Eukaryotic Gene Expression

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Module No.# 03

Lecture No.# 07

Eukaryotic Gene Regulation: Role of Chromatin

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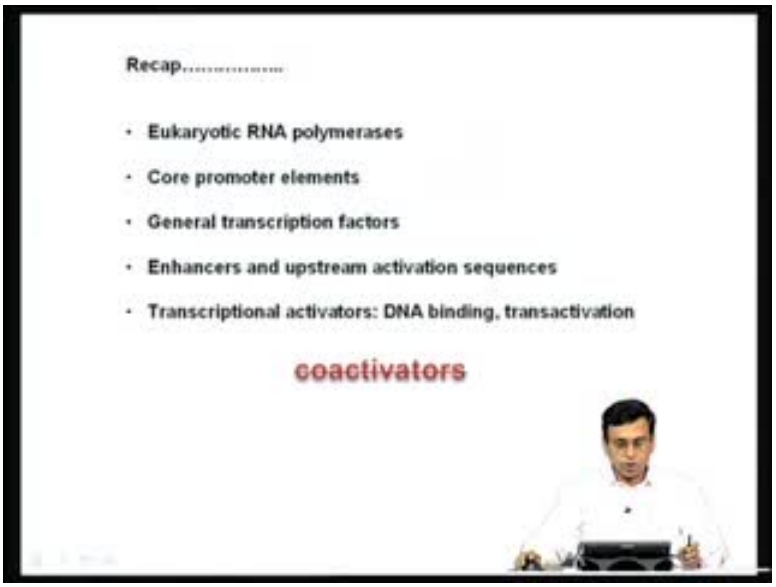

Welcome to this seventh lecture in this course on eukaryotic gene expression. Today, we are going to talk about the role of chromatin in the regulation of eukaryotic gene expression. Just to recapitulate what we have been discuss in the last few classes, we began our discussion with eukaryotic RNA polymerases.

We then went ahead to discuss about the core promoter elements around which the pre-initiation complex is assembled, which consists of general transcription factors and RNA polymerase II, and then, we discussed about the various general transcription factors and how they function, and then we went on and then discussed, in addition to the core promoter elements, there are also many upstream elements, which play a very important

role in the activation of gene expression in eukaryotes. These can be proximal promoter elements, distal promoter elements, enhancer elements, silencers, and so on and so forth.

Then, we discussed, the actual transcription activation takes place by transcription factors, sequence-specific transcription factors, which go and bind to some of these enhancer sequences, and binding of these transcription factors to these enhancer sequences then enhances the rate of recruitment of RNA polymerase of the core promoter region. This is how enhancement of transcription takes place in eukaryotic cells.

We also discussed, very briefly, the structure of the various transcription factors, what kind of DNA binding domains this transcription factor contain, what kind of transcription activation domains these kinds of this transcription factors contain, and so on and so forth.

Now, the major mechanism by which these transcription factors regulate gene expression is when they bind to the sequence elements, to the, to the specific sequences in the promoter regions, they somehow have to interact with the general transcription machinery. This interaction what facilitates faster recruitment of RNA polymerase leading to enhancement of transcription.

So, the last class I mentioned to you, one of the major mechanisms by which these transcription factors do this, is by recruiting what are called as coactivators. It is these coactivators, which serve as a bridge between transcriptional activators on one hand, and general transcription factors on the other hand, and I told you in the last class that we will discuss about coactivators and what exactly is their role in regulation of gene expression.

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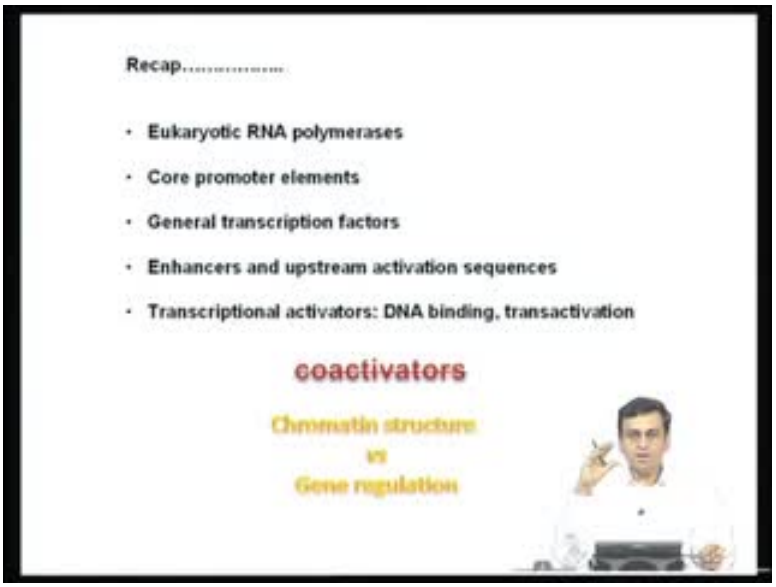

But what I will do today is, before I discuss with you about the role of coactivators, I thought, it is important for us to now to understand the role of chromatin. So, what I will do, today, is to really see what is the link between chromatin structure and gene regulation. This is, so far all our discussion, the last 6 or 7 classes, has been about how the core promoter elements are organized, and how the RNA polymerase and general transcription factors are assembled on the core promoter, and how transcription factors go and bind to upstream sequences, and how binding of these can result in the transcription activation.

We also discussed how, what, how various promoter elements can be mapped by techniques such as DNase 1 foot printing, gel mobility shift assay, etcetera, and how you can actually use either transfection assays in cell lines or cell-free transcriptions systems to study various promoter elements.

But, one thing we have not discussed so far is, in vivo, in reality, the eukaryotic cells, the DNA is not naked. DNA is actually wrapped around nucleohistones in the form, and is organized in the form of nucleosomes.

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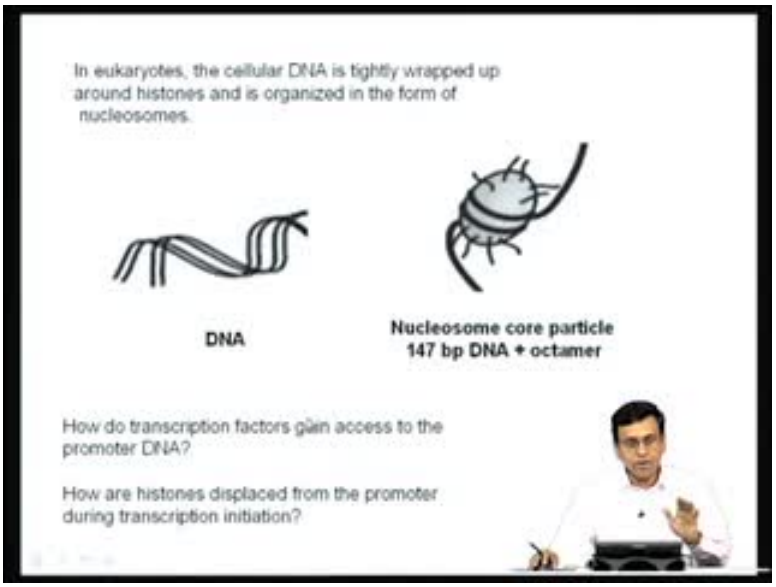

So, this cartoon I have shown here, in eukaryote the cellular DNA is tightly wrapped up around histones and is organized in the form of nucleosomes. So, there is about 147 base pairs of DNA which is wrapped around this each nucleosome core particle, and the DNA gets nicely around, and each this DNA plus the histone octamer– that is what is called is forms a nucleosome. So, the one thing that we have to now discuss is, how do transcription factors gain access to the promote DNA?

