**Eukaryotic Gene Expression**

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**Module No.# 03**

**Lecture No. # 10**

**Eukaryotic Gene Regulation: Chromatin Remodelling**

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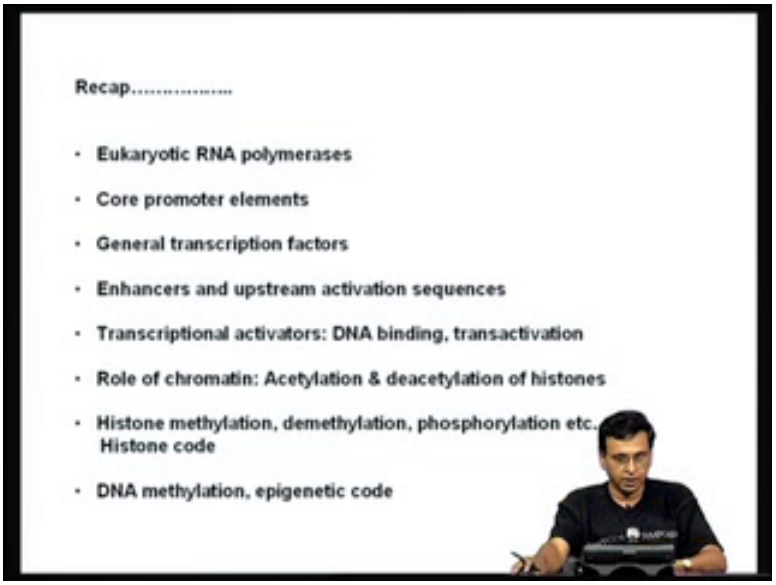


Today's topic is primarily focused on chromatin remodelling; how chromatin remodelling affects gene expression in eukaryotic cells is what is going to be discussed in this particular lecture, today.

Again, to recapitulate what I have discussed so far, again, I will not start all of them. We will start it from eukaryotic RNA polymerases, then discussed about core promoter elements, then we discussed about various general transcription factors, how they, in conjunction RNA polymerase, form a pre initiation complex and results in basal levels of transcription.

Then, we discussed about enhancers in upstream activation sequences, and how transcriptional activators and transcriptional repressors through the DNA binding

domains, and is the transcription activation domains or transcription repression domains, bind to specific sequences in the upstream regions of promoters, and bring about either increasing levels of transcription or decreasing levels of transcription.

Then, we very briefly discussed about the role of chromatin, and discussing the role of chromatin gene regulation, we focused primarily on how post translational modifications of histones play a very important role, when, for example, histones are phosphorylated, or in histones are acetylated, you results in positive regulation of gene expression, and even histones are deacetylated and histones are methylated, it results in condensation of chromatin and results in repression of transcription, and based on the studies of histone methylation, demethylation, and phosphorylation, we had discussed what is called as a concept of a histone code, where specific post translational modifications can be a signature motif for predicting certain gene functions.

We can clearly say, for example, there are, if is for example, if a particular lysine residue is methylated or acetylated, you can predict whether that chromatin is going to be euchromatin or heterochromatin. This is what is people are now talking about histone code; it is still not perfected.

So, many people think that we are still not ready to talk about histone code, and then we discussed in the last class about another important area of gene regulation, about how DNA methylation plays a very important role in the regulation of gene expression and how, among the four bases of DNA, cytosine can actually be methylated by enzymes called DNA methyltransferases, and methylation of the cytosine, then, is methylated cytosines are recognized by specific methyl CPG binding proteins, and these methyl CPG proteins, when they bind to the cytosine residues of the DNA, can now attract negative regulators of gene expression like the histone deacetylases or histone methyltransferases, and this results in repression of gene expression.

And most importantly we discussed, DNA methylation, in conjunction with histone modifications, constitute what is called as an epigenetic code of gene regulation. This is what the crux of what I have discussed, so far.

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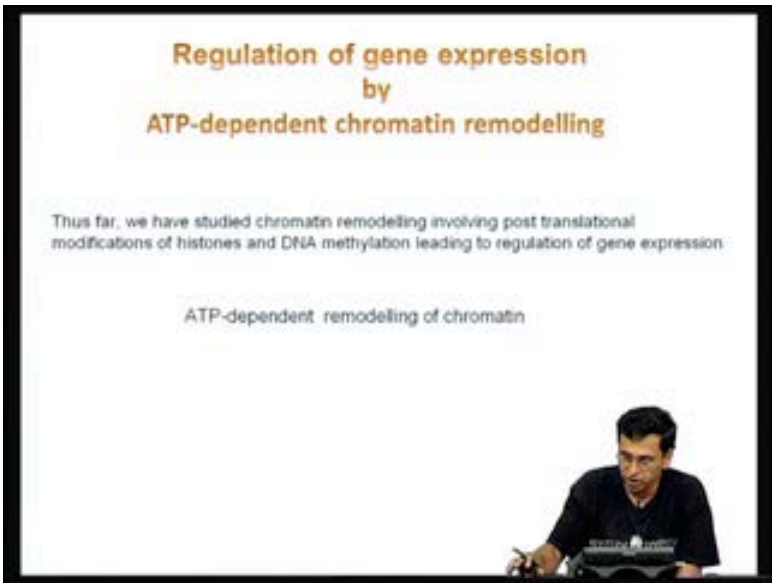


And today, we are going to discuss about another very important and a new area of investigation in the area of eukaryotic gene regulation, that is, chromatin structure can also be modified, or can also be modified not only by modification of the histones or histone tails, but also by an ATP-dependent chromatin remodelling mechanism. This is what is going to be the focus of today's discussion.

So, we, so far, we have studied chromatin remodelling involving post translational modifications of histones and DNA methylation, and how DNA methylation, in conjunction with chromatin modifications of histones, can result in regulation of gene expression. This is what discussed.

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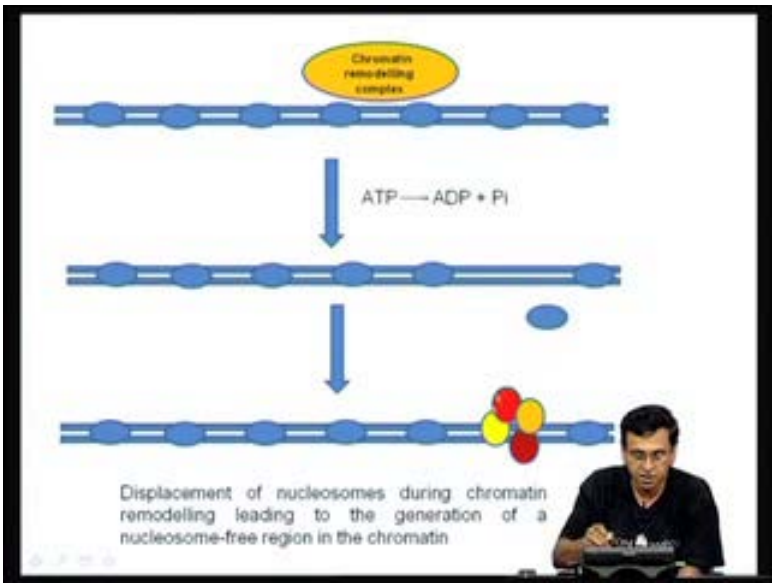


Today, we are going to discuss how there is also what is called as a ATP dependent remodelling of chromatin, which is very different from covalent modifications of histone tails. This has nothing do with covalent modification of histone tails, but there is an ATP-dependent chromatin remodelling machinery, which actually can also bring about, which can alter chromatin structure, and therefore, bring about chromatin regulation.

Let us see what happens now. In this cartoon, let us say, we have a double stranded DNA and we have histones, which are regularly placed in a normal double stranded DNA. Now, there are proteins there are actually multi, multiprotein complexes, which together are now called as chromatin remodelling complexes, and when these chromatin remodelling complexes bind, are recruited to the chromatin, and these chromatin complexes in an ATP dependent manner, when ATP is converted into ADP and P i, energy is released. This energy derived from the APT a ATP hydrolysis, which actually used by chromatin remodelling complex to actually displace histones from a DNA, and as a result, you generate what is called as a nucleosome-free regions, and these nucleosome-free regions can now expose binding sites for transcription factors or the pre initiation complex assembly, resulting in activation of transcription.

So, this ATP-dependent chromatin remodelling has become another important mechanism by which transcription regulation is brought about in eukaryotes. So, displacement of nucleosomes during chromatin remodelling leading to generation of a

nucleosome-free region in chromatin, resulting in the exposure of certain DNA binding sites, so the transcription factors can now come and bind, and then initiate gene regulation, is another very important area in the regulation of gene expression in eukyarotes.

