Eukaryotic Gene Expression
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Lecture No. # 13
Eukaryotic gene regulation: Synthesis of ribosomal RNA by RNA polymerase 1

The last 12 lectures that we discussed in this course namely, lectures 1 to lecture 12, we primarily focused about regulation of messenger RNA synthesis. How RNA polymerase II transcription is regulated? We studied in depth the way general transcription factors that are involved in polymerase II transcription. We also studied how chromatin plays a very important role in the regulation of RNA polymerase II gene expression, and we also studied how post translation modification of histones, and as well as DNA methylation plays a very, very important role in the regulation of polymerase II transcription, and we also studied what are the various RNA processing events that takes place namely mRNA capping, mRNA splicing and polyadnelation, and how all these activities are integrated.

So, the first 12 lectures, we confined ourselves to the regulation of transcription by RNA polymerase II. How messenger RNA synthesis inside the cells is regulated and how RNA polymerase II is able to synthesize messenger RNAs at different levels? How different protein coding genes are transcribed at different levels? Some are housekeeping genes; some of them code for proteins, which are all the time synthesized, which are constituently expressed; there are some genes which are induced all the time and we studied how transcription factors through the DNA binding domains, go and bind to specific extreme sequences and through their transcription activation domains, interact with chromatin modifying proteins or histone modifying proteins and as a result, enhance the recruitment of RNA polymerase to the promoter and that is how the rate of transcription is initiated.

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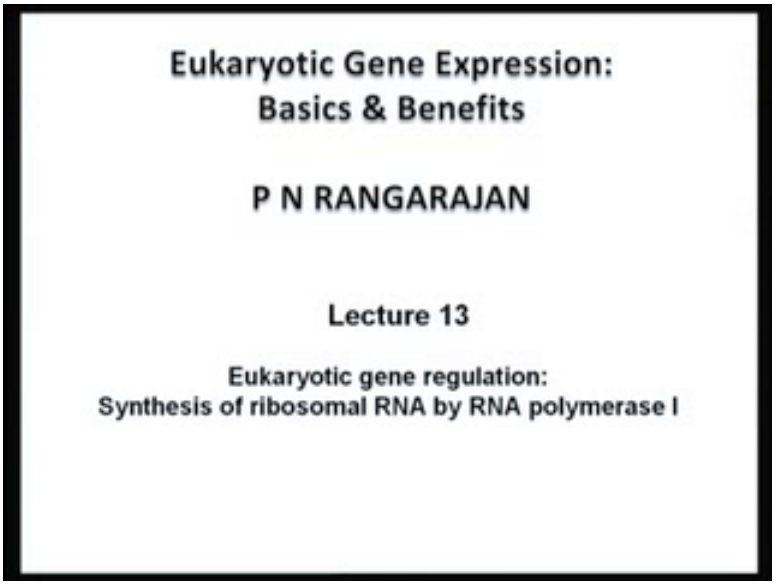

So, the first 12 lectures, we confined ourselves to only RNA polymerase II and regulation of protein coding genes. So, in this class, today, we are going to talk about the regulation of ribosomal RNA synthesis. How ribosomal DNA is transcribed or how RNA polymerase I activity is regulated inside the cells. So, eukaryotic gene regulation synthesis of ribosomal RNA by RNA polymerase I. How RNA polymerase I makes ribosomal RNA and how its activity is regulated inside the eukaryotic cells is going to be the crux of today's lecture.

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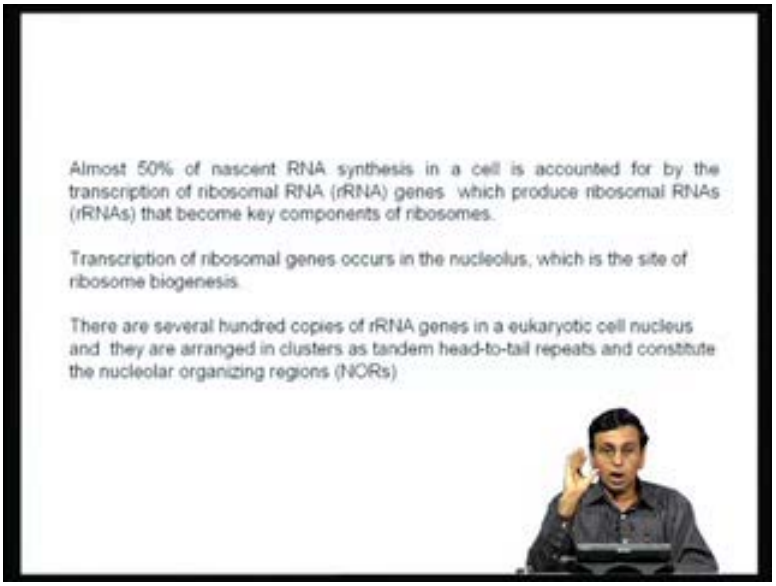

Now, although we spent about 12 hours discussing the regulation of messenger RNA synthesis and regulation of protein coding genes, remember, messenger RNA accounts for a very, very small fraction of the total RNA. If you ask me what are the most abundant RNA inside the cells, if you isolate RNA and run it on a gel, you actually see three major bands 28S RNA, 18S RNA and 5.8S RNA; these are nothing but, ribosomal RNA's

In fact, almost 50 percent of the nascent RNA, which is synthesized in the cell, is accounted by for the transcription of ribosomal RNA genes. So, rRNA transcription or rDNA transcription accounts for more than 50 percent of the total RNA inside the cells. So, messenger RNA is only a very small fraction. Ribosomal RNA followed by tRNA and mRNA accounts only a very small fraction of the total RNA. So, it is important for us to understand how do the cells synthesize such large amounts of ribosomal RNA. So, regulation of RNA polymerase I transcription is also as important and as vital as studying and understanding the regulation of RNA polymerase II.

And in the next class, we will also study the regulation of RNA polymerase III, how tRNAs are synthesized, how pol III makes transforming inside the cells. Now, transcription of ribosomal genes occurs inside the nucleolus, within the nucleus and nucleolus is actually the site of ribosomal biogenesis. So, in order to make this huge amounts of ribosomal RNA because they constitute the ribosomes and the cell has actually identified a very specific region inside the nucleus, which is the nucleolus and is inside the nucleolus that the ribosomal DNA transcription takes place, ribosomal RNA is synthesized, processed and ribosomes are assembled inside the nucleus and they come out.

So, there are several 100 copies of ribosomal RNA genes inside a eukaryotic cell nucleus because we have to make such huge amounts of ribosomal RNA, we require multiple copies of the ribosomal genes. Therefore, there are several hundreds of such ribosomal genes inside the eukaryotic cell nucleus and all these genes are arranged in the clusters as tandem head to tail repeats and what constitute what is called as a nucleolar organizing regions. So, what I am trying to say from the slide is that ribosomal DNA transcription constitutes the majority of major transcription activity inside the cells.

Ribosomal RNA constitutes more than 50 percent of the total RNA inside the cells. The ribosomal DNA transcription takes place inside the nucleolus of nucleus. Not only ribosomal RNA is synthesized, it is processed and ribosomal assembly also takes place inside these nucleolus and the to account to make this huge amounts of ribosomal RNA, organisms have evolved or eukaryotes have evolved to have multiple copies of this ribosomal DNA genes and therefore, many of these rDNA genes, many hundreds of copies of this rDNA genes are present in a head to tail fashion, a tandem fashion inside the genome and they are actually present inside the nucleolus.

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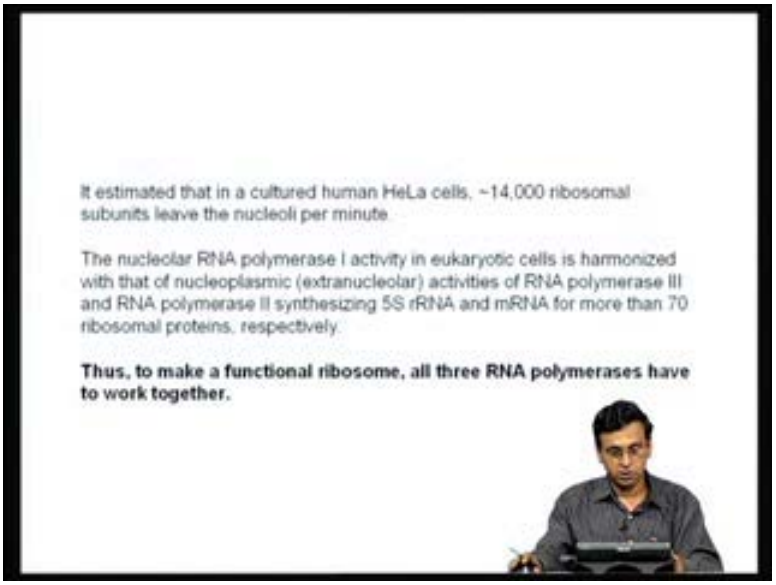

So, nucleolus is the site of ribosomal DNA transcription and ribosomal RNA synthesis and it is estimated that in the human HeLa cells for example, about 40000 ribosomal sub units leave the nucleoli per minute. So, just imagine the kind of transcription activity and ribosomal assembly that is taking place inside ourselves. 40000 ribosomal subunits leave every minute from the nucleolus.

Because unless they come out and They are the protein synthesizing machines and unless they are present in large amounts, proteins cannot be made. So, in order to synthesize proteins in large amount, especially in active growing cells, the ribosomal transcription is very, very essential. So, the transcriptional regulation of ribosomal DNA takes place depending upon the physiological status of cells. If a cell is differentiated, if it does not actively divide, the ribosomal transcription is very low, but in case of actively growing

cells and actively dividing cells, the ribosomal DNA transcription is very, very high because protein have to be made in large amounts.