You should be remember, so far, we have not got this issue at all. We discussed, so far, as if DNA is naked and the DNA sequences are freely available, so that the TF2D can go and recognize the TATA box or other core promoter elements, and transcription factors can go and bind to the upstream sequences, and then they interact, and it can result in transcription activation. But this is not the case, in reality.

In reality, the DNA is striped, organized in the form of nucleosomes, and that is what we call as a chromatin. So, transcription activation actually has to take place not on naked DNA, but it has to happen on chromatin templates. Which means, the histones which are wrapped around the DNA and form the nucleosomes, these histones have to be displaced from the promoter in order for transcription initiation to take place.

So, this is what you are going to next discuss in the next few classes– how do these transcription factors displace histones from the promoter region, so that the pre-initiation

complex can be assembled and transcription initiation can take place? There is a very nice history, so, I would like to take you through a very brief history how chromatin got involved in gene regulation.

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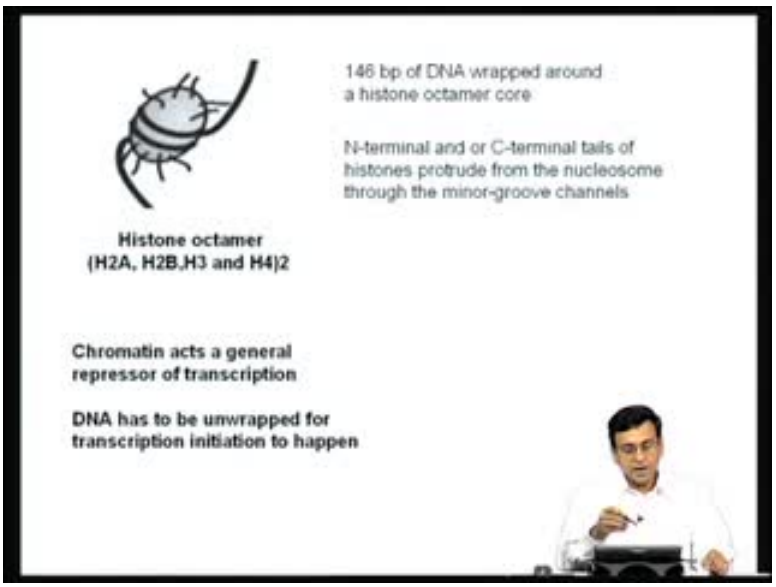

Now, for a long time, people who have been studying chromatin structure were a different kind of a group and people who have been studying gene regulation were considered as a different kind of group. They never thought they actually have something in common. Many times, people thought the only function of histones is, histone is to compact DNA; package DNA in the form of chromatin and chromosomes, and it only has a structural role.

But, it soon became very clear that histones may have many other role other than a structural role. In fact, it may have a very important functional role in gene regulation. Let us now discuss some of these issues in detail. So, I told you the nucleosome core particle, which consists of core histones, what are called as H2A, H2B, H3, and H4. These 4 histones, 2 subunits each, together form what is called as a histone octamer, and the DNA is now wrapped around this kind of net. So, the DNA is arranged in the form what is called as a beads on a string.

Be in between the two nucleosomes you have what is called as H1 histone, which act as a linker histone, and what is very important for our gene regulation is that, when these

histones form this histone octamers, you can see, there are here thread-like structures which are depicted here. These are nothing but the N-terminal and C-terminal trains of each one of this histones.

So, you have that histone octamer, but the N-terminal and C-terminal of each of these histones are actually hanging around, and this N–termini and C–termini are now going to have a very important role in the next few minutes. And these N and C-termini are the ones which are now, are available for, what are called as, post translation modifications, and these post translation modification of the N and C-termini of histones play a very important role in gene regulation. This is what we are going to discuss about in detail today.

So, remember, because the DNA is tightly wrapped around histones and the DNA is organized in the form of chromatin, the chromatin actually acts as a general repressor of transcription, because DNA is very highly nicely packaged with these histones. And therefore, the chromatin acts as a repressor of transcription. So, if transcription initiation has to happen, this DNA, which is now wrapped nicely around these histones, it has to be unwrapped first.

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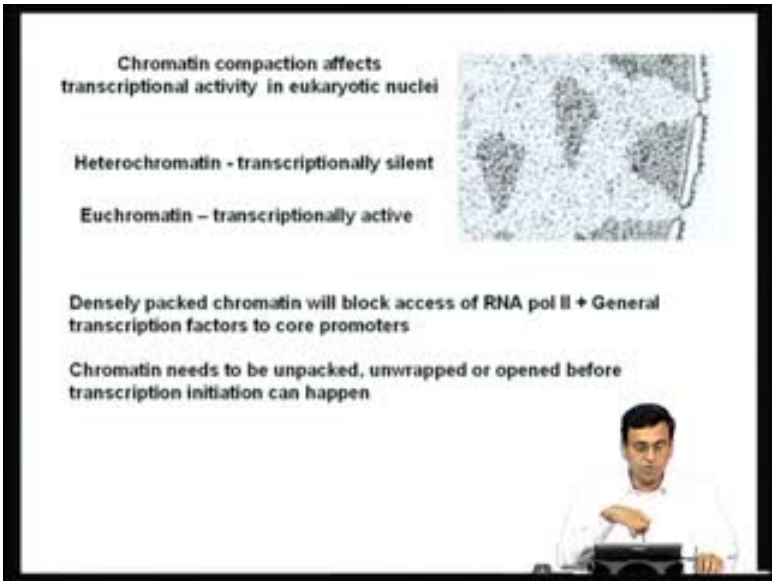

So, this is the first step that has to happen, and as people started looking at the chromatin structure and gene regulation, these two groups are working in parallel for long it for a

long period of time, circumstantial evidences clearly indicated that chromatin may have a very important role in gene regulation. One such circumstantial evidence is, psychologists and cytogenesists, who actually are observing on organization of chromosomes or organization of chromatin by using a number of staining techniques, they realized that, when they stained the eukaryotic nuclei, or even the drosophila polytene chromosomes or lampbrush chromosomes, etcetera, they realized there are some regions of DNA which get densely stained, and some regions of DNA or chromatin which is gets very thinly stained. So, these densely stained regions of chromatin are often referred to as the heterochromatin, whereas the lightly stained regions of nuclei or the chromatin is referred to as the euchromatin.