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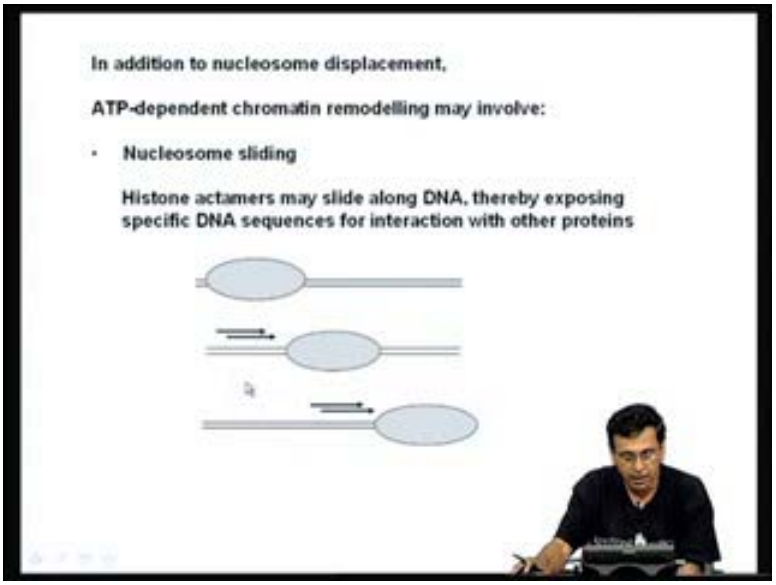


In addition to displacement of nucleosomes, chromatin remodelling utilizing ATP can also involve several other mechanisms. For example, instead of actually displacing nucleotides, there are certain chromatin remodelling proteins, which actually slide nucleosomes little bit further.

You can see here; histone octamers may actually slide along DNA, therefore, exposing specific sequences for interacting the other (( )). For example, here, there is an histone octamer, and by the influence of a chromatin remodelling complex, this nucleosome is actually pushed further down, and it can be even be slided further down.

So, by sliding these histones little bit away, in can even be as little as one base pair; it can even be slide as little as five base pairs; by just moving or sliding histones little bit, you may be exposing a transcription factor binding site. Therefore, these sites, which were not accessible to certain transcription factors, now, can become accessible to transcription factors. Therefore, genes can either be activated or repressed depending upon an activator or repressor has been recruited.

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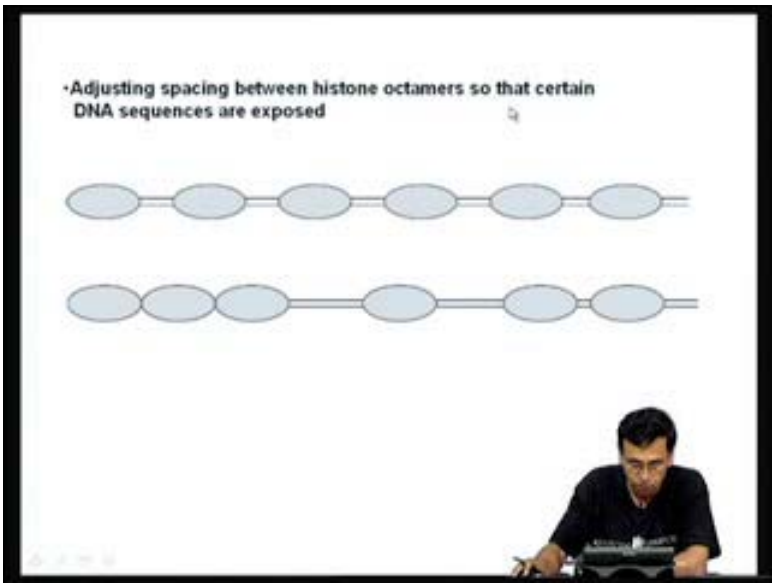


So, nucleosome displacement or nucleosome sliding, and even adjusting the spacing between histone octamers, all these are major mechanisms, by which chromatin remodelling proteins bring about changes in chromatin structure, which affecting, which affect gene regulation.

Here is a cartoon. For example, here is a DNA, in which nucleosomes are regularly spaced, and there are chromatin remodelling machinery, which actually alter this nucleosome spacing, therefore, exposing certain sites, which was previously not accessible to transcription factors or transcription machinery. Therefore, proteins, which could not bind when the nucleosomes are regularly spaced, can now bind after the chromatin remodelling machinery has altered the nucleosome spacing.

So, there are number of mechanisms by which the nucleosome positions can be altered, in an ATP-dependent manner, by certain protein complexes, and these are known as chromatin remodelling machines. So, what we are going to discuss today is that, what are these chromatin remodelling machines and how they influence gene regulation.

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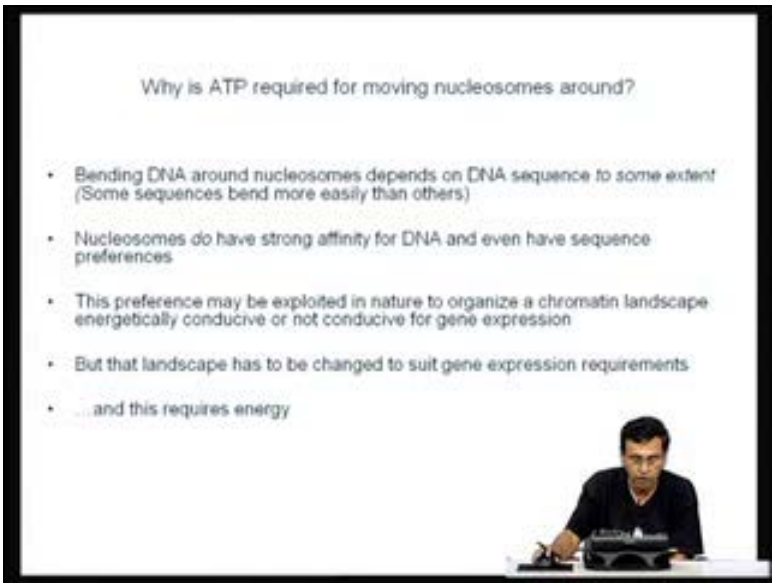


Now, the question we are asking is that– why do we need ATP for moving this nucleosome around, either for sliding nucleosomes or for displacing nucleosomes? Why do you require ATP? As you know, bending DNA around nucleosomes depends on the DNA sequence, to most of…, to some extent, and it is been very well shown by a number of experiments that there are certain sequence, depending upon the DNA sequences, certain DNA sequence can much, bend much more than other sequences.

In fact, this DNA bending has become a very important aspect of gene regulation, because when we have long stretches of DNA, there have been examples where certain transcription factors can actually bind several thousand kilobases upstream region, but can still affect the rate of initiation at the promoter region.

Now, the question is, how transcription factors, which are binding, so far, upstream, can actually influence the rate of transcription initiation. It actually turns out, this DNA which is in-between, can actually loop out, and as a result, this transcription factor, which is finding very far, can actually brought in a very close proximity to the general transcription machinery. Therefore, you can interact with some of the general transcription factors influencing regulation.

So, this DNA bending plays a very important role in the transcription initiation, and many a times, the nucleotide sequence of DNA plays a very important role, whether the DNA can be bent or not.

Now, nucleosomes, we all know, have a very strong affinity for DNA, because histones are positively charged, DNA is negatively charged. And therefore, histones bind DNA very tightly and many a times, some of the histone-DNA attractions may also be sequence-specific.

So, this specific, this limited specificity of histone-DNA interactions has been utilized by nature to organize chromatin in a specific manner. Now, otherwise, you cannot organize nucleus. There is what is called nucleosome spacing; there is a hundred and twenty three base pair DNA which is wrapped on neuron; there is a nucleosome-free region, and nucleosomes are regularly organized in a very specific manner inside the DNA.

Therefore, there is some kind of an organization of chromatin, and this may be primarily mediated by the histone DNA contacts and histone DNA interactions, and in nature, the nature has divide this mechanisms in such a way that a chromatin landscape is energetically conducive; sometimes it may be conducive for gene expression, sometimes it may not be conducive for gene expression, OK?

Now, if this landscape remains as such, then modulation is not possible. It is like a traffic movement in a traffic junction; you need to have these traffic lights, because depending upon the traffic movement, you have to either give a green light or a red light.

So, depending upon whether gene has to be expressed in high levels or a gene has to be expressed in low levels, chromatin structure has to be altered. If the chromatin structure remains as such all the time, then modulation of gene expression is not possible. That is what we have decided in the, we had discussed in the last few lectures.

So, this chromatin landscape, which is determined by specific histone-DNA interactions, has to be modulated by a number of mechanisms, including histone post transcription modifications which we discussed earlier.

So, the landscape, the chromatin landscape, has to be changed to suit gene expression requirements. That means, the histone DNA interactions has to be disrupted, and this requires energy.

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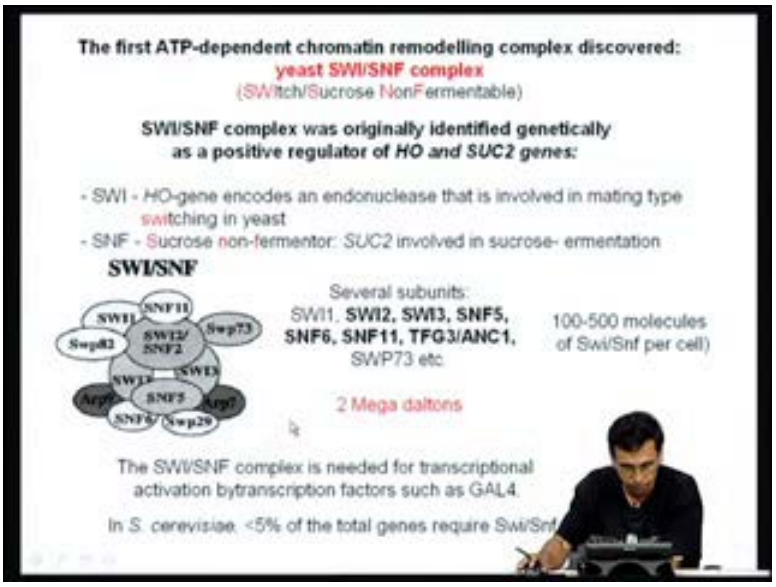


So, if we have to move around or replace histones on different regions, in addition to adding negative charge or positive charge on the histones by post transcription modifications, if we have to remove these histones or move around histones, you need to consume energy to move these nucleosomes around.