Now, the nucleolar RNA polymerase I activity inside the eukaryotic cells is harmonized with that of nucleoplasmic, that is, extra nucleolar activities of RNA polymerase III and RNA polymerase II. This is also very, very important point. Now, if you want to make functional ribosomes, it is not just RNA polymerase I which is involved, it also involves RNA polymerase II and RNA polymerase III because the ribosomes consists of not only 28S, 18S and 5.8S RNA, which are actually made from the ribosomal DNA, it also contains 5S RNA, which is made by RNA polymerase III and at least 70 different ribosomal proteins have to now combine with this ribosomal RNAs and all these proteins have to be synthesized from messenger RNA.

So, if you want to have a functional ribosome, you require all the 3 RNA polymerases have to work together. So, to make functional ribosomes, all the 3 RNA polymerase have to work together. RNA polymerase I makes 28S, 18S and 5.8S rRNA; the messenger RNAs for at least 70 different ribosomal proteins are made by RNA polymerase II and 5S rRNA is actually made by RNA polymerase III.

So, all these 3 RNA polymerase have to work together in order for it to get a functional ribosome. Let us now stick today about the synthesis of 28S, 18S and 5.8S rRNA from the ribosomal DNA by RNA polymerase I. How does the synthesis takes place and how does the regulation takes place.

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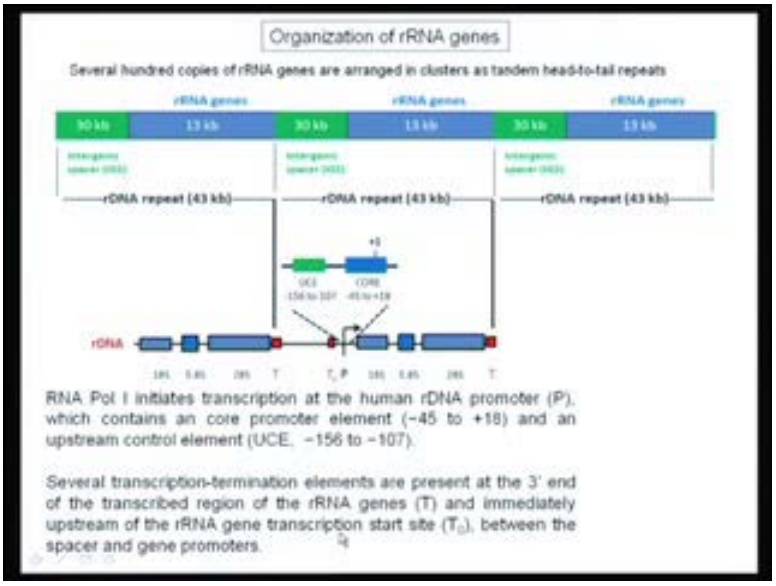

As I told you, in order to synthesis this huge amounts of ribosomes which the cell requires to make proteins, the ribosomal DNA has to be present in multiple copies and as I mentioned earlier, several hundred copies of ribosomal RNA genes are arranged in clusters as tandem head to tail repeats in the genome and they are all present inside the nucleolus.

And what I have shown in this cartoon is how these rDNA clusters are arranged and we can see this green and blue together represents one rDNA repeat and is about 43 kilo basis long and such rDNA repeats are arranged in a head to tail fashion in the genome and several hundred copies of such rDNAs are present at a given stretch in the inside the genome and all are they are all transcribed within the nucleolus.

So, each rDNA repeat is approximately about 43 kilo basis in the length and it consists of two important regions: one is called as an intergenic spacer; another is called a region which actually codes for the ribosomal RNA genes - a non-coding region and a coding region. The blue 13 kb region actually codes for the ribosomal genes and the green one, which is about 30 kb, is actually called as an intergenic spacer or IGS, which does not code for anything.

And let us now see how exactly this ribosomal DNA unit is there and how exactly the regulation of synthesis take place and this cartoon actually tells you the coding region. The 13 kb actually contains genes that code for 18S, 5.8S, 20S ribosomal RNA and this

is the intergenic spacer and this is the promoter from which a transcription starts site, the arrow indicates the transcription start site and flanking the transcription start site, we have the promoter and the promoter extends both in the transcribe region, the downstream of transcription start site as well as the upstream of transcription start site and extends usually from minus 156 to plus 18 of the transcription start site.

So, the ribosomal DNA transcription or the ribosomal, the rDNA promoter consists of about minus 156 to plus 18 and this is a key regulatory region for ribosomal DNA transcription. This is where all the action is and this is what we are going to discuss what kind of transcription factors actually are present or interact between the minus 156 region to plus 18 region and within this region, there are two important cis-acting elements, just like we have the tata box, the near upstream elements, far upstream elements and transcription factor binding sites, in the case of the RNA polymerase II promoter which we have discussed extensively.

In the case of RNA polymerase I promoter, we have two important cis-acting elements: one is called as a core region; another is called as the upstream control region. The upstream control region extends from minus 156 to minus 107, whereas the core region extends from minus 45 to minus 18.

So, this core region is similar to the initiator or the tata box region of the RNA polymerase II promoter and the upstream control region is similar to the enhancer or the upstream binding sequences on the RNA polymerase II promoter. So, just as we have a basal transcription factor, which are assembled near the tata box are initiated in the case of RNA polymerase II promoter, similarly, a number of basal transcription factors in the RNA polymerase I - there it was polymerase II; here, it is RNA polymerase I - along with certain basal transcription factors assembled near the core promoter, whereas to enhance the rate of transcription initiation by this pre initiation complex assembled near the core region, a number of protein factors bind to the upstream control [rare] element and enhance the rate of recruitment of RNA polymerase I. Therefore, the rate of transcription initiation can be enhanced in the RNA polymerase I promoter.

So pol I initiates transcription in the human rDNA promoter, which is this, made as P here, which contains the core promoter element extending from minus 45 to plus 18 and it also contains upstream control element or UCE, which extends from minus 156 to

minus 107 region. There are several transcription termination elements which are present at the 3 prime end of the transcribed region of rRNA which are designated in the red colour, as designated as T here and they are also present immediately upstream of the rRNA gene transcription site, which are designated as T0 between the spacer and gene promoters. So, there are two different kinds of transcription termination elements: one immediately after the transcriber gene and one just upstream of the promoter region; they are designated as T as well as T0.

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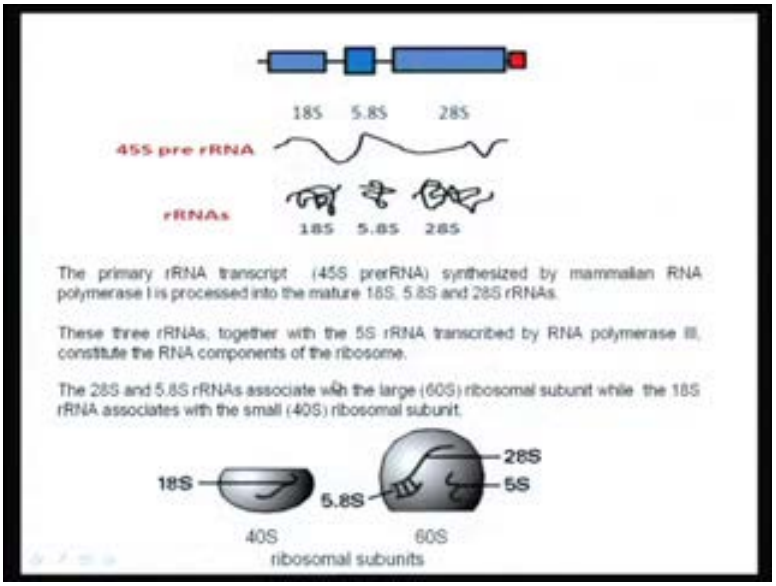

Now, as such this is the coding region or the transcribe region of the RNA polymerase II, rDNA unit, which contains genes and code for the 18S rRNA, 5.8S rRNA and 28S rRNA and all these three are recognized as a percussion molecule, which is called as a 45S pre ribosomal RNA and this 45S pre ribosomal RNA is then cleaved to get the three individual rRNA namely, the 18S rRNA, 5.8S rRNA and the 28S rRNA.

So, a single percussive rRNA is synthesized from the ribosomal DNA unit by RNA polymerase I and this rRNA is post transcriptionally cleaved to the three different ribosomal RNA molecules namely, 18S rRNA, 5.8S rRNA and 28S rRNA. So the primary transcript 45S pre rRNA synthesized by mammalian RNA polymerase I, is processed or post transcriptionally cleaved to mature 18S, 5.8S and 28S rRNAs and all these three RNAs together with a 5S rRNA transcript by the RNA polymerase III constitute the RNA components of the ribosome.

So, a ribosome is a ribonucleo protein particle; it contains proteins, it contains RNAs and of the four different RNA molecules, which are present in the ribosome, three of them namely, the 18S, 5.8S, 28S are made by polymerase; they come from the ribosomal DNA and 5S RNA is actually rRNA transcribed by the polymerase III and together they constitute the RNA component of the protein.