People soon realized, if you look at the transcription activity in the euchromatin and heterochromatin regions, most of the transcriptional activity is present in the euchromatin region, and most of the DNA, which is localized in the heterochromatin region, is transcriptionally silent.

So, these kinds of observations clearly indicated that the organization of a DNA into chromatin has a very important role in gene regulation. So, the genes have to be actively transcribed, they have to be in the form of euchromatin, and if the DNA is very tightly packaged in the form of heterochromatin, and then the probability that genes in that region are actively transcribed are very low.

So, these kind of evidences clearly indicated there is some link between chromatin and gene expression. So, the densely packed chromatin will block access of RNA polymerase II to general transcription factors of the core promoters, and that is why, probably, the genes which are localized in the heterochromatin regions are not actively transcribed.

So, if these genes have to be transcribed, these promoters or genes, which are present in heterochromatin regions, has now been converted into euchromatin. That means, the histones have to be removed and the chromatin has to loosen. Then only, the proteins involved in gene regulation can go and bind to the promoter sequences and activate transcription.

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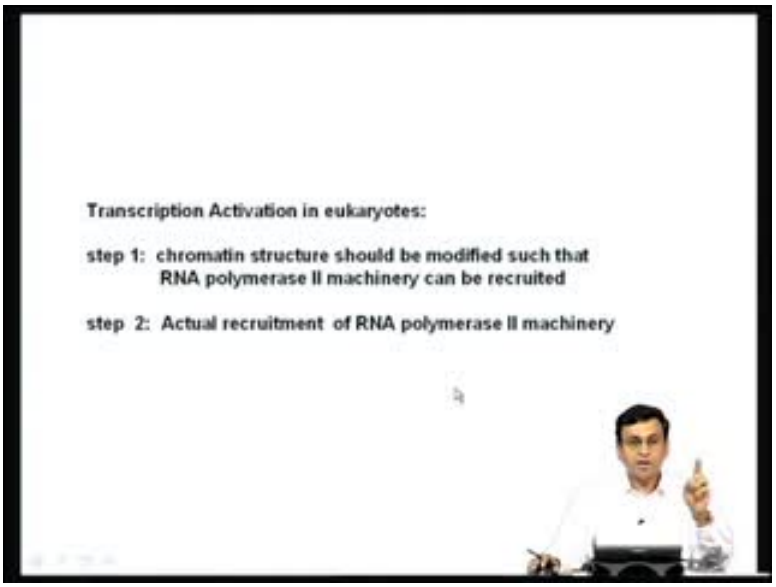

So, the chromatin in these regions, promoter regions, need to be unpacked, unwrapped, or opened up before transcription initiation has to happen. So, the important point that we are going to discuss today is that if transcription initiation has to happen in eukaryotic nuclei, 2 things have to happen.

The first step is, the chromatin structure should be modified, such that the RNA polymerase II machinery can now gain access to the promoter region, and then the next step is the actual recruitment of RNA polymerase II machinery to the transcription start site.

So, it is like if somebody wants to perform either a dance or perform music, now the first, the stage has to be clear and the stage has to be set; only then the people can come in, and then make their performance. The same way, the chromatin, if it is (( )) that if the histones pack the DNA tightly around, and ordered in the form heterochromatin, then there is no place for the players to come in and act.

So, the first thing that has to happen is the stage has to be clear. That means, the histones have to be removed and the promoter DNA has to be made available, so that, then these general transcription factors or other transcription activators can come and then perform their job.

So, the transcription initiation, probably, involves 2 steps: the first is modification of the chromatin structure to facilitate the various factors to gain access, so that they can come, and then recognize their sequences and initiate transcription. Remember, these 2 steps are very important for all our future discussions now. Modification of chromatin structure is very essential for the regulation of gene expression in eukaryotes. This is the most important point that we are going to discuss today.

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Just like I told you, the euchromatin-heterochromatin studies clearly indicated that the chromatin structure has a role in gene regulation. The same way, cell-free transcription studies also gave some clues that chromatin structure plays a very important role in regulation of gene expression.

For example, we discussed in the last classes, the way the cell-free transcription studies were performed, initially, people started using crude nuclear extracts, (( )), in which templates can be put in and their transcription activity can be assayed.

Then, once people started purifying general transcription factors, purified the RNA polymerase II and transcriptional activators, people started developing what are called as reconstituted systems, wherein you add individual purified components to the cell-free transcription systems, and then add your DNA template, and assay your promoter activity.

But then, as people started cloning many of these general transcription factors, like the components of TF2D, TF2B, TF2A, and so on and so forth, people stopped adding the recombinant general transcription factors to the cell free systems, and then started seeing whether you can demonstrate activation of transcription from these DNA templates in vitro.

Now, very importantly, (( )) initial studies on the gene regulation were all again performed in the naked DNA templates; this is what we actually discussed so far. That means, you take a DNA– promoter DNA– we hook into either a gene-free cassette or something like that, and then add your nucleotides; and add either cloned transcription factors or purified general transcription factors; add RNA polymerase II; add nucleotides, one of which is radio labeled, and then you measure the promoter activity.

People started realizing that naked DNA templates may not really reflect the real situation that is happening in the in vivo systems. People started using what are called as chromatin templates. That is, people who were studying chromatin structure and chromatin organization, so on and so forth, have developed protocols or purifying various histones like H1, H2A, H2B, H4, and so, and so on.

So, people take took all these purified histones, and then add them to the naked DNA, so that you can actually assemble these nucleosomes and you can actually prepare what are called as chromatin templates, in which you actually have nucleosome structures.

So, there was a paradigm shift in the thinking of people working on gene regulation, that if you really want to interpret the results of the cell-free transcription studies, so…, what actually happening in vivo, naked DNA templates may not be the right way of doing experiments. You actually have to demonstrate how the transcription factors assemble on chromatin templates, and how transcription activators actually activate transcription on chromatin templates.

So, people, whoever actually doing all these experiments naked DNA templates, switch to what is called as chromatin templates, where you take your plasmid DNA templates containing a promoter and a reporter, and then actually add histones, so that it forms a chromatin template, and then on this chromatin template (( )) RNA polymerase, general

transcription factors, and see whether you can now demonstrate transcription activation in a cell-free system.

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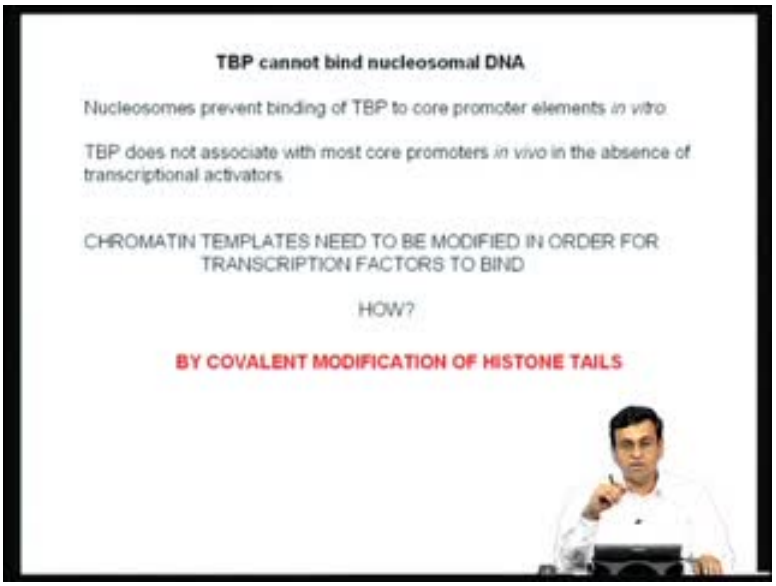

Now, when these studies were done, a surprising result that came out, that if many of the activator-dependent transcriptions could not be demonstrated when chromatin templates are actually used. In fact, very surprisingly, the TATA binding protein could not really bind the chromatin templates on its own.