Now, the first ATP-dependent chromatin remodelling complex was discovered in the budding yeast Saccharomyces cerevisiae, and this actually called as SWI/SNF complex. What is this SWI/SNF complex? SWI actually means, it was actually discovered in yeast mating type switching. SWI stands for switching of mating types in yeast.

So, there is a gene called HO, which encodes an endonuclease, which plays a very important role in regulation of mating type switching in yeast, and, in fact, for this mating type switching, they found out if a mutations in this SWI gene, then mating type switching is impaired, clearly indicating that there are some kind of a little link between this SWI proteins and mating type switching in yeast, and that is how this SWI complex was actually (( )).

Another important component is called SNF. SNF actually stands for Sucrose NonFermentable average there is sucrose fermentation, people have realized, again, if we have mutations in this kind of a chromatin re[modeling]- com[plexes]- -[re]modeling complexes again, genes involved in a sucrose fermentation metabolism of sucrose fermentation is affected because of the gene expression changes. Therefore, the first ATP-dependent chromatin remodelling complexes were designated as SWI/SNF complex, and this SWI/SNF complex encodes genes which affect regulation of yeast mating (( )) and genes involved in sucrose metabolism.

And people have then went ahead and purified the all the components of these chromatin remodelling complexes, and as we can see these chromatin (( )) complex, namely SWI/SNF complexes are a multiprotein complexes, and often, the complex is very huge; it can go up to 2 mega Daltons, and it contains a number of subunits, which are actually designated as y one's, y two's, y three's, y five, and so on and so forth.

So, chromatin (( )) complexes are multiprotein complexes which play a very important role in the regulation of gene expression by modifying chromatin structure, but the number of chromatin remodelling complexes may not be very high; it is only about 100 to 500 molecules of SWI/SNF may be present per cell.

And as a result, only about five percent of the total genes in the yeast cells are actually, expression is regulated by these SWI/SNF complexes. So, these protein ATP chromatin remodelling complexes are involved in, have a limited role in the regulation of gene expression. Not all the genes are regulated by chromatin remodelling complexes; only the subset of genes are actually affected by this chromatin remodelling mechanism.

For example, if you talk about the regulation of genes involved in galactose metabolism, we all know, for example, the GAL 4 transcription factor plays a very important role in switching on genes involved in galactose metabolism, and the…, this GAL 4, if we, it has to activate genes surrounding galactose metabolism, requires this SWI/SNF complex.

So, there are certain transcription factors, if they have to act or activate gene expression, they primarily do it by recruiting chromatin remodelling complexes.

So, certain transcription factors bring about gene activation by recruiting histone modifying enzymes, whereas certain transcription factors regulate gene expression by recruiting chromatin remodelling complexes, and there are many instances a combination of both histone modifications as well as chromatin remodelling machinery together bring about regulation of gene expression of a particular promoter.

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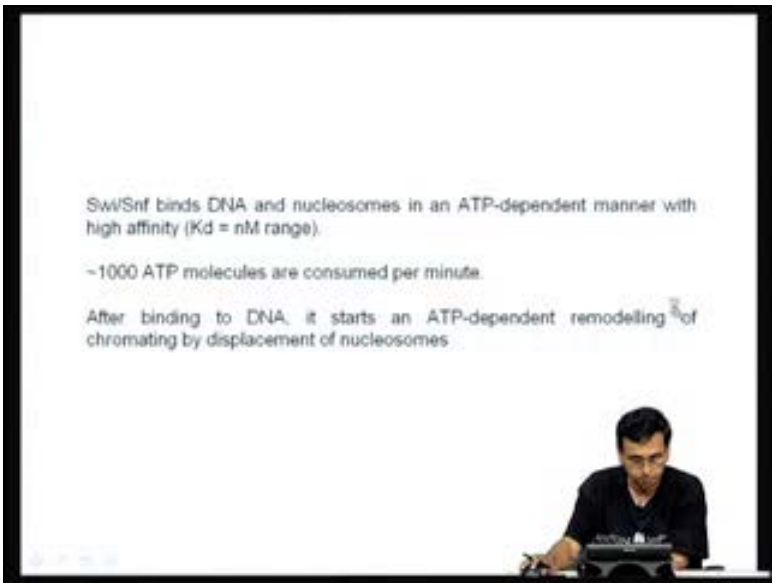


Now, the SWI/SNF binds DNA and nucleosomes in a ATP-dependent manner, with a very high affinity the Kd of SWI/SNF complex for DNA and nucleosomes in the nanomolar range. So, they bind very tightly.

So, about 1000 ATP molecules are shown to be consumed per minute. So, it is a very energy demanding reaction. After binding DNA, the SWI/SNF complex starts in ATP dependent remodelling of chromatin, by actually moving around histones or even displacing histones.

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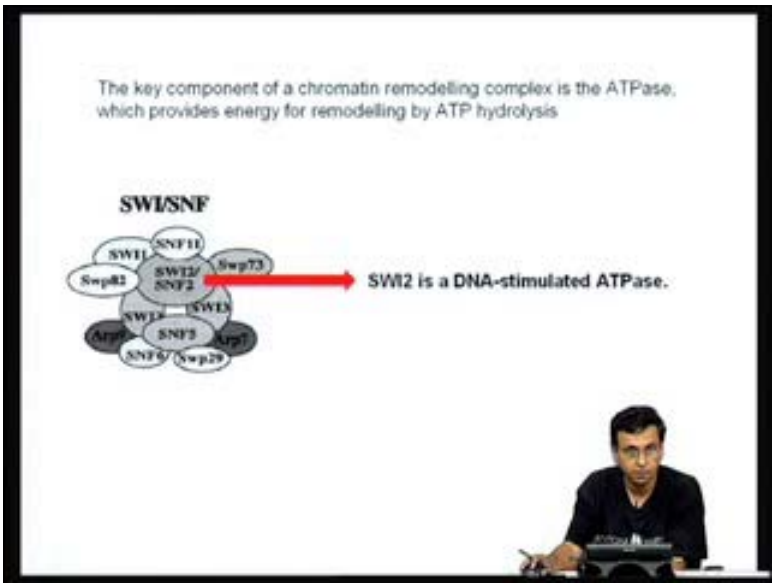


The key component of these chromatin remodelling complexes, I told you, there are number of protein complexes in these chromatin remodelling complexes. One of the key components of this chromatin remodelling complex is a protein which contains a ATPase activity; that means, it is capable of hydrolyzing ATP to ADP and P I, and since the entire process requires energy, this ATPase activity is very important for the entire remodelling process.

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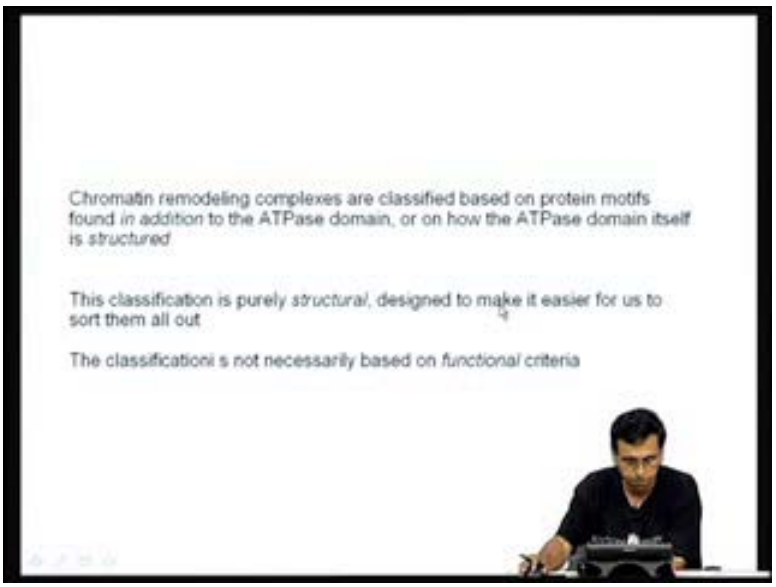

So, one of the key components of the chromatin remodelling complex is an ATPase, for example, this SWI/SNF complex the protein called as SWI2 is actually DNA stimulated ATPase, which actually plays a very important role in the entire process of chromatin remodelling.

Now, chromatin remodelling complexes are classified based on the protein motifs found in addition to the ATPase domain. So, they also contain number of protein motifs.

And also, how the ATPase domain itself is structured? Remember, if you have to, if you once you start isolating or identifying this chromatin remodelling complexes in a number of organisms, you have to organize them in a special format, so that you can understand. You have the group them based on, you need to show whether the human enzymes are related to yeast enzyme, or an yeast enzyme is related to Drosophila enzyme, and so on and so forth.

So, you need to identify certain pattern. Therefore, these chromatin remodelling complexes from different organisms have been grouped depending upon specific structural motifs they have, and also depending upon the ATPase domain.

If, for example, a Drosophila chromatin remodelling complex ATPase domain is homologous to that of a human, then we put them under the same family, so on and so forth. So, this classification is purely structured, designed to make it easier for us to sort them all out, otherwise it becomes very difficult to understand, and, and relate the various studies being carried out in different organisms, and this classification is not necessarily based on any functional criteria.