And in addition, messenger RNAs corresponding to at least 40 different ribosomal proteins have to be transcribed by polymerase II, transcribed into proteins and these proteins then interact with the RNAs and then to get a functional ribosome particle. So, the 28S and 5.8S rRNAs associate with the large ribosomal subunit; this is what is shown in this cartoon. The 5.8S and the 28S, 5.8S and 5S are associate with the large sub unit of the ribosomal particle is 60S, while 18S rRNA associate with the small sub unit of the, 40S subunit of the ribosomal particle. So, together 60S and 40S together form the functional eukaryotic ribosome

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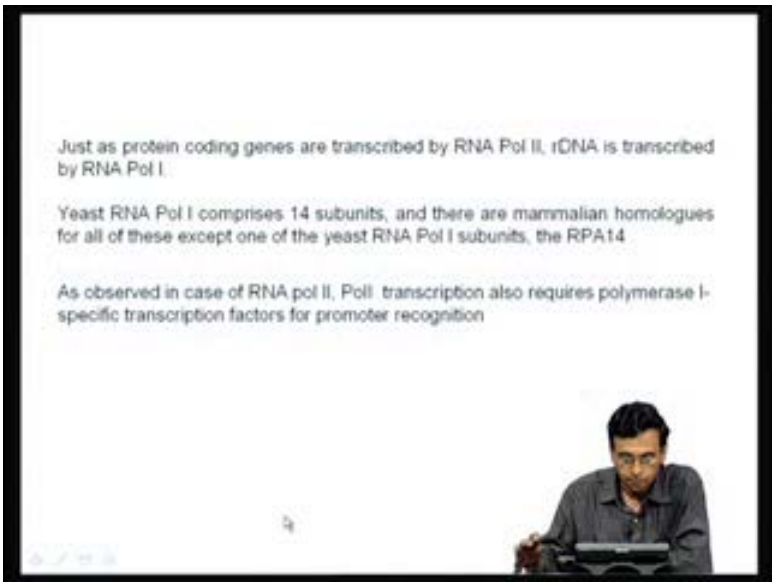

So, just as the protein coding genes are transcribed by RNA polymerase II, the rDNA, ribosomal DNA is transcribed by RNA polymerase I, which we all know very well. The yeast RNA polymerase I - we knew from our previous 12 lectures that the eukaryotic polymerase II is a multi-protein complex consists of a number of subunits; the same way, the polymerase I also consists of a number of subunits at least 14 of them are there in the case of yeast cells and there are mammalian homologues for all these 14, which have

been first identified in the yeast cells except for one of the yeast homologues is missing in the humans namely, the RPA14.

What I am trying to say is that just like RNA polymerase II, RNA polymerase I also is multi subunit complex; it is a huge protein and as observed in the case of RNA polymerase II, pol I transcription also requires pol I specific transcription factors for promoter recognition. So, the mechanisms are more or less similar, but the players are different. So, just like the RNA polymerase II require the basal general transcription factors like TF2D, TF2E, A, B, E, D and so on, so forth, the same way, the RNA polymerase I also requires certain general transcription factors for accurate initiation transcription from the rDNA promoter. These general transcription factors, which are required for RNA polymerase I are designated as TF1, whereas in the case of RNA polymerase II they would be designated as TF2; TF2A, 2D and so on, whereas in the case of polymerase I, they are designated TF1 A, E and so on, so forth. Similarly, in the case of RNA polymerase III, they are designated as TF3 A, B, C or TF3 1, 2, 3 like that.

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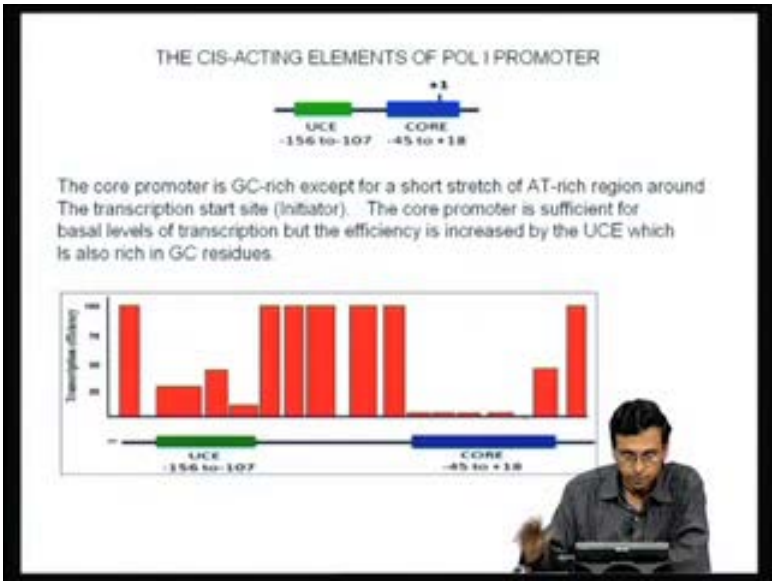

Now, one of the cis-acting elements of RNA polymerase I promoter, as I mentioned there are 2 major cis-acting elements: 1 is called as a core region, which extends from minus 45 to plus 18 and transcription start site is included within this and they also have what is called as a called as a upstream control region, which extends from minus 156 to minus 107 and in between, you have what is called as the spacer region.

The core promoter is GC-rich except for a short stretch of AT-rich region around the transcription start site, which is the equivalent to the initiator of the RNA polymerase II promoter and the core promoter is sufficient for basal levels of transcription, but the efficiency of transcription can be further enhanced if the upstream control element is present upstream, which is also rich in the GC residues.

Just as people have mapped important nucleotides, important regions required for pol II transcription; in the case of pol I transcription also, if you make mutations within the core region or within the upstream control region, there is a dramatic decrease in the transcription efficiency, clearly indicating that these two regions play a very, very important role for transcription initiation by RNA polymerase I.

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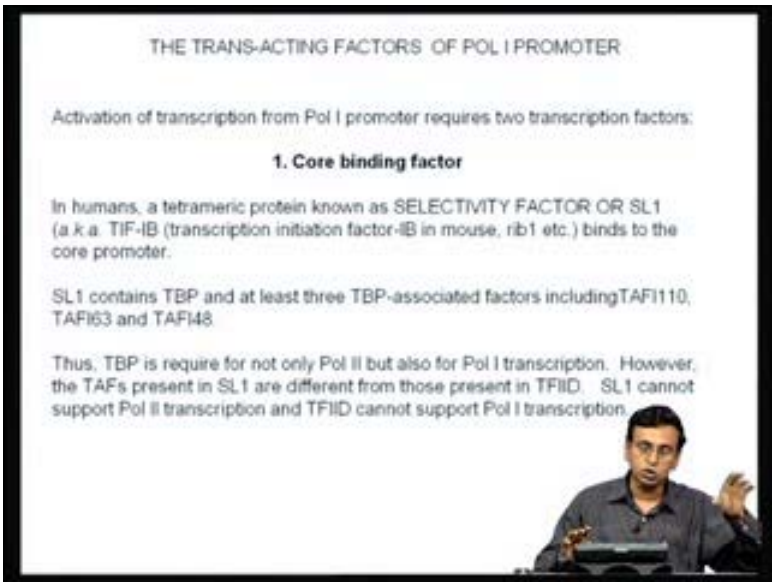

Now, So, two major cis-acting elements are present in the RNA polymerase I promoter: the core region as well as the UCE or the upstream control region. What are the transacting factors? We know from our pol II example that if there are cis-acting elements, certain protein factors have to go and interact the cis acting elements; that is how the regulation takes place. In the case of pol II transcription, with the RNA polymerase II general transcription factors and many transcription factors act as transacting factors. In the case of Similarly, in the case of polymerase I also, there are transacting factors which interact with two major cis-acting elements of rDNA promoter.

One of the transaction transacting factors or transcription factors involved in pol I transcription is called as a core binding factor. This is the protein which interacts with the core region of the RNA polymerase I promoter. In the case of humans for example, this core binding factor is known as selectivity factor one or SL1, whereas the same protein factor in the case of mouse is known as TIF1B; that is transcription initiation factor 1 B. It is also varies known as rib1 etcetera and so forth.

Just remember, the human core binding factor is called as SL1, whereas the same thing in the mouse is called as TIF1B. It is this SL1 factor, which actually binds to the core sequence of the RNA polymerase I promoter. What is this SL1? What is this selectivity factor 1? SL1 actually contains the tata binding protein and at least, 3 TBP associated factors, which are designated as TAF1110, TAF1163 and TAF198, 48. Remember, just like in the case of pol II transcription also, tata binding protein is involved; in the case of pol I transcription also, tata binding protein is involved and above all the general transcription factors, which we have studied may be TF2D, B, E, A and F, it is the tata binding protein which plays a very important role in the transcription of not only polymerase II, but also by polymerase I as well as by RNA polymerase III.

So, tata binding protein plays a very important role in the transcription of all 3 RNA polymerases and just as the tata binding protein, in the case of pol II transcription is associated with specific TAFs, we use to call as TAF(II)250 and so on and so forth. In the case of pol I transcription also, there are specific TAFs, that is the TBP associated factors which are associated, but these are designated as TAF1 with the instead of TAF2, we call them as TAF1 depending upon the molecular weight, TAF1110 means it is a 110 kilodalton protein, TAF163 means 63 kilodalton protein, TAF148 means 48 kilodalton protein.

The TAFs which are required for pol I transcription is different; the TAFs II which are required for pol II transcription is different. So, the TBP is required not only for pol II, but also for pol I transcription. However, the TAFs present in SL1 are different from those present in TF2D.

So, the TBP in association with specific TAFs forms TF2D and this TF2D is involved in pol II transcription, but the same TBP in association with the TAF1 specific factors, this is called SL1; that is involved in pol I transcription. SL1 cannot promote pol II

transcription; TF2D cannot promote pol I transcription; these are distinct. So, TBP by interacting with either pol I specific factors or pol II specific factors can promote pol II transcription or pol I transcription respectively.