So, very clearly sending that when we have when DNA is packaged in the form of nucleosomes or when you have chromatin templates, somehow, these nucleosomes prevent the binding of TBP to the core promoter elements in vitro.

Somehow, it became very clear, in order for the TBP to recognize the core promoter elements, you require transcriptional activators. So, there were the thinking change, indicate that one of the mechanisms by which the transcription activators may be facilitating the assembly of the general transcriptional machinery in the transcription start site, is actually by removing the nucleosomes or removing the histones in and out of the region, so that the general transcription factors can go and bind, then activate transcription.

So, chromatin templates needs to be modified in order for the transcription factors to bind. This was the most important observation that came out of some of these early

studies, clearly indicating that there is…, you need to now demonstrate how actually, what is the role of chromatin in the regulation of gene expression?

But, the question is– how do this chromatin templates function? How are transcription factors recruited on chromatin templates? A very important outcome of many of their… a number of 5 years of research that came out, is that the chromatin templates are actually modified and the general transcription factor are actually brought to the core promoter elements, and one of the important mechanisms by which this is brought about is actually by covalent modifications of the histone tails.

Now, I told you, in the nucleosomes, which contains the H2A, H2B, H3, and H4, the amino and carboxyl terminals of these histones– they are actually free. They are actually hanging around and they are actually of, therefore, amenable for post translation modifications like phosphorylation, acetylation, deacetylation, and so on so forth.

So, soon became clear covalent modifications of the histones may actually have a very important role in the removal of histones, or in the tight packaging of histones in another promoter regions, and this may have a very important role in the regulation of gene expression. Now, let us see how actually this was discovered.

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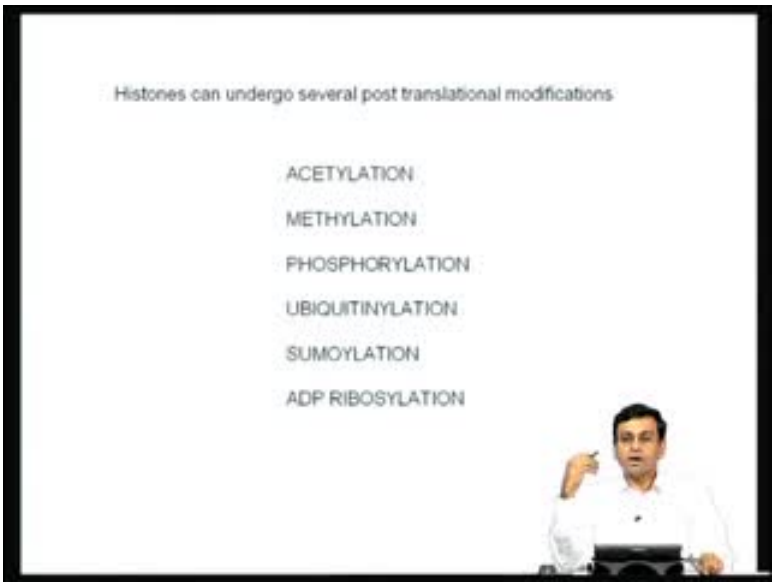

So, a number of studies, people who have actually is looking at histones started looking; then people realized that histones actually undergo a number of post translational

modifications. These include acetylation, methylation, phosphorylation, ubiquitinylation, sumoylation, and ADP ribosylation; and there are many other modifications that are that happened to these histones, just like for any other proteins.

So, just like many proteins are subject to post translation modifications, histones are also subjected to a number of post translational modifications. The question is, what is the relevance of these post translational modifications, or these post translational modifications affect only the chromatin structure, or they may also have a functional role? This is what was investigated.

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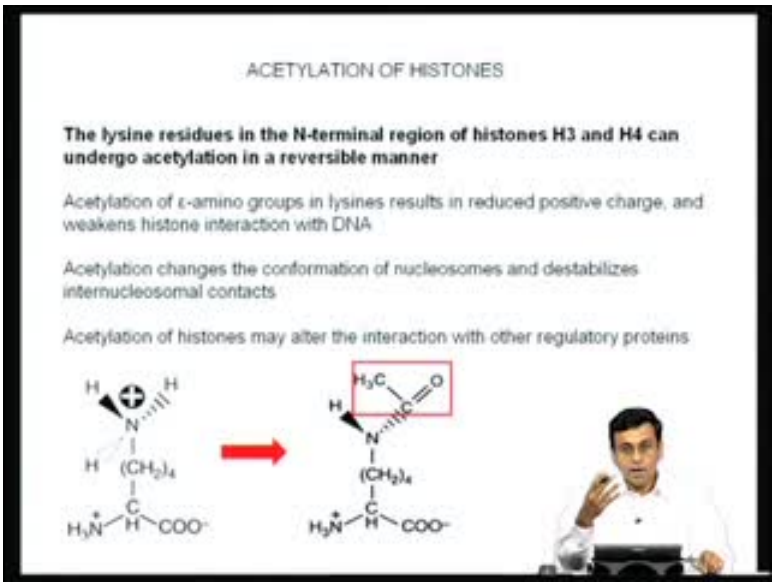

Let us now go one by one, and then really see what kind of what is the effect of some of these post translational modifications on histones, and let me begin with, what will be the effect of acetylation of histones on in a gene regulation?

Now– histones– we all know they are basic proteins. They are highly enriched in basic amino acids, especially the lysine and arginine residues. What… that is why they are all positively charged, and that is why they can very tightly go and bind to DNA? Because DNA is negatively charged. Histones are positively charged, therefore, the interactions can be very strong.

What became very clear is the lysine residues in the N-terminal region of histones H3 and H4 can actually undergo acetylation in a reversible manner. You remember,

whenever we talk about regulation in biological systems, most of these modifications have to happen in a reversible manner; then only regulation can takes place. So, most of the changes that we see in biological systems are often reversible. When you add a phosphate, you can also remove the phosphate; when you add an acetyl group, you will also able to remove acetyl groups.

So, there are enzymes, which it may add acetyl groups, then they also have to an enzyme which will remove the acetyl groups; then only the on-off mechanism will actually work. So, an important observation that became evident is that the lysine residues in the N-terminal regions of the nucleosome core particle may, especially which contains of H3 and H4, they can actually undergo acetylation in a reversible manner.