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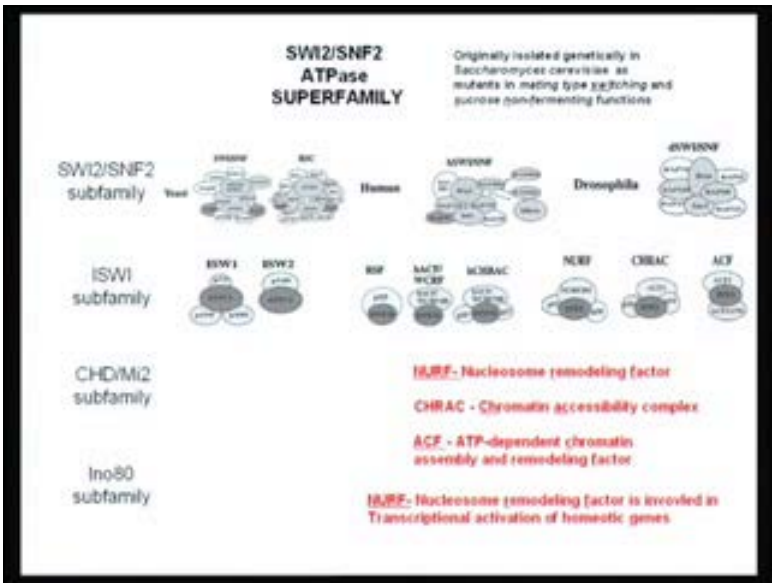


I just given one example of showing how the various chromatin remodelling complex have been classified into different families. For example, this is what is called as a, if you take, for example, this SWI/SNF2 ATPase super family. Remember, the SWI/SNF chromatin remodelling complex are the first chromatin remodelling complexes, which was discovered in Saccharomyces cerevisiae, and this family now consist of a, called as a SWI2/SNF2 subfamily. There is something called as ISW1 subfamily, CHD or MI2 subfamily, Ino80 subfamily, and so on and so forth, and each subfamily, their homologos have been identified in yeast, human, Drosophila, and so on and so forth.

And there is also all kinds of nomenclature. Again, I do not want to confuse you at this stage about this nomenclature. Just to give an example, what these things actually mean, for example, when you…, when you call a chromatin remodelling complexes NURF, which actually means nucleosome remodelling factor, nucleosome remodelling factor, NURF, that is what it means.

Similarly, there is a nucleosomeremodelling complex called c, h, r, a, c- CHRAC. It actually means chromatin assembly complex. CHR stands for chromatin assembly complex. So, these are all the abbreviations given to identify a different chromatin remodelling complex in different organisms. Similarly, ACF stands for ATP-dependent chromatin assembly and remodelling factor- ACF.

Now, in different organisms, different chromatin remodeling complexes play different roles, for example, if you take this NURF remodelling complex, the nucleosome remodelling factor is involved in the transcription activation of certain homeotic genes, in the case of Drosophila. This has been very well studied.

So, different chromatin remodelling complexes are involved in the regulation of different sets of genes in different organisms, and these names, these abbreviations are actually mean, that they have a certain meaning.

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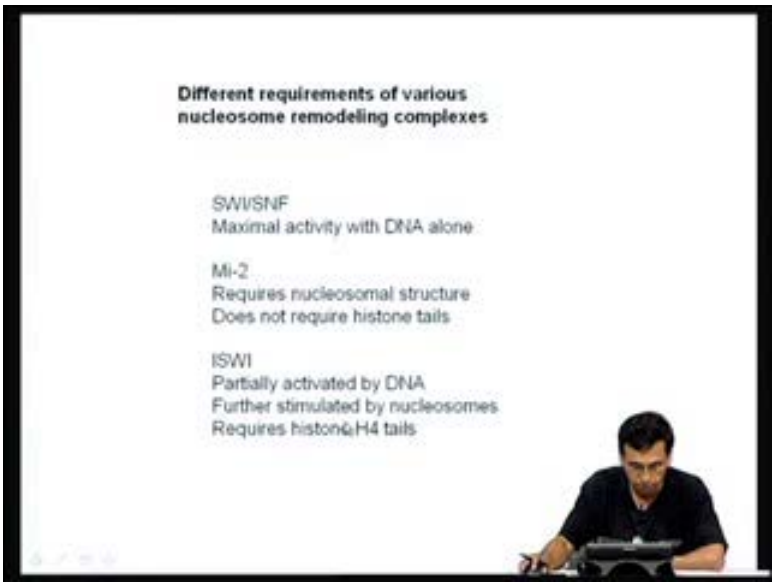


The different there are also different requirements for various nucleosome remodelling complexes. For example, if you take this SWI/SNF complexes, it has maximal activity in the DNA alone, without, not necessarily chromatin, even DNA alone, they can, they have a maximal activity.

If you now take, for example, MI2, it requires a nucleosomal structure for its function, and it does not require any histone tails, whereas if we take ISW1, it is partially activated by DNA, in the case of SWI/SNF, the DNA itself can activate the ATPase activity, whereas here, it requires a nucleosomal structure, whereas here it is partially activated by DNA and the DNA ATPase activity is further stimulated by nucleosomes, and it reverse histone tails.

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So, different chromatin remodelling complexes have different requirements for their remodelling activity. Now, just give three examples here. Now, how these chromatin remodelling complexes move around histones or nucleosomes, also vary from one chromatin remodelling complex to another.

For example, if you take the ISW1, it actually moves nucleosome from the central part of the nucleosome to the terminal position. They move the histones from the central part of nucleosome to the periphery, whereas if we take CHRAC, which also contains ISWI, it actually facilitates the movements of histones from terminal to the central region of the histones.

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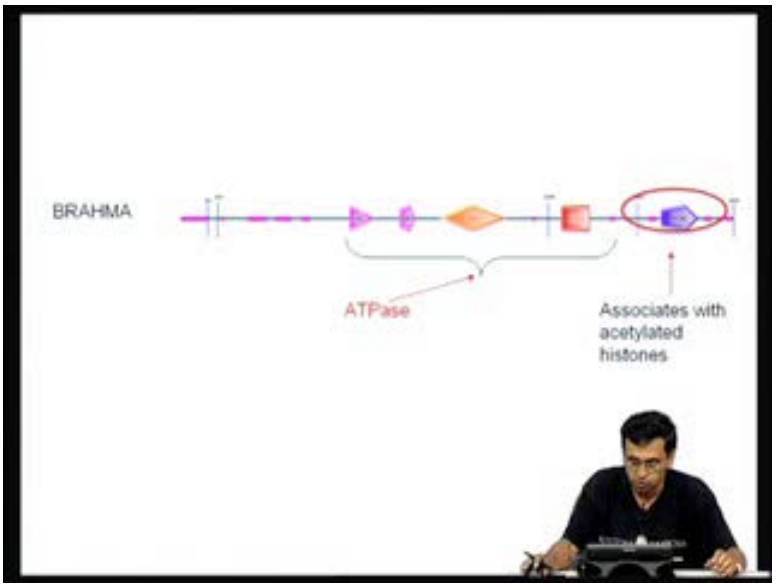


So, how they actually rearrange histone and nucleosome also depends upon one chromatin remodelling complex to another. The structure of all these chromatin remodelling complexes and their members have been very well studied. I just given one example here. For example, if you have a, a, there is a chromatin remodelling complex called BRAHMA, if you look at one after them, if you look at various components of it, it has an ATPase domain, and as I mentioned, the ATPase domain is very important for the remodelling activity, and it also has a bromodomain.

And if you go back and recall some of our histone modification studies, the bromodomains are very important for recognizing acetylated histones. So, the bromodomain proteins, one of the important activity of HATs is to recruit bromodomain-containing proteins. So, proteins recognize acetylated histone through their bromodomains. So, HATs actually contains bromodomains.

So, it also contain associates acetylated with, because this, this remodelling complex also contains the bromodomain, it can actually interact with the acetylated histone. So, anyhow, the chromatin remodelling histone acetylation can be brought together on a specific gene expression process.

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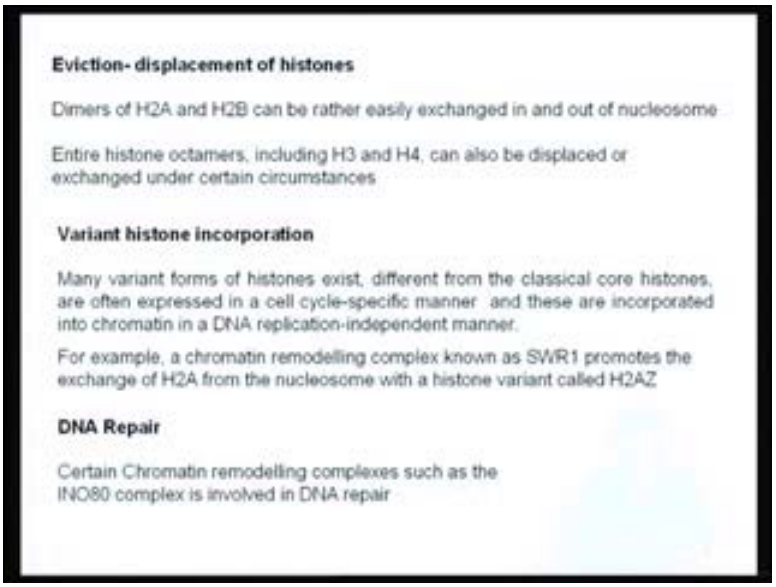


What are the mechanisms by which this ATP remodelling is brought about? There is a very nice article in Journal of Cell Science. These models are little bit complicated and very difficult to explain without figures, but since there are certain copyright issues in bringing some of this model figures into these lecture series, I am not been able to discuss in detail about the mechanistic insights of many of these models.