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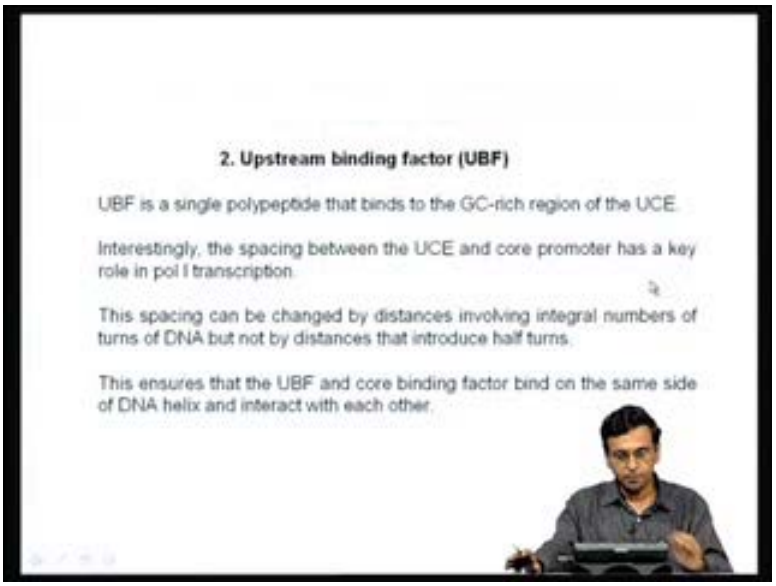

The second important transacting factor is called as the upstream binding factors or UBF which actually interact with the UCE region of the pol I promoter. Now, UBF is a single polypeptide that binds to the GC-rich region of the upstream control element or the UCE of the pol I promoter. Interestingly, in addition to this core promoter and UCE, the spacer between the UCE and core promoter plays a very, very important role in pol I transcription.

The spacing can be changed by distances involving integral numbers of turns of DNA, but not by distances that introduce half turns. It is very, very important because the factors that bind to the UCE and the factor that bind to the core (( )) have to be on the same side of the helix and then only pol I transcription takes place and if you now change the spacer by half turns, then this proteins binding to them cannot be on the same side of the helix and therefore, pol I transcription cannot happen. So, this distance between the core UCE and core promoter is very, very important and this ensures the protein that bind to core region, protein that bind to UBF are on the same side of the helix.

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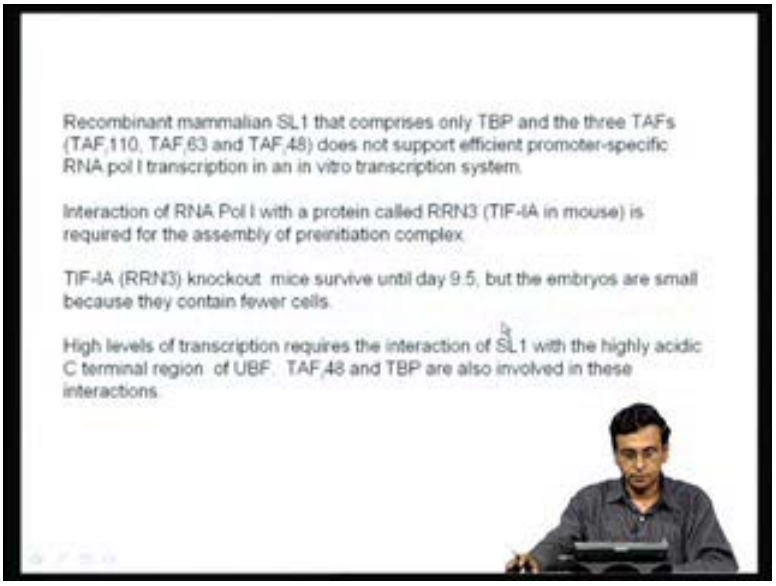

Now, recombinant mammalian SL1, which consists of TBP and the 3 TAFs cannot support efficient promoter specific RNA pol I transcription in an in vitro transcription system. So, just like, we had TAF2D, TBP alone could not be sufficient to initiate activate determinant transcription in the case of pol II transcription, in the case of pol I transcription, TBP and TAFs alone or not enough to support basal transcription. In the cell-free transcription system, in addition to the TAFs and TBP, you also require another protein, which is known as RRN3. This RRN3 is actually required for efficient formation of pre initiation complex, in the case of the pol I promoter.

This RRN3, which is actually known as TAF-IA in the case of the mice, if you knock out this RRN3, the mice can survive only up to 9.5 days of embryonic development and after that they die and even in these embryos, they are these embryos are very, very small because they contain very few cells. So, if you do not have RRN3, the embryos die after day 9.5 and the cells are also very, very small in these embryos clearly indicating that in addition to TBP and these 3TAFs which are pol I specific, this RRN3 plays a very, very important role for efficient transcription of the pol I rDNA promoter by RNA polymerase I.

So, the SL1 in combination with RRN3 is required for the basal transcription and these factors, the SL1, when it interacts with the UBF, which is binding with the upstream control region, now we get much higher levels of transcription and in addition to this

SL1, the TAF 148 and TBP are also involved in interaction between the UBF and the basal transcription in the case of RNA polymerase I.

So, the core promoter region and the transcription factors, which bind to the core promoter region consist of what is called as SL1 or selectivity factor 1. In the case of mouse, it is called as TAF1B. So, the SL1 consists of tata binding protein and TAF 110, TAF 163, TAF 148. In addition to these three, you require another protein factor called as RRN3 for basal transcription of RNA polymerize I promoter. This is about the core region or core promoter region of the RNA polymerase I.

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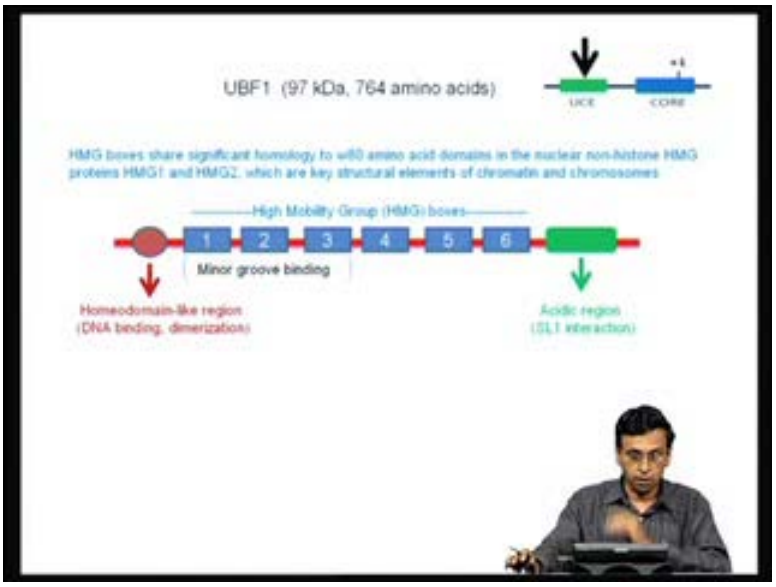

Now, if we move to the upstream region, the upstream control element binds to a protein called as UBF1. The UBF1 or the upstream binding factor 1 is the 97 kilodalton protein and consists of about 764 amino acids and this is a cartoon that depicts the structure of the UBF1. It basically consists of three important regions. It contains what is called as a homeodomain like region, which contains, which is involved in both DNA binding as well as dimerization.

We have studied extensively about what is homeodomain in our previous classes. When we discussed about RNA polymerase II transcription, what kind of DNA binding motifs the transcription factors involved in RNA polymerase II transcription contain - zinc finger motifs, zinc finger motif using zipper helix lophilates, helix turn halides homeodomain and so on, so forth. So, this transcription factor, which involves pol I also

contains a region which is involved in DNA binding and dimerization and it consists a homeodomain like region.

In the C- terminus, it contains a highly acidic region; that means, it is rich in glutamic acid and aspartic acid and this region is actually involved in interaction with the core binding factors namely, the SL1 factors. In addition, it contains a number of good regions called as high mobility group boxes or HMG boxes. Now, what are these HMG boxes? These HMG boxes share significant homology to what is called as a w80 amino acid domains and the nuclear non-histone HMG proteins - HMG1 and HMG2, which are key structure elements in the chromatin and chromosomes. So, these regions have high mobility HMG boxes containing homology 2 proteins involved in chromatin structure and chromatin modulation.

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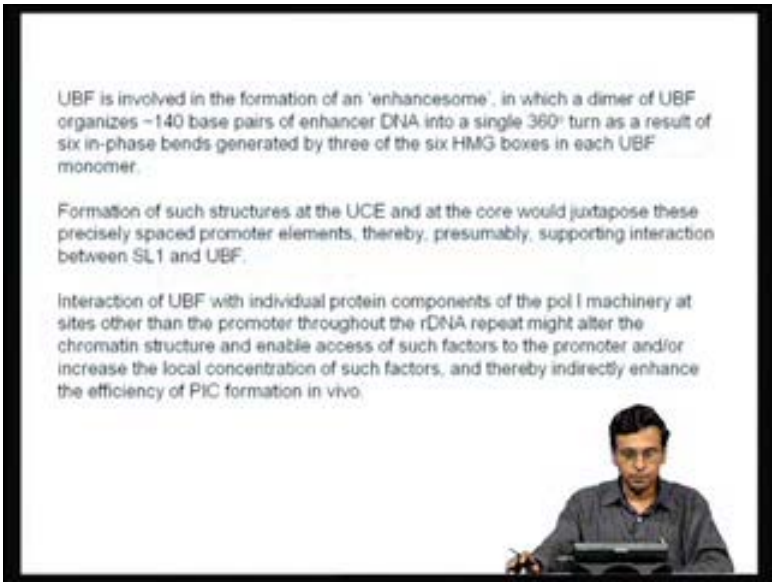

The UBF is actually involved. The reason why it contains these kind of HMG boxes is that the UBF actually plays a very important role in regulating rDNA transcription in the chromatin context. In fact, the UBF is actually involved in formation of what is called as an enhanceosome, in which a dimer of UBF organizes 140 base pairs of the enhance DNA into a 360 degree turn as a result of which six in phase bends are generated by three of the six HMG boxes in each UBF monomer.

We can see what is the functional significance of HMG boxes of the UBF factor and formation of such structures at the upstream control element region under the core region

would juxtapose these precisely spaced promoter elements thereby supporting their interaction between SL1 and UBF. So, these HMG boxes play a very important role in positioning these factors in such a way that they can interact and the spacer also plays a very, very important role in this.

Interaction of the UBF with the individual protein components of pol I machinery at sites other than the promoter throughout rDNA repeat might alter the chromatin structure and therefore, enable access of such factors to the promoter and increase the local concentration of such factor thereby indirectly enhance the efficiency of pre initiation complex formation. So, the UBF is just like the enhancer binding factors or transcription activators which are studied in the case of pol II transcription.