So, acetylation of epsilon amino groups in the lysines result in reduced positive charge, and therefore, weakens the histone interaction with DNA. As I told you, one of the key mechanisms why by which histones interact with DNA is– histones are positively charged; DNA is negatively charged; and the acetylating this N-terminal trace of histones you are actually bringing up additional negative charge. Therefore, you are decreasing a overall positive charge, and therefore, the interaction between histones and DNA weakens. And therefore, it may not result in a… it may result in loosening of the histones in the chromatin region.

So, acetylation changes the conformation of nucleosomes and destabilizes the inter-nucleosomal contacts, and acetylation of histones, actually, may alter interaction with other regulatory proteins. So, these are some of a key observations that came out when people started looking at post translational modifications of histones, and their relevance to gene regulation, and this cartoon just tells you, this is lysine residue of a histone, which is shown here, and this is how the methyl, the acetyl group is actually added, resulting the acetylation of the histones.

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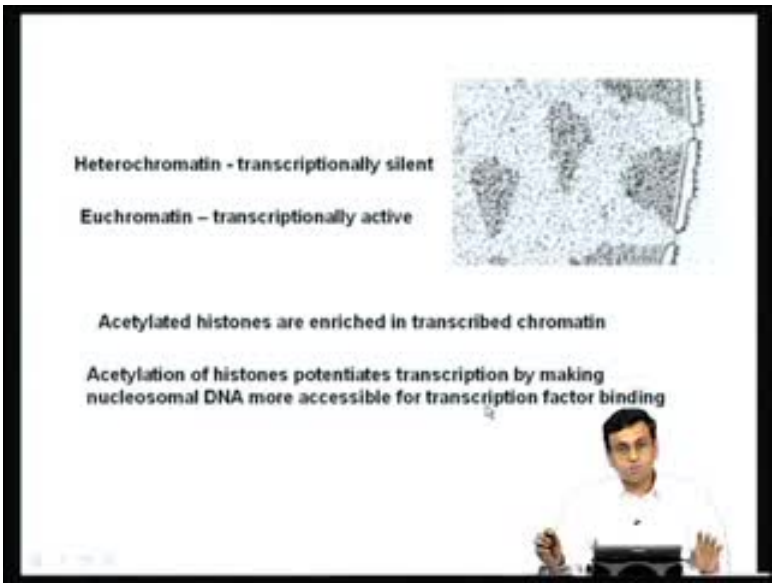

So, now we can really see, people went up to see how exactly you can correlate these observations to some of the observations they made earlier? Now, I told you earlier, heterochromatin, which is highly condensed chromatin, is transcriptionally silent; and euchromatin, which is kind of a lightly stained region of chromatin, is transcriptionally active. And when they actually looked at the acetylation of histones in euchromatin regions and heterochromatin regions, they, in fact, found acetylated histones are actually enriched in the transcribed chromatin or in the euchromatin regions of nuclei, whereas deacetylated histones are actually enriched in the heterochromatin regions or densely packed regions of chromatin, clearly indicating that there is a correlation between transcriptional activity and acetylation is of histones. So, acetylation of histones somehow potentiates transcription by making nucleosomal DNA more accessible for transcription factor binding. This was some of the important outcomes that came by study of acetylation of histones.

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So, then now the question comes– you found that histones are actually existed as acetylated proteins and acetylated histones are actually present at the euchromatin regions of the… of euchromatin regions in the nuclei. But then, the question comes– what are the proteins or what are the enzymes which add this acetyl group to histones? Where you need to identify the mechanism by which histones are actually getting acetylated.

A very important, very important, very surprising finding that actually came from some of the studies is– many proteins involved in transcriptional factors activation such as transcription factors, or coactivators, or even the TBP associated factors or TAFs, they actually contain histone acetyltransferase activity. This was one of the most important outcomes of all this. With the convergence of people studying histones and people studying gene regulation, that an important observation that came of this of convergence of this 2 groups is that many transcription factors actually can serve as enzymes, and what is the enzyme activity? Their enzyme activity is their ability to acetylate histones.

So, you can see, this, I would actually call, is one of the most significant results in the chromatin biology and in the regulation of gene expression– identification of histone modifying activities or enzymatic activities in the transcription factors.

So, we are now going to use a terminology called HAT, which actually means histone acetyltransferases. So, let us now examine some of the important observations that led to this conclusion.

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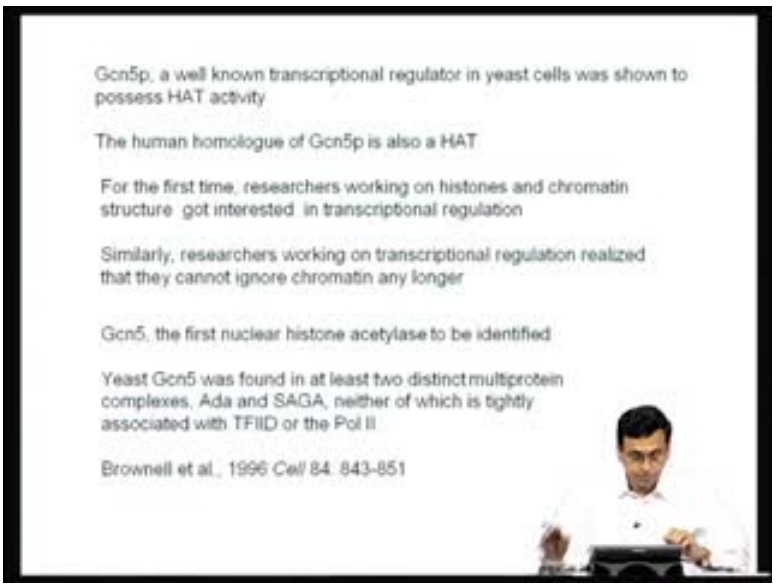

The first and foremost observation that a transcription factor actually possesses a histone acetyltransferases activity came from a transcriptional activator called Gcn5 protein, which is a very well known transcriptional activator in yeast cells. So, and this Gcn5p actually was shown to contain histone acetyltransferase activity. This was one of the most significant observations in the area of eukaryotic gene regulation, and soon they realized that the human homolog of the Gcn5p also has histone acetyltransferase activity, indicating that this enzymatic activity is conserved from yeast to humans.

So, for the first time, researchers working on histones and chromatin structure, both got interested in transcription regulation. Remember, till then, people who are been working studying histone structure, chromatin structure, were considered as a different group, and people who are studying gene regulation, gene expression, were considered as different group. But for the first time, they realized that they have now work together because the chromatin seems to be playing not only in compaction of DNA and have a structural role, it also seems to play a very important functional and regulatory role in the regulation of gene expression.

So, people who are working on chromatin structure and histones got interest in gene regulation. Similarly, researchers working on transcription regulation realized that they cannot ignore chromatin structure any longer; they have to come together and start working together if they really want to understand the mechanism by which eukaryotic gene expression is getting regulated.