So, I have just, very briefly, mentioned. For example, there is something called a twist defect diffusion model where, in these models, small or local alterations from the mean twist defects propagates new, propagate around nucleosome; that is what this model talks about. Very difficult to explain these models without figures, but I suggest you refer this article in Journal of Cell Science, which very nicely describes all these models of ATP remodelling using very nice cartoons and very nice figures.

There is also what is called as cis-trans chromatin remodelling. Now, the nucleosomes can indeed not be moved around only within a particular chromatin fiber, if there are also what is called as trans remodelling, where histone or a nucleosome from one chromatin or one DNA template can be transferred to another chromosome or another chromatin fiber. So, in trans. So, a cis remodeling, trans remodelling, all these are possible when you talk about ATP-dependent chromatin remodelling.

There are also… chromatin remodelling not only just involves moving around histones, it also involves what is called as a displacement of histones. There can be exchange of histones, for example, dimers of H2A and H2B can be rather easily exchanged in and out of nucleosome, and entire histone octamers, including H3 also, can also be displaced or exchanged under certain circumstances. With the specific components of a nucleosomes can be exchanged, or the entire nucleosome octamer can be exchanged, and there are all kinds of variations when you talk about chromatin remodelling in different organisms.

And very importantly, chromatin remodelling also involves, what as called as a variant histone replacement. This also we have discussed in some of the earlier classes, where I have told you, there, there are variants of histones, which actually can be replaced in certain specific stages of cell cycle.

For example, there are, in addition to histone H2A, H2B, H3, and H4, and H1, there are many variations of this H2 histones occur, and many of these histone variants are expressed. For example, in during germ cells, during gametogenesis, certain histones are replaced by what are called testis specific variants, and so on and so forth.

So, many of histone variants actually exist, which are very different from the classical core histones, and they are often expressed in a cell type-specific manner, and these variants of histones can be incorporated to chromatin in a DNA replication-independent manner. For example, a chromatin remodelling complex known as SWR1 promote the exchange of S2A from nucleosome with a histone variant called H2AZ.

So, it is not just shuffling around histones for moving around nucleosomes; the core, the classical histones, can be replaced by very specialized histones by certain chromatin remodelling complexes.

And, in addition to transcription and regulation of gene expression, the chromatin remodelling also plays a very important role in DNA repair. For example, certain chromatin remodelling complex such as the Ino80 is actually involved in DNA repair process as well.

So, chromatin remodelling is a important not only for regulation of gene expression, but also has a very profound role in a number of other cellular process, including DNA repair.

So, the important point that I want to mention here is that, just as we have transcription factors which recruit histone modification enzymes like histone acetyltransferases, histone deacetylases, histone kinases, and so on and so forth, there are also many transcription factors which are capable of recruiting chromatin remodelling complexes. So, by recruiting these chromatin remodelling complexes, it can bring about regulation of gene expression.

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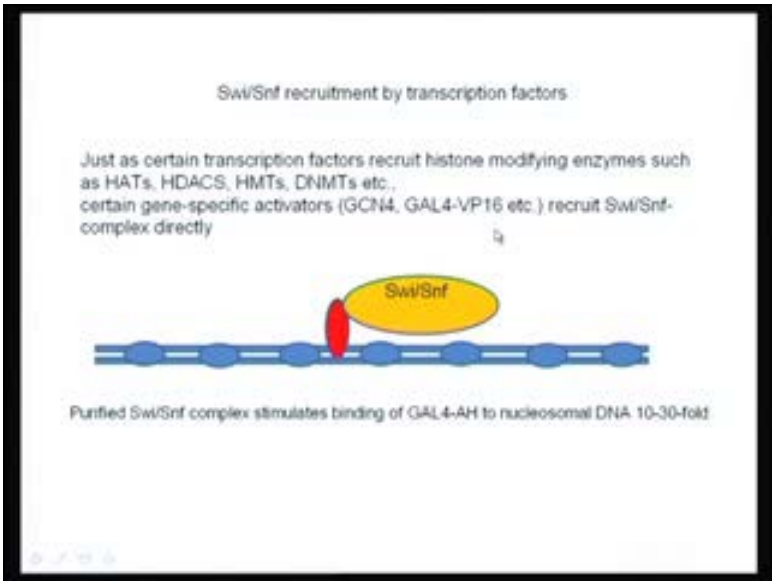


So, just as certain transcription factors recruit histone modifying enzymes such as HATs, HDACs, histone methyltransferases, or DNA methyltransferases, there are certain gene specific activators like GCN4, GAL-VP16, etcetera. Instead of recruiting these histone modifying enzymes, they can actually recruit SWI/SNF complex directly.

So, the chromatin structure can be altered either by a post translation modification of histone tails by recruiting these histone modifying enzymes, or chromatin structure can also be modified by recruiting chromatin remodelling complexes. So, it varies from one particular context to another context. For example, the purified SWI/SNF complex

actually stimulates the binding of GAL4 acidic activation domain to nucleosomal DNA by 10 to 30 fold.

So, the binding of a transcription factor to nucleosomal DNA can be very dramatically enhanced by some of these chromatin modifying complexes, because their affinity to some of this transcription factors.

So, one of the major mechanisms by which transcription factors regulated gene expression, is by direct recruitment of chromatin remodelling complexes. These chromatin remodelling complexes, using ATP, can now move around or shuffle around, or remove nucleosomes facilitating the binding of transcription factors, or assembly of preinitiation complex, resulting in activation of transcription .

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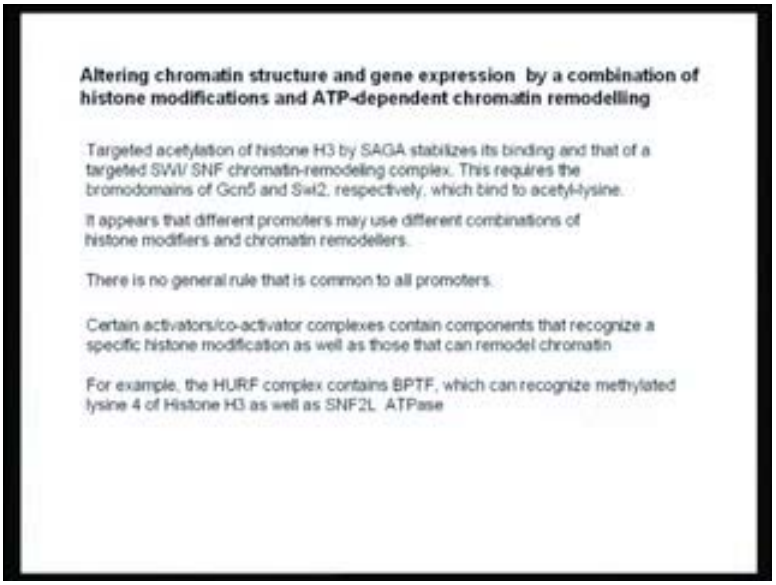


So, altering chromatin structure and gene expression by a combination of histone modifications and ATP-dependent chromatin remodeling. Remember, although we are studying all this processes in a very sequential manner, initially, we talked about histone modification, then we talk about DNA methylation, then we are now talking about chromatin remodelling.

But, in vivo, these things did not happen in a sequential manner. Many of these does not happen in a very concerted manner, and in any given promoter, a for example, transcription activation from a promoter by initially involved in acetylation of histone,

and this acetylation of histone may actually recognize a HAT complex, and this may also then recognize, involve the, lead to the recruitment of a chromatin remodelling machinery; and all these things, in conjunction, can result in the efficient recruitment of a pre initiation complex, resulting in faster turnover of RNA polymerase results in enhancement of rate of transcription.

So, all these process may actually act in concert to promote transcription initiation from a particular promoter, for example, targeted acetlylation of histone H3 by SAGA. As you remember, SAGA is a HAT containing multicoactivator, which was originally identified in the yeast.

So, it is a HAT-containing coactivator. Targeted acetylation of histone acetylation H3 by, acetylation of histone H3 by SAGA, stabilizes its binding and that of it targeted SWI/SNF chromatin remodelling complexes, and this actually requires the bromodomains of Gcn5 and SWI2 respectively, which bind to acetyl-lysine.

So, some of the members of this SAGA, Gcn5 is a member of the SAGA complex, and the Gcn5, actually, and SWI2, can now recruit a chromatin remodelling complex. Therefore, histone acetylation, in combination with chromatin remodeling, can actually involve in the regulation of gene expression by the SAGAcomplex.

So, it appears, different promoters may use different combinations of histone modifiers and chromatin remodellers. Like I said, if a particular gene has to be repressed, then a histone deacetylase in combination with specific chromatin modifier may bring about tighter assembly of histones, and therefore, transcription can be shut off.

On the other hand, if a particular gene has to be activated, a histone acetylase or histone kinase can, in conjunction to a specific chromatin modifier or remodeler, can bring about activation of gene expression by moving around or removing histones from the promoter. The take home message is that there is nothing like a general rule.

