects of RNA polymerase III transcription research.