So, the Gcn5, the yeast Gcn5 protein was the first nuclear histone acetylase transferase, histone acetylase to have been identified and, very interestingly, that yeast Gcn5 was found to exist in at least 2 distinct multiprotein complexes– one is called as Ada and another is called SAGA. I will discuss these multiprotein complexes in detail in a couple of lectures down the line.

Neither of these Ada or SAGA is tightly associated with either TF2D or polymerase II, indicating that this multiprotein complex containing a histone acetyltransferase is another important player in gene regulation, in addition to other multiprotein complexes like TF2D and RNA polymerase II.

Now, remember, already we told in the beginning that transcription initiation requires at least 50 to 60 proteins, which consists of RNA polymerase and subunits, general transcription factors, and each one of the general transcription factors, in turn, are multi subunit complexes. Now, we are bringing another player into the field; another multi subunit complex such as SAGA and Ada, which actually contain histone transferase or histone acetyltransferase or HATs as one of their components.

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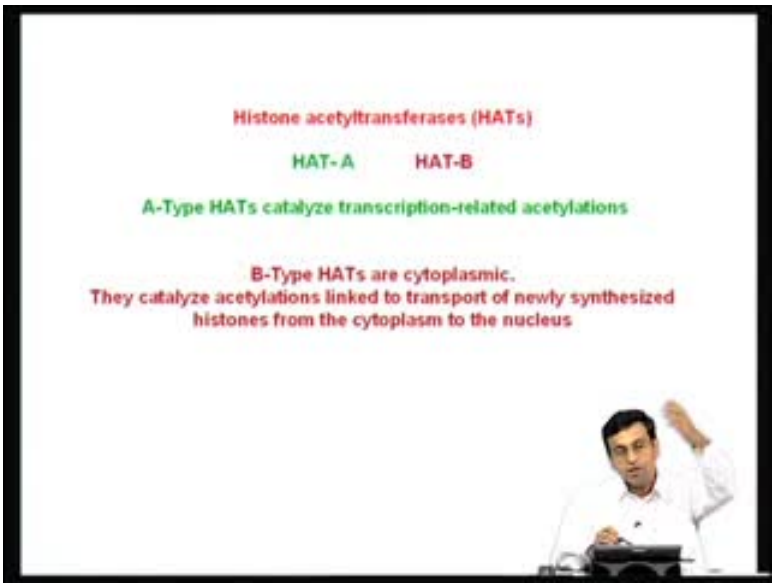

So, the number of proteins involved in gene regulation is increasing, and it soon became clear there are actually 2 types of HATs. The 2 types of histone acetyltransferases, one is called as HAT–A, another is called HAT-B.

Now, the A-type HATs actually catalyze transcription-related acetylation, is so they have very important role in the regulation of gene expression. The B-types HATs are actually cytoplasmic, and they actually catalyze acetylation is linked to transport of newly synthesized histones from cytoplasm to the nuclei.

So, newly synthesized histones, they get acetylated, and then transported to the nucleus by HAT, by B type HATs, whereas the A-type HATs are actually staying in the nucleus, and they are involved in the modification or acetylation of histones at the chromatin. And so, the observation that a transcription factor like Gcn5p actually contains the histone acetyltransferase activity opened a Pandora box.

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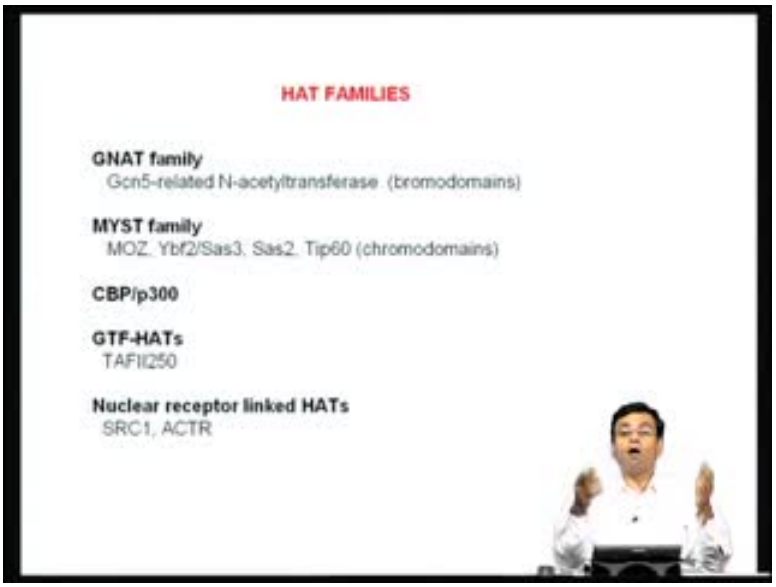

Say, number of people who are actually purified a number of transcription factors coactivators, and so on so forth, started looking, and then see, whether any of this transcription factors have histone acetyltransferase activity, and very surprisingly, they found many of the transcription factors or coactivators that they have identified, in fact, are capable of acetylating histones.

So, this opened up what is called as a HAT family of proteins, some of which I have mentioned here, like the GNAT family which consists of Gcn5 related N-acetyltransferases, and you have what is called as a MYST family, which consists of MOZ, Ybf2, Sas2, Tip 60, etcetera. There is another important, this is coactivator called CBP or p300. Again, we will discuss in detail next few classes.

Many of the general transcription factors, especially the TBP associated proteins also, were found to contain histone acetyltransferase activity, and many coactivators which are actually associated with nuclear receptors like SRC1 and ACTR also were shown to process histone acetyltransferase activity.

So, this linking, or this convergence of histone modification or histone acetylation to transcription regulation, is one of the important discoveries in the area of gene regulation. So, many transcription factors were actually found to contain histone

acetyltransferase activity, and this led to a very new thinking, new line of thinking on regulation of gene expression.

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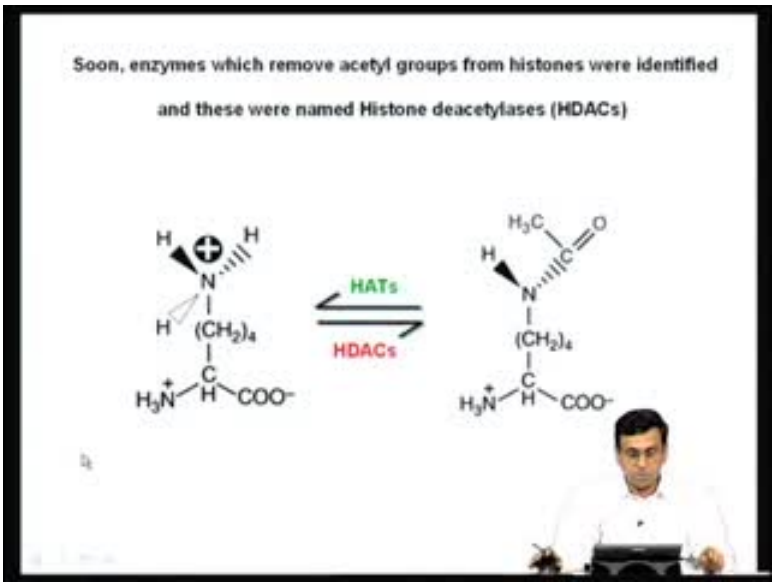

Now, if the histones are getting acetylated, there also have to be guides who will remove the acetyl groups from the histones; only then, regulation can take place, right?