Now, we have, what kind of a combination of histone modifying enzymes and histone chromatin remodelling factors are working together, varies from promoter to promoter. In fact, the reason I am emphasizing this point is that, it is, if you now go to the literature, read some of the research articles, you may be flooded with examples and

examples and examples, each describing different combinations of histone modifying enzymes, different combination of chromatin remodelers, how they work together and bring about activation or repression of transcription.

And it is impossible to discuss all these examples. So, maybe we will discuss some specific examples as we go on this course, but there are so many variations, so many combinations. It is very difficult to discuss all these things. So, remember, a combination of histone modifying enzymes and chromatin remodellers together work, and this combination, ultimately, may decide whether the gene has to be activated or gene has to be repressed.

Certain coactivators or coactivator complexes contain components that recognize a specific histone modification, as well as those that contain, can that can remodel chromatin, for example, the HURF complex, which is a chromatin remodelling complex, contain what is called as a BPTF, which can recognize a methylated lysine of histone H3, as well as SNF2 ATPase.

So, here is a multiprotein complex, a coactivator, and one component of this coactivator is a histone, which can recognize methylated histones. Another member of this coactivator complex is actually a chromatin remodelling complex.

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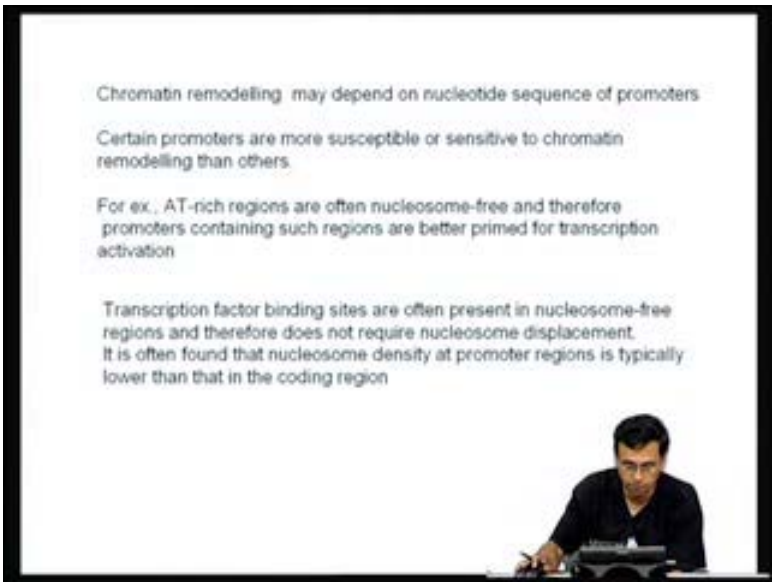

So, you can see, there are multiprotein complexes, which contain both histone modifying activity, as well as, or, a histone. It can recognize both the histone modification, and also containing component of a chromatin remodelling complex. So, all this things act in concert, ultimately, to bring about the regulation of gene expression.

The chromatin remodelling may also depend upon the nucleotide sequence of a particular promoter, and certain promoters are more susceptible or sensitive to chromatin remodelling than other promoters. The one best example that I can give you is that, certain AT rich regions are often nucleosome free, and therefore, promoters containing such AT regions are better primed for transcription activation. So, the nucleotide sequence may also, there are certain nucleotide regions, or certain promoter regions, or certain nucleotide sequences, which should be more amenable for chromatin remodelling than other promoter sequences or promoter, promoter nucleotide sequences.

Transcription factor binding sites are often present in nucleosome-free regions, and therefore, does not require chromatin nucleosome displacement. So, there are many instances, actually, where the transcription factor binding sites, actually, contain what is called as a DNA zone hypersensitive sites. That is, these sites are actually nucleosome free, and therefore, we take nuclei and treat them with DNase, certain promoter regions are can be very efficiently cleaved by DNase 1, because there are no nucleosomes there, and therefore, transcription factor can actually bind them very (( )).

Therefore, these are nucleosome-free regions. Therefore, no chromatin remodelling is necessary here. Transcription factors can clearly bind, and these promoters can be activated very efficiently without any chromatin remodeling, without any need for chromatin remodeling, and it is often found that nucleosome density at promoter regions is typically lower than that in the coding region.

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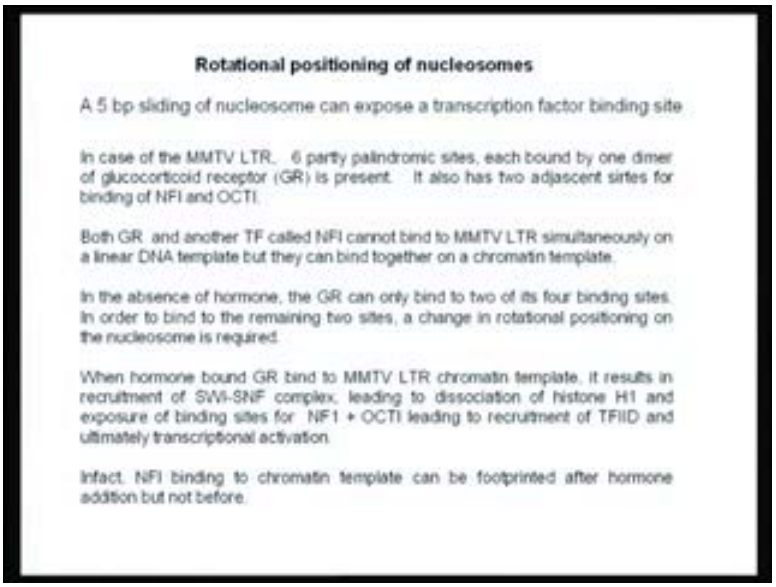


Remember, any statement I make like this, there will always be exceptions, but we are talk about very general or at this concerns are released. In majority of the case, that has been found, if you look at the nucleosome density in a promoter region versus a coding region, normally, the nucleosome density in a promoter region is much less, compared to the nucleosome density in a coding region.

Now, there are number of examples in the literature to actually describe how chromatin remodelling can actually bring about regulation of gene expression. I have just given one example here. Just, I will tell how a simple five base pair sliding of the histones along a nucleosome can expose a transcription factor-binding site, resulting in transcription activation. It is a very nice example; we can read up little bit more because it is a very nice example to very well studied example, to understand chromatin remodelling and regulation of gene expression.

Now, we all we all have studied in the previous about few lectures about a nuclear receptor called glucocorticoid receptor. We have discussed very well how, in response to glucocorticoid hormone, a glucocorticoid receptor which is present in the cytoplasm, now undergoes conformational change dissociates from Hsp90, and this glucocorticoid receptor then dimerizes, then goes inside the nucleus, bind to specific sequences– upstream sequences called as glucocorticoid respone elements, and then this results in activation of transcription.

So, all genes which are responsible to glucocorticoid hormone contain this glucocorticoid respone elements, and glucocorticoid receptor now goes and binds there, and in the place of glucocorticoid hormone receptor, glucocorticoid hormone, the transcription activation domain now interacts, recruits a specific coactivators, resulting in activation of transcription.

Now, very beautiful experiments have been conducted to actually understand how the glucocorticoid receptor functions in a chromatin template. For example, in the case of mouse mammary tumor virus called as MMTV, it has what is called as a long terminal repeat. These these retroviruses and this mouse mammary tumor virus, contain what is called as a long terminal repeat, which actually contain promoter regions for transcription of all the viral genes.

Now, this MMTV LTR contain six partly palindromic sites, and each of them is bound by one dimer. Remember, glucocorticoid receptor recognize 2 half sites– AGAACA, three nucleotide spacing, TGTTCT. This is the binding site for a glucocorticoid receptor.

So, if a promoter has AGAACA, three nucleotides, TGTTCT, remember, AGAACA, TGTTCT is a palindrome. TGTTCT, if we read in the opposite strand, is AGAACA. So, we have AGAACA and AGAACA are the two opposite strand, and TGTTCT on the half sides. So, I have two half sides AGAACA, TGTTCT separated by three nucleotide spacer, and this serves as a binding site for one dimer. So, one monomer recognize AGACCA on one strand, and another monomer recognizes the AGATCA on the other strand and the receptor binds as a dimer, ok?

So, there are six such sites, six palindromic sites, then 2 plus 2, 4, act as a binding site for a 2 glucocorticoid receptor dimer. Now, see, let us me read this slide, it is very interesting experiments, in addition to this binding glucocorticoid receptor, the MMTV LTR also has two adjacent sides for the binding of nucleotide factor 1 and OCT 1. These are also 2 important transcription factors, which are required for transcription activation of the MMTV promoter.

Now, things out, both GR and this nucleotide factor 1 cannot bind to MMTV LTR simultaneously on a linear DNA template. Now, if we have a linear DNA template, and you have this glucocorticoid response elements, and we have the binding sites for

nuclear factor element, but once the glucocorticoid receptor will binds, because of steric hindrance, the adjacent site for nuclear factor 1 cannot be occupied by nuclear factor 1. Therefore, transcription activation is not very efficient on a linear template.

But, and, in the absence of hormone, glucocorticoid receptor can only bind 2 of its 4 binding sites. So, in order to bind to the remaining 2 sites, a change in rotational position on a nucleosome is required; that means, the nucleosome, the histones has to slide a little bit. So, the other two sites can also be exposed.