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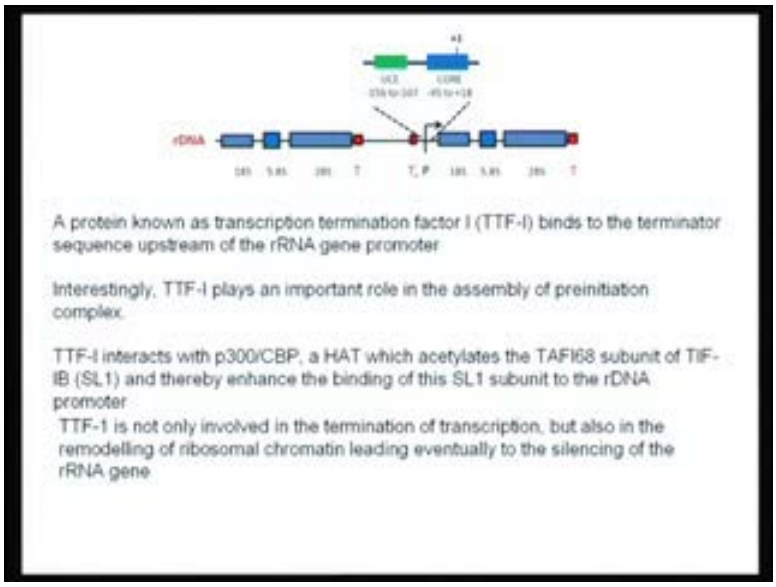

Now, very interestingly a protein known as transcription termination factor or TTF-I binds to the termination sequence of upstream region of rDNA promoter. As with seen in the case of pol II transcription, once trans is pre-initiated, it also has to terminate. There are sequences in the case of pol I transcription, which actually serve as terminator sides and to this termination sequences, specific proteins called as TTF-I actually binds and brings about termination of transcription, but one unique feature about this transcription terminator protein or the transcription termination factor I is that it plays a very important role not only in termination of pol I transcription, it also plays a very, very important role in the assembly of pre initiation complex.

It is a very, very rare example where a protein designation of a transcription termination factor actually promotes transcription initiation. How does it do it? It turns out that TTF-I actually interacts with a histone acetyltransferase, which we again studied it extensively in our previous lectures called p300 or CREB binding protein, which actually has a histone acetyltransferase activity.

So, you can see the HATs are not only involved in the regulation of pol II transcription, but they are also involved in regulation of pol I transcription and we know this HAT actually assimilates one of the sub units of the core binding factors namely, TAF 168 of the SL1 and thereby enhancing the binding of the SL1 subunit to the rDNA promoter. So, here, we have very interesting example, where a transcription termination factor recruits a histone acetyltransferase so that this histone acetyltransferase can actually acetylate one of the subunits involved in the core transcription factors and therefore, enhance the rate of pre initiation complex formation.

In addition, the TTF-I is also involved in what is called as a remodeling of the ribosomal chromatin leading to eventual silencing of the rDNA gene. We will come to this little bit later during this talk. I will not like to confuse about the role of TTF-I in chromatin remodeling. We will discuss the discuss this particular aspect little bit later.

Remember, the TTF-I which is involved in transcription termination because of rDNA transcription also plays a very, very important role in enhancing the rate of transcription initiation because it recruits a histone acetyltransferase and makes it acetylate, one of the core binding factors namely TAF 168 of the SL1.

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So, how does the transcription proceed in the case of RNA polymerase I on a rDNA promoter. As we have seen in the case of the pol II promoter, the first step is the formation of pre initiation complex and this results in the initiation of transcription, then you have promoter escape and promoter clearance. The initiation complex now starts elongation, ultimately transcription initiation and then the polymerase I again has to come back and with the help of initiation factors, starts pre initiation and that is how the polymerase I transcription cycle continues.

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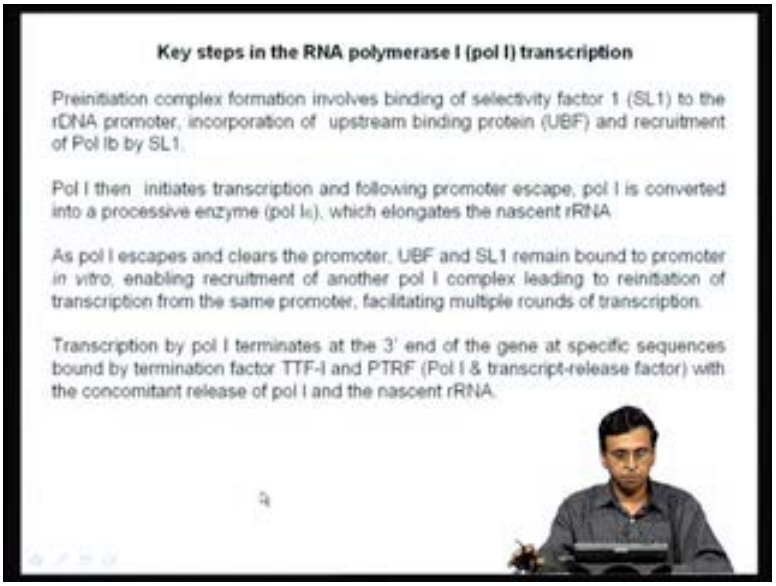

So, let us now discuss what are the key steps, which are actually involved in the RNA polymerase I transcription? The pre-initiation complex formation in the case of pol I promoter transcription involves the binding of the SL1 to the rDNA promoter, incorporation of the upstream binding protein and recruitment of pol Ib by SL1.

So, these two factors namely, the SL1 and UBF1, when they go and bind to the respective regions and recruitment of RNA polymerase I is one of the first step in the initiation of transcription by RNA polymerase I. Pol I then initiates transcription and following promoter escape, pol I is converted into a processive enzyme called pol I epsilon, which is involved in the elongation of the nascent RNA. Just like in the case of RNA polymerase II, the A is converted into O or the phosphor or non phosphorated form is converted into phosphorated form.

In the case of polymerase I also, it is converted into a specific polymerase I epsilon, which is now involved in carries out the elongation of the nascent ribosomal RNA transcription and once the pol I escapes and clears the promoter, the UBF and SL1 remain bound to the promoter in vitro. Therefore, it can recruit one more molecule of pol I and initiate another round of transcription and in fact, it is because of this; that means the same UBF and SL1 can recruit multiple, initiate multiple rounds of transcription and therefore, multiple RNA polymerases can start trans from the rRNA gene.

And finally, the transcription RNA polymerase I terminates at the 3 prime end of the gene at specific sequences and this is carried out by the termination factor TTF-I and also, another factor called as PTRF or pol I and transcript region release factor and as a result of these interactions, the pol I is released and therefore, it can go back, again interact UBF and SL1 and initiate one more round of transcription. This is how in brief the pol I transcription cycle proceeds.

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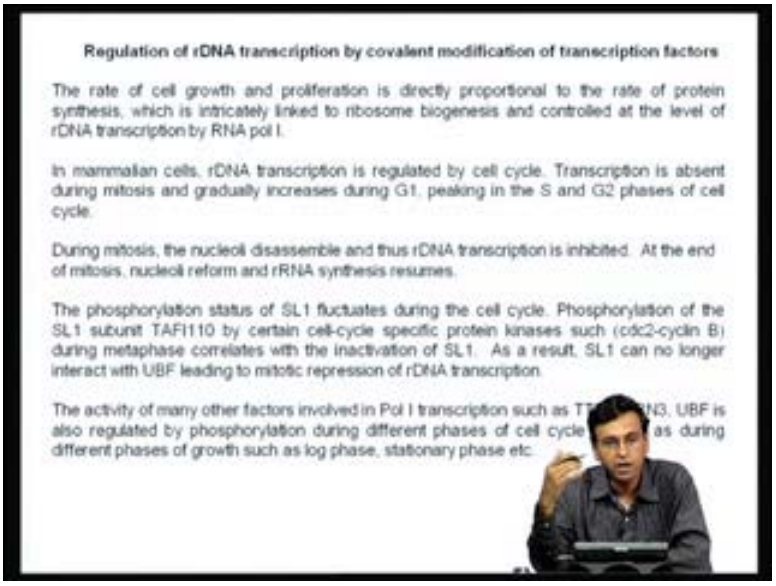

Now, the important aspect that we have to discuss, Just now we spent considerable amount of time discussing the mechanism by which pol II transcription is regulated. Let us now try to understand how is RNA ribosomal DNA transcription is regulated. How does RNA polymerase I activities regulated inside the cells? It is very, very important because I told you 50 percent of the total RNA inside the cells is accounted for by ribosomal RNA.

Now, you do not require this sort of ribosomal RNA all the time inside the cells. So, only in actively growing cells, you may have to require more protein synthesizing capability. Therefore, you may require more amounts of rDNA transcription, whereas cells which are not metabolically active, you may not require that kind of a protein synthesizing machinery and therefore, it is a waste to have a continuous rDNA transcription. So, depending upon the physiological status, depending upon the metabolic status of the cell, the rDNA transcription is very finely regulated.

Now, let us now take 1 or 2 examples and see how does the rDNA transcription is regulated inside the cells. Now, the rate of cell growth and proliferation is directly proportional to the rate of protein synthesis, which is intricately linked to the ribosome biogenesis and therefore, control the level of rDNA transcription by RNA polymerase I. Now, cells have to grow actively, they have to make lots of protein and if cells have to make lots of proteins, they require lots of ribosomes. If more ribosomes are required,

then more ribosomal RNA transcription has to take place and more of the machinery are involved in ribosomal protein subunits, rDNA.