When an acetylation is happening on histones and genes have been activated, and then unless you remove the acetyl group and inactivate this histone acetyltransferase activity, gene expression will, transcriptional activation will go on and on, which is not good for the cell.

So, if there are proteins which are adding an acetyl group to histones, there have to be protein which are removing the acetyl group from the histones; and soon, people recognized there are, in fact, enzymes called histone deacetylases or abbreviated as HDACs, which actually are capable of removing the acetyl groups added by the HATs. So, these are called as HDACs or histone deacetylases.

So, you can see here, you have histone molecules and there are enzymes called HATs, which actually add a acetyl group to the histones, and these acetylated histones are actually then deacetylated by group of enzymes called HDACs, and there is an equilibrium between the HDAC activity and HAT activity in a…, at any given time inside the cell.

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So, HATs such as Gcn5p are actually found to be present in huge multiprotein complexes. Remember, one of the important functions of this HAT is they have to be associated with promoter regions or components of transcriptional machinery.

So, many of these histone HATs or the HDACs were found to be multi subunit complexes, and there are actually component of other proteins which together form a huge multiprotein complexes, and as I told you before, these multiprotein complexes are different from those who we have already studied, such as TF2D or RNA polymerase II, etcetera, saying that there is another distinct entity of multiprotein complexes, in which the HATs or HDACs are an integral component.

For example, a multiprotein complex called SAGA actually contains Spt-Ada-Gcn5-Acetyltransferase, if which, on which Gcn5 is the acetyltransferase; and similarly, the human Gcn5p was also found to be in a similar complex called STAGA. What the point I am trying to tell you is that many of this histone acetyltransferase is exist as multiprotein complexes with other important regulatory proteins, which play a very important role in regulation of gene expression.

So, these multiprotein complexes, in which HATs are one of the components, they were found to interact with both TBP on one hand and acidic activators on the other hand. See, this is the reason I did not bring coactivators into the picture, because many of these

coactivators, whose job is to link the transcriptional activators on one hand and the general transcription machinery on the other hand, and many of these coactivators like the SAGA complex, actually found to be histone acetyltransferase activity. And they, on one hand are interacting with transcriptional activators, on another hand they are interacting with components of the basal transcription machinery. So, there are many such proteins like PCAF, TFTC, which actually stands for TBP-free TAF-containing complex were found to be members of this GNAT family of histone acetyltransferases.

Now, very interestingly, the free Gcn5 protein, when it is not present as a multiprotein complex, when it on it is own, can acetylate only free histones. It cannot acetylate histones which are bound to DNA in the form of chromatin, but as a part of the SAGA complex, it could actually acetylate histones. Now, we can see the significance of these HATs present as a multiprotein complexes, and these multiprotein complexes gives the HAT the ability to acetylate histones bound to chromatin, whereas the free Gcn5 can only acetylate free histones.

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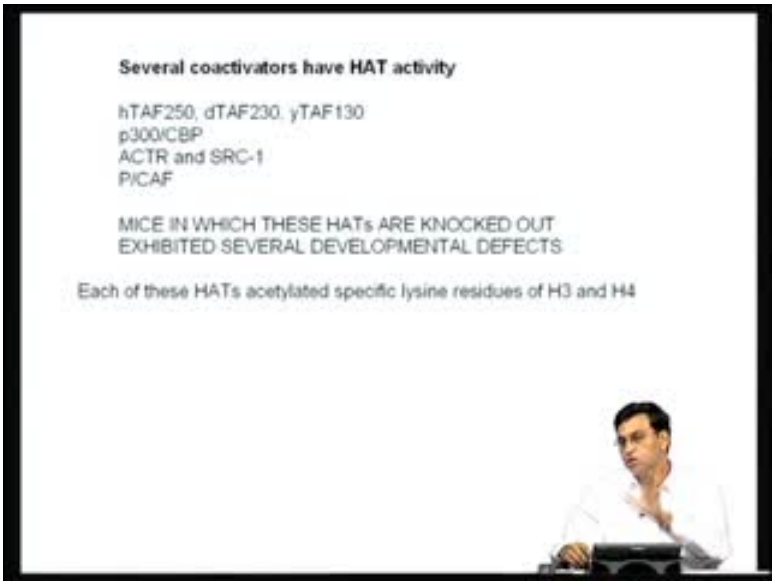

So, people should realize that many proteins, which were actually identified as coactivators, that is these proteins or protein complexes, which bridge between, which acts as a bridge between transcriptional activators and general transcription factor. They all contain HAT activity, and I have listed some of the examples here, like human TAF

250, which is one of the subunits of the TF2D, and you have TAF 230, TAF 180. These are all TBP-associated factors; they are all turned out to be histone acetyltransferases.

p300/CBP, a very important coactivator for many transcription factors we will discuss in detail at later classes, and very importantly, when you knock out some of this HATs in mice, and these mice developed several developmental defects, clearly indicating that the histone acetyltransferases are playing a very important role in regulation of gene expression during development, and if you knock of the HAT activity or you can knock of the genes encoding the HATs, you end up with many developmental abnormalities, and many of them are actually lethal.

So, the embryos do not even develop at all, indicating that histone acetylation plays a very role in developmental regulation of gene expression, and these HATs actually have the capability to acetylate specific lysine residues in H3 and H4. As I told you, histones are basic proteins, and each of this H3 or H4 histone contains a number of lysine or arginine residues, and be interestingly, many of these HATs, when I mentioned some of this set, each of them HATs recognized a specific lysine residue or a specific arginine residue in either H3 or H4.

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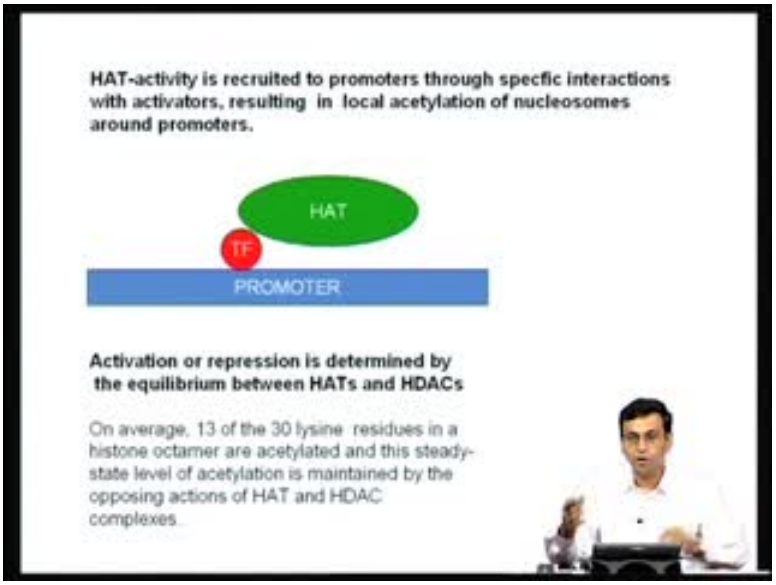

So, these are highly specific acetylation reactions involving select lysine or arginine residues of H3 or H4. So, you can now see, a concept is now emerging. What I have

shown, so far, in order for transcription initiation to or activation to take place, first, the core promoter region, the general transcription machinery has to be assembled. Then, you get basal level of transcription. Then, I told you, transcription factors go and bind to upstream sequences, and somehow, the binding of transcription factors upstream region can facilitate faster recruitment of this basic transcription machinery promoter, therefore, promoting multiple role of transcription initiation, and thereby enhancing the rate of transcription.