So, when hormone bound glucocorticoid receptor binds to MMTV LTR chromatin template, instead of a naked DNA template, where it bounds to a chromatin template, it results in the recruitment of SWI/SNF complex. So, the hormone induces a conformational change the in the transcription activation domain or glucocorticoid receptor, resulting in the recruitment of a SWI/SNF complex. This results in chromatin remodeling in such a way that the histone H1 is dissociated, and as a result, the binding sites for NF1, OCT1, is now exposed on the other side of the chromatin, and this results in the binding of NF1, OCT1 can now bind along with glucocorticoid receptor, and this result the recruitment of TFIID, and ultimately transcription activation.

So, you can see, we have glucocorticoid receptor and we have NF1. They cannot bind to their binding sites on a naked DNA template because of the steric hindrance. But, they can bind very efficiently when the same DNA is organized in the form of chromatin, and by recruitment of a chromatin remodelling complex and displacement of histone, you can now access, both these factors can access binding site, resulting in very efficient transcriptional activation.

In fact, there are very nice experiments that I have been done to actually show, NF1 binding to a chromatin template can be footprinted only when you have hormone, whereas, the absence of hormone NF1 cannot be bound, but the moment you have hormone, the hormone-bound glucocorticoid receptor now recruits a chromatin remodelling complex, and therefore, facilitates the binding of NF1.

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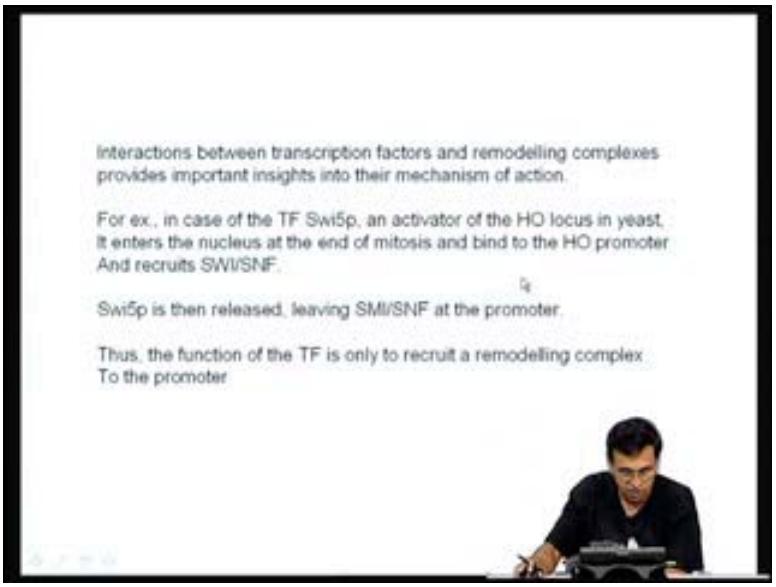


So, just to tell you that how, moving nucleosomes around can actually expose certain transcription factor binding sites, and therefore, facilitate synergistic action of transcription factors, which in concert can result in activation of transcription from chromatin templates.

And this, another example here, interactions between transcription factors and remodelling complexes provides very important scientific mechanism of action. This is one mechanism what I told you, so far, how the glucocorticoid receptor binding and recruitment of chromatin remodelling complexes now facilitates the removal of histones, and exposes a site for an adjacent transcription factor to bind, and together, it results the recruitment of TFIID and transcription activation.

And in the case of yeast cells, for example, there is a transcription factor called Swi5p, which is not a chromatin remodelling complex, which are regular transcription factor, and this activator is actually required for transcription activator of the HO locus. Remember, HO is a gene that encodes endonuclease, which is required for mating type switching in yeast cells, ok?

In this particular case, when this transcription factor enters the nucleus at the end of mitosis, and binds to the promoter of the HO, gene it results in the recruitment of the SWI/SNF complex. Now, very interestingly, the job of Swi5p seems to be just

recruitment of a chromatin remodelling complex to the promoter. Once the chromatin SWI/SNF complex is recruited to the promoter, the Swi5p is released, leaving this SWI/SNF at the promoter. So, the function of this transcription factor, in this case, is just to recruit a chromatin remodelling complex, that is all.

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So, you can see, there are diverse examples to actually show there are transcription factors like glucocorticoid receptor, which recruit a chromatin remodelling complex, but they continue to bind there, and then that facilitates the binding of another transcription factor, leading to recruitment of TFIID and activation of transcription. In this case, the only function of the transcription factor like Swi5p seems to be, once it bounds, it recruits the chromatin remodelling complex, and then the Swi5p dissociates and the chromatin complex take over, brings about changes in chromatin, resulting a activation of transcription.

And one can ask the question– what is the relevance of studying all this chromatin remodeling? Does it really have any bearing on the human health? Just as I told you, that mutations in DNA methyltransferases can result in the manipulation of genetic disorders, clearly indicating the DNA methyltransferases play very important role in number of genetic disorders, and so on and so forth, similarly, there are also several genetic diseases which have been identified, which have been associated with chromatin remodeling. There is a very nice review in Current Opinions in Genetics and Development, which

talks about the various genetic diseases, which are associated when you have mutations in gene encoding chromatin remodelling complexes.

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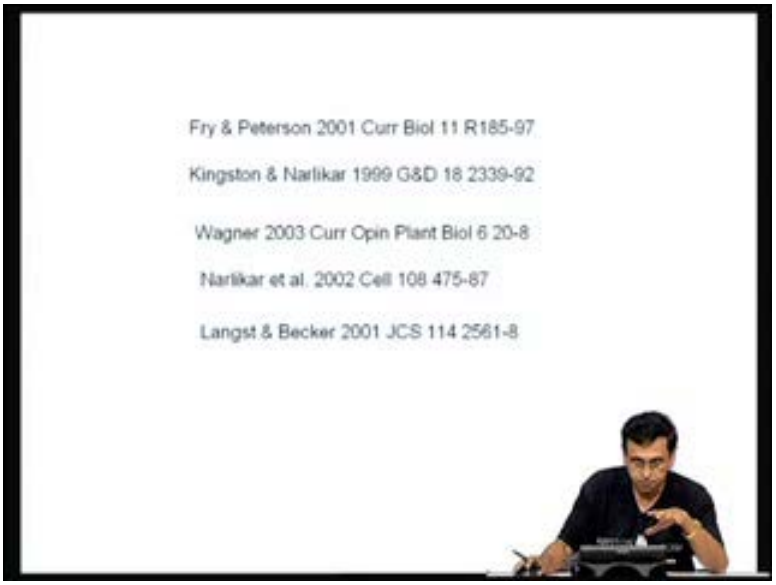


Very clearly indicating that, chromatin remodelling affects a number of important processes, and when you have mutations in proteins encoding, mutation of genes encoding the chromatin remodelling protein, it can result in genetic diseases, saying that it has a very important physiological function. There are also number of papers I have listed here, which discuss, in great detail, the role of chromatin remodelling and their effect on gene regulation. So, I am not going to the details of this.

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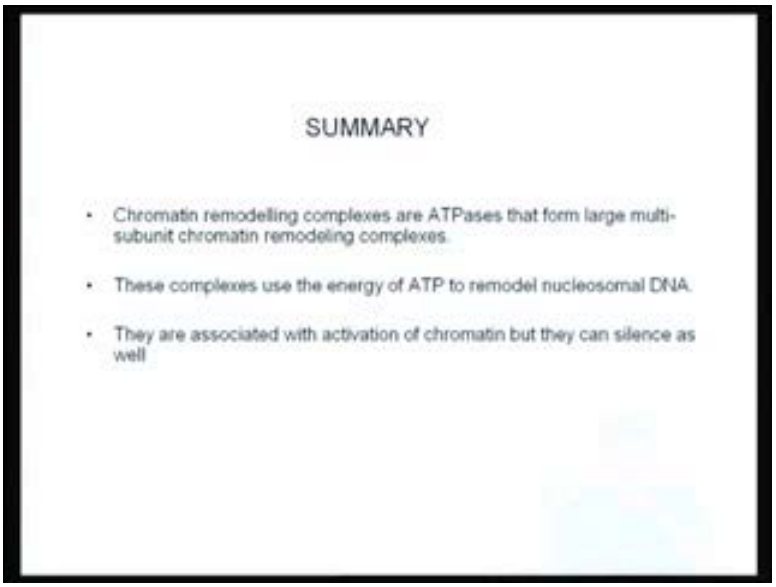


So, what I am, what I would like to summarize here, so far, what I have told you is that chromatin remodelling complexes are nothing but, they are ATPases that form a large multi-subunit complex. These complexes actually use ATP– the energy derived from ATP hydrolysis, to remodel nucleosomal DNA, and these are associated with activation of chromatin. But, in some cases, chromatin remodelling complexes are also involved in silencing of gene expression. So, in addition to altering chromatin structure involved in post translational modification of histones, we have now bought in a new, new player into the regulation of gene expression, namely, chromatin remodelling.

Whereas this post translational modifications of histones does not require any ATP hydrolysis, whereas these chromatin remodelling machines, which are actually huge multiprotein complexes, they actually use energy derived from ATP hydrolysis to alter nucleosome positioning. There the nucleosomes can slide, or nucleosomes can actually be displaced, or certain classical histones can be replaced with certain variants of histones. Now, all these possible combinations can result in creating what is called as a nucleosome-free region in chromatin region, and as a result, transcription factors can come and bind to this chromatin templates, resulting in activation of transcription or repression of transcription.

The other important point I have mentioned here, is that, just I have mentioned in the previous class, that histone acetylation, DNA methylation, and many other modification,

they act in concert. There are also examples, where in, chromatin remodelling complexes, in conjunction to histone modifiers, act in conjunction, to either activate or repress the transcription for the particular promoter.