So if you to have an actively protein synthesizing cell, you need to enhance the rate of rDNA transcription. So, depending upon what stage of cell cycle the cells are, the rDNA transcription is also very finely regulated. So, in addition to the requirement of the protein synthesizing capability, depending upon the cell cycle stage also, rDNA transcription is very finely regulated. For example, transcription is completely absent, the rDNA transcription is completely absent during mitosis, then gradually increases during G1 phase of cell cycle, it is the maximum during S phase and G2 phase of the cell cycle. So, once the cell enters mitosis, the rDNA transcription is completely shut off.

Why does happen? That is because the nucleolus disintegrates. When cells enter mitosis, the nuclear membrane breaks them, there is no nucleolus and therefore, there is no rDNA transcription taking place. Since there is no nucleus, rDNA transcription is completely shut off. So, during mitosis, there is no rDNA transcription and once the mitosis over and the nucleus reappears, again nucleolus is formed and then rDNA transcription is started.

So, remember this is a very, very important point. During mitosis, since there is no nuclear membrane, there is no nucleus and no nucleolus; rDNA transcription is completely shut off during mitosis and then gradually as the nucleus reorganize and the nucleolus is reformed, rDNA transcription resumes and the phosphorylation status, how does it happen? The phosphorylation status of SL1 also fluctuates during cell cycle. Phosphorylation of the SL1 subunit of TAF1110 by the cell cycle specific protein kinases during metaphase correlates with the inactivation of SL1.

So, if you have to shut off rDNA transcription, it turns out there are certain specific cell cycle specific kinases, which when phosphorylated the TAF 110 subunit of the SL1 and as a result is inactivated and therefore, SL1 can no longer interact with UBF leading to mitotic repression of rDNA transcription. So, during mitosis, a specific kinase called cdc2-cyclin B goes and phosphorylates with TAF-I 110 subunit of the SL1 transcription factor and when this is phosphorylated, it can no longer interact with the UBF transcription factor and as a result, pol I transcription cannot takes place.

So, one of the mechanisms by which ribo rDNA transcription is regulated is by cell cycle specific phosphorylation of one of the subunits of the SL1. When it becomes

phosphorylated, it is inactivated and therefore, during mitosis rDNA transcription is shut off. Similarly, many other factors involved in pol I transcription like TTF1, RRN3, UBF etcetera are also regulated by phosphorylation during different phases of cell cycle, but I do not want to give too much details at this stage and confuse you.

Remember, one of the major mechanisms by which rDNA transcription is regulated is by phosphorylation of individual components of the transacting factors involved in rDNA transcription such as SL1, TTF1, RRN3, RBF. All these are subjected to phosphorylation, dephosphorylation by specific ionizers and it is by this phosphorylation, dephosphorylation, rDNA transcription is regulated during different stages of cell cycle.

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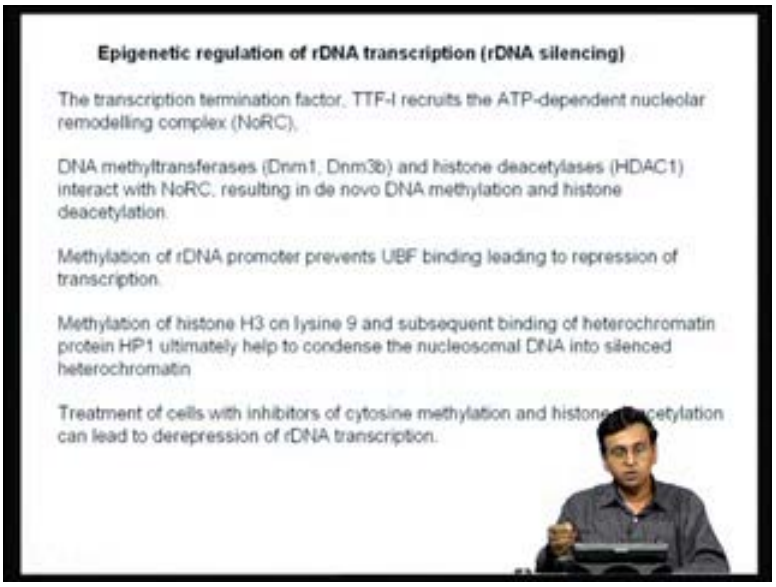

In addition to this mechanism, in addition to the regulation of rDNA transcription by differential phosphorylation of transcription factors, another major mechanism by rDNA transcription is regulated by what is called as a epigenetic regulation of rDNA transcription. Just as we have seen in the case of pol II transcription, phosphorylation of transcription factors is one mechanism by which pol II transcription is regulated and in the previous lectures, I gave an example of for example, CREB, where I told you when you treat cells with cyclic AMP, cyclic AMP activates an enzyme called protein kinase A, protein kinase A now, goes and phosphorylates a transcription factor called as CREB, cyclic AMP response binding element protein which then, once it is phosphorylated, the

phosphorylated form CREB interacts with the histone acetyltransferase called cbps p300 and this results in recruitment of RNA polymerase II and enhancement of transmission. This is one mechanism. The same case here also. We just discussed that depending upon the phosphorylation status of SL1 and other transcription factors, the rDNA transcription will be regulated and we also studied in addition to those kinds of mechanisms, histone modifications and DNA methylation can also regulate pol II transcription and we discussed this as epigenetic regulation of DNA transcription.

In the same way, pol I transcription is also subjected to the epigenetic regulation of the gene expression and histone phosphorylation or histone modifications as the DNA methylation plays an important role not only in the regulation of pol II transcription, but also in the regulation of pol I transcription. For example, the transcription termination factor, TTF-I recruits an ATP dependent nuclear remodeling complex called NoRC and DNA methyltransferases called Dnm1 and Dnm3b, as well as histone deacetylases (HDAC1) interact with these nucleolar remodeling complex and as a result, it results in DNA methylation as well as histone deacetylation.

So, what is the mechanism by which rDNA transcription is regulated epigenetically? It actually involves the transcription termination factor 1, it actually recruits a chromatin remodeling factor called NoRC, nucleolar remodeling complex and this nucleolar remodeling complex now, interacts with certain specific DNA methyl transferases as well as a histone deacetylases called HDAC1 and as a result, the rDNA promoter is methylated in vitro of the histones in the rDNA promoter are deacetylated and this results in the prevention of binding of the UBF to the methylated promoter and as a result, rDNA transcription is suppressed.

So, phosphorylation of transcription factors involved in rDNA transcription as well as DNA methylation in histone deacetylation are two major mechanisms by which rDNA transcription is shut off, but epigenetic, this mechanism what we discussed now is actually involved in what is called as a rDNA silencing and is actually a long term rDNA repression. Methylation of the histone H3 on lysine 9 and subsequent binding of heterochromatin protein HP1 ultimately helps the condensation of nucleosomal DNA into silenced chromatin.

So, rDNA silencing basically, involves methylation of the H3 on the lysine 9 residue and these methylate lysines are recognized by these HP1 protein, which again we discussed in detail, when we discussed about histone phosphorylation. Histone methylation in the previous classes and once the HP1 bonds, it initiates hetero chromatinization of the promoter region leading to shut off of the transcription and in fact, what is the evidence that epigenetic regulation of transcription takes place in the case of pol I?

If you actually treats cells which inhibits status in methylation as well as inhibits histone deacetylation, it actually can result in enhancement of rDNA transcription clearly indicating that cytosine methylation as well as histone deacetylation play a very important role in the regulation of rDNA transcription and this is the major mechanism by which rDNA transcription is regulated in a epigenetic manner.

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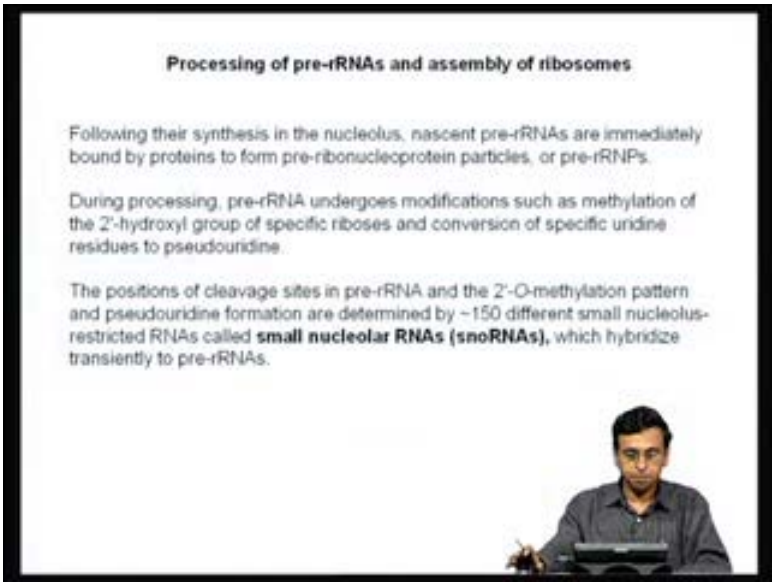

Now, let us spend some time to understand how is the precursor rRNA is processed. Like I told you, a single 48S rRNAs is synthesized from the ribosomal DNA and then this is then cleaved into 28S, 18S and 5.8S rRNA. How does it take place? So, following a synthesis in the nucleolus, nascent pre ribosomal RNAs are immediately bound by proteins to form pre-ribonucleoprotein complex called pre-rRNPs.

Again, you can see all these are analogous to what we discussed in the case of the pol II transcription. Just as in the case of pol II transcription, you have capping factors, you have splicing factors and polyadnelation factors which all go and interact with the RNA

and form RNPs, ribonucleo proteins, in the same way, immediately after the synthesis of the ribosomal RNA, these ribosomal RNA also interacts specifically with protein molecules resulting in the formation of ribosomal ribonucleoproteins or rRNPs.

So, these proteins actually process these pre-RNPs so that you get 28S, 18S, 5.8S rRNA. During this processing, pre-RNA undergoes several post transcriptional modifications just like the mRNA undergoes mRNA capping, the cytosine is modified as 5 methyl cytosine they are in the 5 prime end. Similarly, pre rRNA undergoes several base modifications such as methylation of the 2 prime hydroxyl group of specific ribosugars as well as conversion of specific uridine residues into pseudouridine residues.

So, specific base modifications also takes place in the case of the as a part of RNA processing in the case of pre-rRNA processing. The positions of the cleavage sites in pre-rRNA as well as the 2 prime O methylation pattern and pseudouridine formation are determined by at least 150 different small nucleolus restricted RNAs and these are called as small nucleolar RNAs or snoRNAs. So, just like we have all these unsnRNPs, which are involved in unsrnRNP in the case of splicing, similarly, you have what are called as snoRNAs which are actually involved in the processing of pre-rRNA to get matured ribosomal rRNAs.

I will not go into the exact mechanism by which these kind of pre-rRNAs processing takes place. It is a little bit advanced; so, we do not have to worry about it. Just remember, pre-rRNA processing involves base modifications such as conversion of uridine to pseudouridines as well as the modification of the ribo sugars. Also, it involves specific cleavage of the 48S rRNA percursors so that you get 28S, 18S, 5.8S RNA and these entire reactions are catalyzed by specific RNA molecules known as small nucleolar rRNAs, which are present uniquely inside a nucleolus and these snoRNAs are uniquely involved in the processing of pre-ribosomal rRNA molecules.

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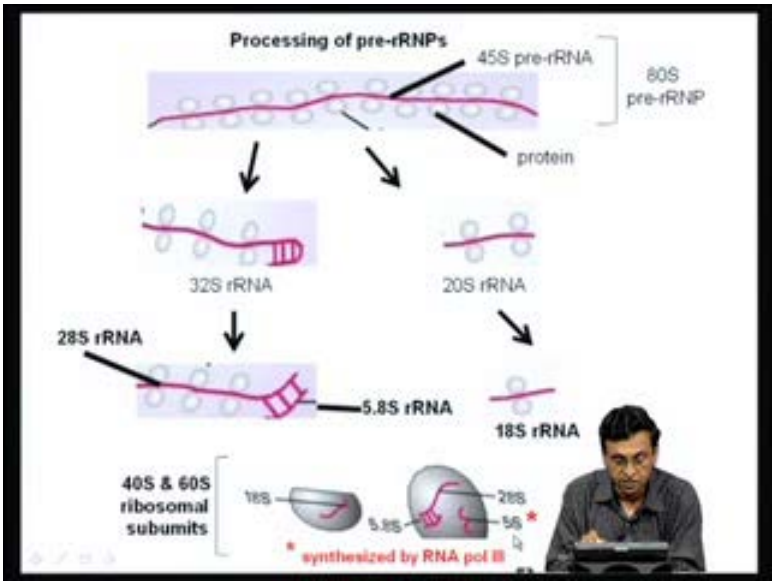

So I will just put a cartoon here just to register in your mind, what are the key steps involved in rRNA processing. For example, pre-rRNP processing involves, as soon as the 48S RNA is transcribed, it immediately associates with the number of proteins so that the rRNA now becomes a 80S pre-r ribosomal ribonucleo protein or pre-rRNP 80S about a molecular weight of 18S. So, the 80S pre-rRNP contains both 48S rRNA shown in red here as well as protein components.

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So, ultimately these 5.8S rRNA, 80S rRNA and 28S rRNA form the components of the ribosomal sub units. The 18S rRNA, for example, is a component of the 40S ribosomal sub unit, the 28S and 5.8S rRNA form the components of the 60S ribosomal sub unit. In addition, the 5S rRNA, synthesized by the polymerase III also joins and becomes a component of the ribosome. So, the RNA components of the ribosome, three of them come from ribosomal DNA and one of them come from the pol III transcription.

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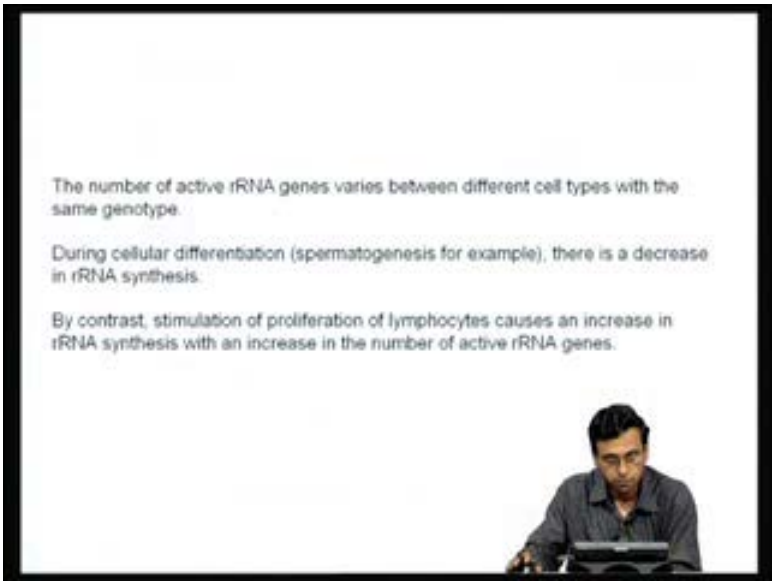

So, let us now spend some time to understand what is the significance of study of ribosomal DNA transcription. Why should we understand the regulation of rDNA transcription. So far, I discussed what are the major cis-acting elements involved in pol I transcription and we also discussed what are transcription factors which bind to the core promoter region as well as the upstream control region and how rDNA transcription is regulated in a cell cycle specific manner and we know that during mitosis because the nucleus and nucleolus disintegrate, there is no rDNA transcription.

And during this process, during mitosis, certain factors like the SL1 also undergoes or other factors like UBF, they are also phosphorylated in a cell cycle specific manner leading to their inactivation. So, phosphorylation, dephosphorylation of these transacting factors is one mechanism by which rDNA transcription is regulated and I also discussed what is called as a rDNA silencing, which involves epigenetic mechanism of regulation, wherein DNA methylation as well as histone deacetylation plays a very, very important role. In hetero chromatinization, the rDNA transcription leading to rDNA silencing and then we discussed the RNA processing, how rRNA is processed into 28S 18S and 5.S RNA and unique RNA molecules called small nucleolar RNA or snoRNAs play very very important role in rRNA processing and generation of these three different rRNA molecules, which together with a 5S rRNA synthesized by RNA polymerase III contribute to the RNA component of the ribosome.

This is the major mechanism by which ribosomal RNA is synthesized and how rDNA transcription is regulated. Now, let us see in the context of physiological context, what is the significance of study of ribosomal DNA transcription. The number of active rRNA genes varies between different cell types even within the same organism. So, even if you take the ribosomal DNA transcription from liver, it is different from that of muscle, brain and so on and so forth because it depends on the metabolic status of the cell type.

Similarly, during cellular differentiation, for example, spermatogenesis there is a dramatic decrease in ribosomal RNA synthesis. In the contrast, when you stimulate cell proliferation, for example, if you take lymphocytes and add molecules like Phytohaemagglutinin (PHA), it enhances cell proliferation and during sub cell proliferation, there is a dramatic increase in the number of rRNA gene transcriptions as well as rRNA synthesis. So, when cells undergo differentiation, there seems to be decrease in rRNA synthesis or rDNA transcription, but when you stimulate cell proliferation, there seems to be an increase in the rDNA transcription.

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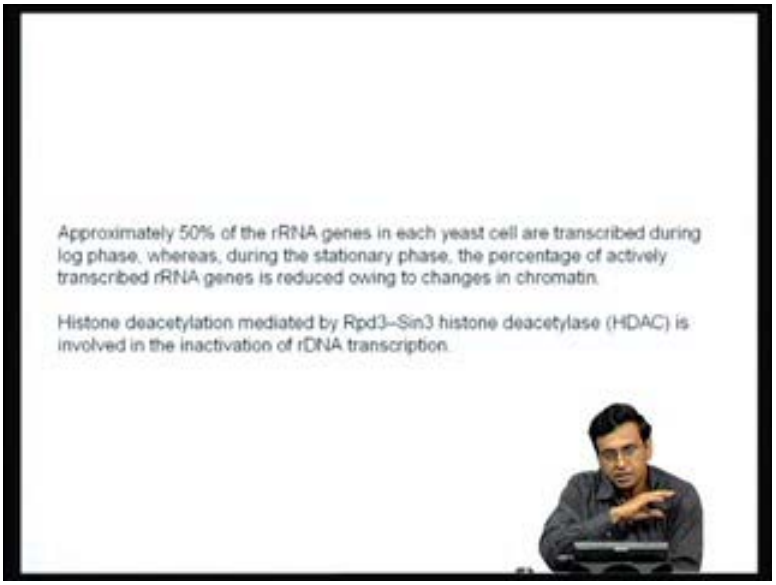

Now, although there are hundreds and hundreds of copies of rDNA genes inside the nucleus, not all these rDNA genes are transcribed at a time. Only about 50 percent of the rRNA genes are transcribed in a yeast cell during log phase, whereas during stationary phase, the percentage of actively transcribed RNA genes is reduced owing to changes in chromatin.

So, depending upon whether cells are actively dividing or if the cells are in resting phase or stationary phase, the percentage of rDNA enzymes are transcribed varies and in fact, histone deacetylation mediated by Rpd3-Sin3 histone deacetylase complex has been shown to be involved in the inactivation of rDNA transcription and as we discussed in the previous slides, this constitutes the epigenetic regulation of rDNA regular transcription.