So, never, we should never get the impression that, just because we are studying each one of this topics in a different classes, does not mean that these all act in isolation. In vivo, all this processes are combined together, and they all occur together and they complement each other, and one process is depend on another, for example, in many cases, the acetylation of histones can actually lead to the recruitment of a chromatin remodelling machinery, along with the histone acetyltransferases.

In many cases, the coactivator complexes may contain both an histone acetyltransferase, as well as a chromatin remodelling ATPase together, indicating that the all this things actually act together, and one process leads to another, and ultimately, together, a combination of these events is what ultimately determines the activation or repression of transcription of specific genes. This is what we have discussed now.

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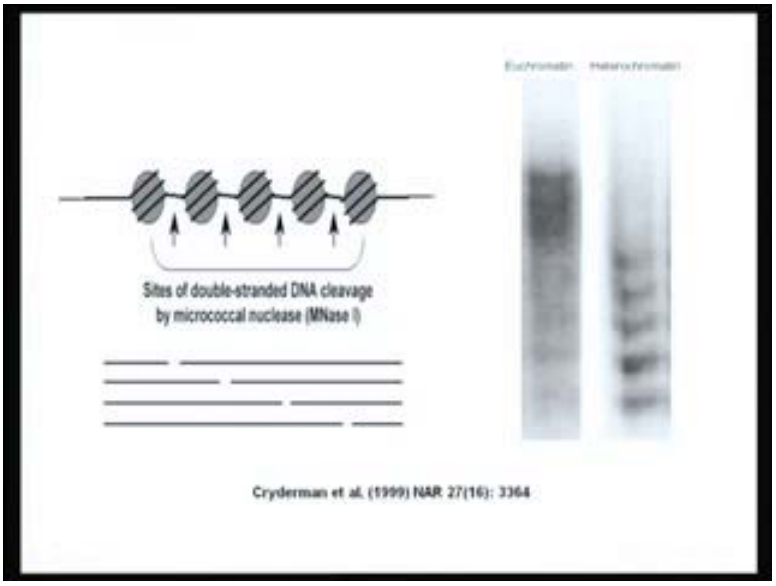


There are also number of experimental techniques that people use for studying this kind of a chromatin structures, and also identifying the role of these chromatin modifying enzymes. Otherwise, I will just mention one important technique which people in the field normally use. If you want to really identify the organization of nucleosomes,

whether the DNA is really organized in the form nucleosomes, you do a, what is called as a very important experimental technique, which is called as the micrococcal nuclease digestion, ok?

I just taken this cartoon from this particular paper, where they actually demonstrated how you can distinguish a euchromatin region in a, in a nucleus, from a heterochromatin region. Suppose, I want to find out, I am interested in a specific promoter or a specific gene, and I want to look, find out whether this promoter region is exist in the euchromatin region or heterochromatin region of the, in the nucleus.

What I do is that, I keep the nuclei, and subject these nuclei to what is called as a digestion with micrococcal nuclease. Now, as we know, the nucleosomes are very finely, very evenly, very nicely organized, and they, when you now take such DNA, and then treat that with micrococcal nuclease, micrococcal nuclease cleaves only the inter histone region, the between the histone octamers, at the, at lower, lower, low concentrations, and as a result, it leaves only this inter histone regions or inter nucleosome regions. Therefore, you create nicks only at these regions, and therefore, you get what is called as a very nice ladder.

So, what do you do is that you do the micrococcal nuclease digestion and then isolate DNA, and then do a southern blotting by from making a radio labelled probe of your promoter region of your interest, and probe this blot with this particular probe, and the same probe is the same here.

But in, there are 2 situations– in one case, where the gene is getting expressed, another case where the gene is not getting expressed, and very interestingly, you see here, in this example, when your gene is getting expressed, your promoter is actually present in the form of euchromatin region, and you can see, no longer see this kind of a ladder pattern here, seeing that nucleosomes structure here is altered, in the euchromatin region, whereas the gene is not expressed. You see a very nice nucleosome pattern here because the MNase cleaves only in the intra atom region. You get a very nice ladder, ladder pattern.

So, using techniques such as these things, you can actually identify whether your gene of interest is present in the euchromatin region, or whether gene is present in the heterochromatin region.

So, what I have, what are the implications of all that I have studied, all we have studied, discussed, so far, is that whether a gene is to be expressed or it should not be expressed, depends upon whether the gene is organized in the form a euchromatin or whether the gene is organized in the form of a heterochromatin inside the nucleus.

And whether the chromatin is the heterochromatin state or euchromatin state depends on a number of factors. It depends on the methylation of cytosines of the DNA; it depends on the coral modifications of the histone; it depends on the activity of the chromatin remodelling complexes. So, all these factors collectively contribute to whether your gene has to be transcriptional active, and your gene has to be transcriptionally inactive.

And, more importantly, there are also now people, people have been studying this kind of a chromatin organization inside the nucleus. Many of you might have only talked about, when you talk about nucleus structure, organization of nucleus, you might have simply studied only about nucleus and nucleolus. Other than these two sub nuclear structures, you probably have not studied anything.

But, recent studies on understanding the organization of chromatin and organizations of genes in the euchromatin heterochromatin have now identified, there are number of sub nuclear structures, and there are number of complexes, which play a very important role in the organization of genes, and this nuclear architecture plays a very important role in the regulation of gene expression.

There are, for example, what are called as speckles, paraspeckles, Cajal bodies; I do not want to explain all these things. We will discuss all this in a later stage, but what I am trying to mention here is that, in addition to nucleolus, which is one of the major component inside the nucleus, which is what most of you might have studied, there are also a number of subunits, such that are being discovered now, and all these things seem to be playing a very important role.

There are, there are many instances where, immediately after transcription, the RNA is not exported out. The RNA is actually stored in specific sub nuclear structures for some time, and then it is taken out. So, in addition to transcription per se, there are also number of post transcriptional processes, which regulate whether the RNA which has been transcribed has to be translated and a protein has to be made or not.

In addition, the other important point that I would like to discuss in the next class, is that, so far, we have been discussing only transcription initiation. Now, what we will discuss in the next class, is that once the RNA polymerase kickstarts, once the RNA polymerase's carboxy terminal domain is phosphorylated, and the RNA polymerase moves, then there is what is called as a transcription elongation.

That means, the RNA polymerase has to go through and transform the entire coding region and the non coding regions as well, and then, in the case of eukaryotes the introns have to be spliced out; the exons have to be joined, and then at the end, there is what is called as a transcription termination, polyadenylation, and poly-A tail has to be added, and what I would like to discuss in the next class is that this transcription initiation, which is primarily carried out by RNA polymerase, RNA polymerase, and the general transcription factors, play very important role not only in transcription initiation, but they also play a very important role in transcription elongation, polyadenylation, mRNA capping, and so on and so forth.

Remember, all the eukaryotic messages undergo what is called as a five-prime capping; there is a five-prime cap. The cytosine is modified the guanine residue at the five-prime is modified, and is actually called as a five-prime cap.

So, the enzymes involved in five-prime capping; the enzymes involved in RNA splicing; the enzymes involved in polyadenylation of RNA; they all interact with the mechanism, the machinery of, involved in transcription initiation.

So, I do not want to you to be under the impression that the function of general transcription factors and RNA polymerase is not just to initiate transcription, but they also have to interact with components of post translational modifications. The machinery involved in transcription initiation, they also interact with capping enzymes; they also interact with splicing enzymes; they also interact with polyadenylation machinery; and

all these things are very highly integrated. Ultimately, if a polyadenylated messenger RNA has to come out of the nucleus, all this (( )) machinery have to function together, and these are all integrated.

So, what we will study in the next class, after, after understanding how transcription initiation takes place, how enhancers act, how transcription factors bind and activate transcription, how chromatin plays a very important role in the regulation of gene expression, and how chromatin modifying enzymes, histone modifying enzymes, DNA methylation, all these things in combination contribute to the regulation of gene expression in eukaryotes.

In the next class, we are going to discuss about how transcription initiation is coupled to transcription elongation, messenger RNA capping, polyadenylation, and how all these events are actually integrated with each other.

So, let us discuss in the next class about the interlinking or interrelationship between the initiation of transcription, elongation, mRNA capping, and polyadenylation, and how all these process are integrated together. This is going to be focus of the next class.

So, I think, with these, about 10 lectures, I have now completed one part of this lecture series. This, actually, forms one major capsule of this entire lecture series on eukaryotic gene regulation, and at the end of these 10 lectures, you probably now have a very comprehensive idea about regulation of gene expression; how are transcription initiation is affected by transcription factors; and what is the role of chromatin in the regulation of gene expression.

So, once we discuss in the next class about the integration of transcription initiation between, for, with RNA capping, polyadenylation, and splicing machinery, we will then move on to understand the mechanism by which RNA polymerase I and RNA polymerase III initiate transcription.

So far, in all our discussion, we have been focused only on RNA polymerase II and protein coding genes. Now, we will then spend 2 classes to understand the mechanism by which RNA polymerase I transcription takes place, is transcribes the genes, and RNA

polymerase III, what are the various factor that are associated, what are the important facets of RNA polymerase I transcription, RNA polymerase III transcription.

Then, we will move into the next phase of our lecture series in understanding signal transactional processes. We will take examples of each and every one of the transcription factors, and study in detail, how a signal emanating from the surface, cell surface, gets in all the way, and then ultimately results in activation or repression of transcription. I think I will end here.