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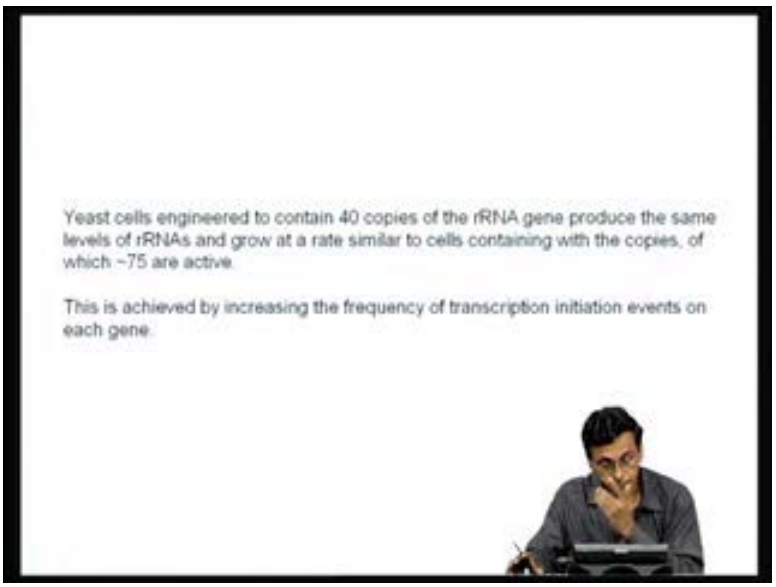

There are very interesting experiments which have been conducted to actually study rDNA transcription, for example, in the case of yeast cells, which have been engineered to contain 40 copies of rRNA genes instead of the normal 75 and they asked the question would they produce same level of RNA; that is, if you reduce the number of rDNA copies, does the rRNA transcription also go down or does it remains the same. Very interestingly, they found although you decrease the number of copies of rRNA genes, they still grow at the same rate as the wild type yeast cells and the amount of RNA produced still was the same and it turns out that is why these kind of experiments actually told that not all the rDNA copies are transcribed at the same time. Depending upon the need, only a percentage of the rDNA genes are transcribed and this can be finely regulated depending upon the need of the cells.

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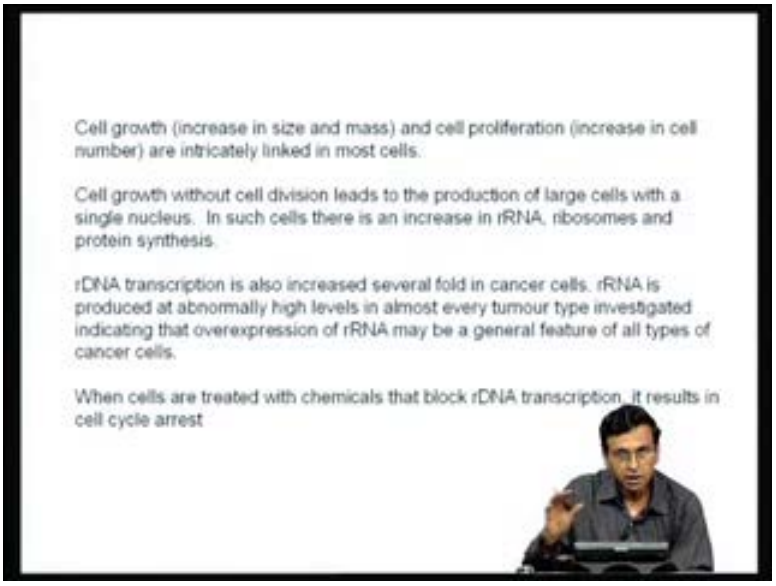

Similarly, cell growth - when cells grow, there is actually an increase in size as well as increase in the mass, whereas when cells divide, there is an increase in the cell number. All these events are intricately linked and cell growth without cell division leads to production of large cells with a single nucleus and in such cells, there is a dramatic increase in the ribosomal RNA, ribosomes as well protein synthesis

So, when you have huge cells with a huge amount of cell volume, there is a high level of ribosomal DNA transcription and the rDNA transcription also increases several fold in cancer cells - in actively dividing cells. The rRNA is produced at abnormally high levels in almost every tumor type investigated indicating that over explosion of rRNA may be a general feature of all types of cancers. In fact, people are even thinking, if you measure the rDNA transcription, it can serve as a prognostic marker for diagnosing cancer because invariably in all cancer cells, there is a dramatic increase in the rDNA transcription. So, the levels of rDNA transcription can serve as prognostic markers for cancer and when cells are treated with chemicals that block rDNA transcription, it results in cell cycle arrest.

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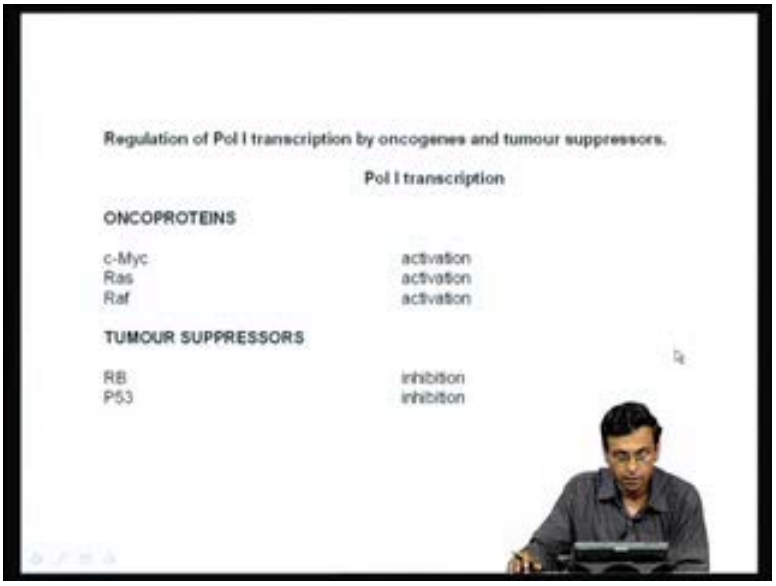

I just give you an example to actually show how pol I transcription actually can be regulated in cancer cells as we know cancer is either because of over expression of oncogenes or lack of tumor suppressor genes. So, just like an automobile has a accelerator and a brake, if oncogenes are over expressed, that is if we want automobile to speed up and lose control, you press accelerator too fast or if you do not want the automobile to stop, if the break fails, then also it can result in accident.

In the same way, cancer can be either because of over expression of oncogenes or lack of exploration of too much of suppressor genes and people have actually found that the protein such as c-Myc, Ras and Raf, which are actually oncoproteins, they all activate pol I transcription, whereas transcription factors like RB and P53, which actually act as brakes, which inhibit cell proliferation, they actually inhibit pol I transcription.

So, there is a direct correlation between protein, which actually enhance cancer, which cause cancer, they activate pol I transcription or protein which inhibits cell proliferation or act as tumor suppressor genes, they seem to inhibit rDNA transcription indicating that there is a very good correlation between cancer phenotype and the level of rDNA transcription.

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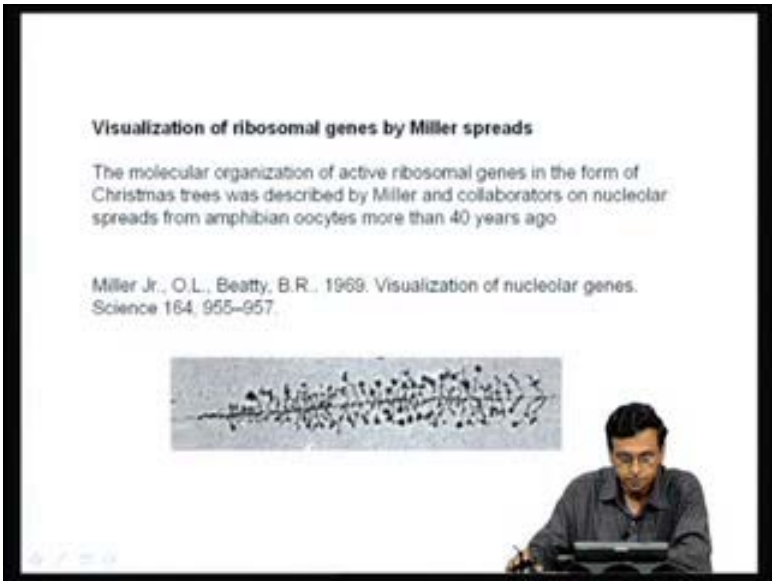

There are very interesting experiments, which have been done to actually visualize rDNA transcription as early as 1969, for example, Miller and Beatty actually demonstrated how we can actually demonstrate the actively transcribing rDNA genes and it can actually demonstrate, these are actually called as Christmas trees. So, the molecular organization of active ribosomal gene in the form of Christmas trees was described by Miller and collaborators on nucleolar spreads from amphibian oocytes more than 40 years ago. In fact, these are popularly known as Miller spreads. So, you can actually see actively transcribed in rDNA genes they can actually demonstrate RNA s actually coming out. these are actually called as Christmas trees.

So, what I have actually done in this particular class is to describe in detail, the mechanism by which the organizers of ribosomal DNA genes, the mechanism by which ribosomal DNA genes are transcribed as well as the regulation of ribosomal DNA transcription and I made it very important to made it a point to emphasize the fact that ribosomal DNA transcription plays a very, very important role because unless there are ribosomes, cells cannot make proteins. Therefore, depending upon the If the cells have to make lot amount of proteins, they have to need large amount of ribosomes. That means the rDNA transcription has to go on very high levels and depending upon the physiological status of the cell, metabolic status of the cell and depending upon the cell cycle, the rDNA transcription is very finely regulated. There are two major mechanisms of regulation of rDNA transcription: one is post transcription, modifications of

transcription factors like UBF, SL1 and so on, so forth; other major mechanism is called as rDNA silencing, which involves DNA methylation, methylation of the rDNA promoters as well as histone deacetylation leading to hetero chromatinization and this results in what is called as a rDNA silencing. I think, with this I have completed both pol II transcription and pol I transcription. In the next class, we will study how pol III transcription is regulated and what is the significance, what is the mechanism by which tRNA genes are transcribed and tRNA synthesis is regulated inside the cells. I think I will stop here.