Now, we can realize, one of the mechanisms by which these transcription factors are actually acting is by recruiting the histone acetyltransferases, which are actually part of many coactivator complex like SAGA and so forth. So, the main purpose, or main mechanism by which the transcription factor are enhancing the rate of transcription, is actually by recruiting these HATs or multiprotein complex-containing HATs. These HATs, in turn, interact with some of the components of the general transcription machinery, and that is how the transcription factor function is now linked to the base general transcription machinery.

So, activation or repression of transcription is determined by the equilibrium between HATs and HDACs. So, when you have acetylation, there is an enhanced expression of genes; when there is deacetylation, there is a decrease in gene expression. And there is an equilibrium between the HAT activity and HDAC activity at any given time, at any given promoter region, and it was estimated that on an average 13 of the 30 lysine residues in a histone octamer are actually acetylated at any given time, and this steady state level of acetylation is maintained by the opposing actions of HAT and HDAC complex.

So, there is a constant acetylation of histones residues, lysine residues have your arginine residues of histones, and they are also getting deacetylated by HDAC, and this acetylation-deacetylation is a very dynamic process, and this equilibrium is what ultimately is involved with the regulation of gene expression.

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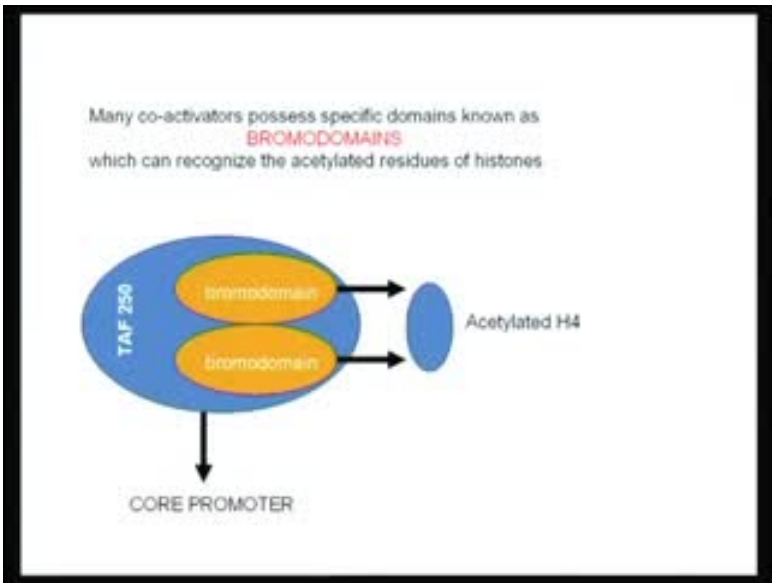

So, many coactivators possess specific domains known as bromodomains, which can recognize the acetylated residues of histones, for example, in this case I have shown in this cartoon how the one of the components of TF2D, namely, the TAF 250, that is, the TBP associated factor 250, through the bromodomains, on one hand they can recognize the acetylated histone H4 residues; on the other hand, the TAF 250, in association with the other component of TF2D, can also recognize the core factor, and therefore, these 2 process can be recognized, integrated, and that is how it brings about activation of transcription.

So, histone acetylation, the acetylated histone H4 is recognized by the member of a general transcription factor complex through the bromodomains, and then in combination with RNA polymerase, can bring about transcription activation.

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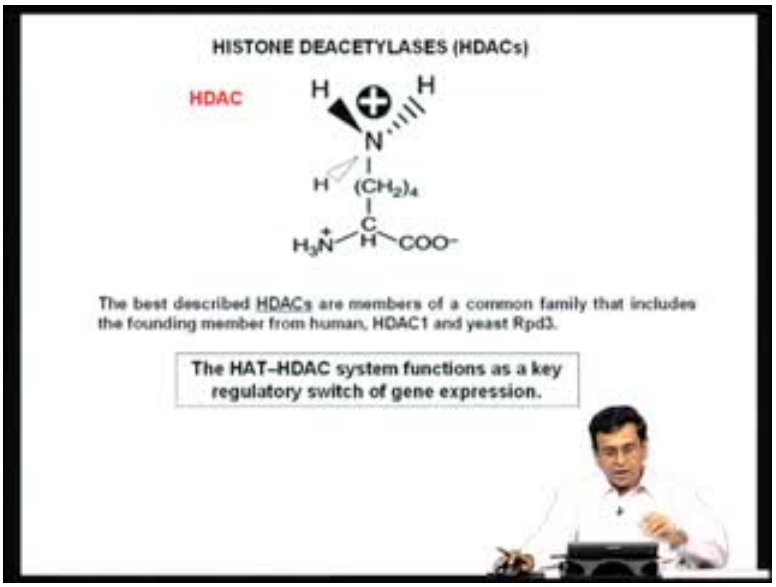

Now, what we will now discuss is that, we will talk on now about histone deacetylases. So, once the acetyl groups are added to the histone molecules, they also have to be removed; otherwise, the genes should be turned down all the time.

So, how histone deacetylation take place and what kind of enzymes are actually involved in histone deacetylation? And what is their role in the regulation of gene expression? As I mentioned, there is an equilibrium between histone acetyltransferases or HATs and histone deacetylases, and the equilibrium in the HATs and HDAC actually determines they, whether genes are in transcription active state or transcription inactive state.

So, I have just shown this here, that you have, for example, a lysine residue of a histone, and you have HAT molecules, which now add acetyl group to the lysine of a histone, and therefore, it becomes an acetylated lysine. And you also have this HDAC or histone deacetylases, which now again convert it back to a deacetylated form, and this would result in transcription repression.

So, there are number of histone deacetylases have been described in the literature, and the best described HDACs are members of a common family that includes the founding member of from humans, namely, the HDAC, and it is homolog in yeast called Rpd3.

So, remember, the HAT-HDAC system functions as a key regulatory switch of gene expression. So, when HATs are active, histones are acetylated and you have activation of

transcription; when HDACs are active, you have a repression of gene expression. Therefore, genes are inactive. So, it is the HDAC-HAT system functions as on-off switch for regulation of gene expression in eukaryotes.

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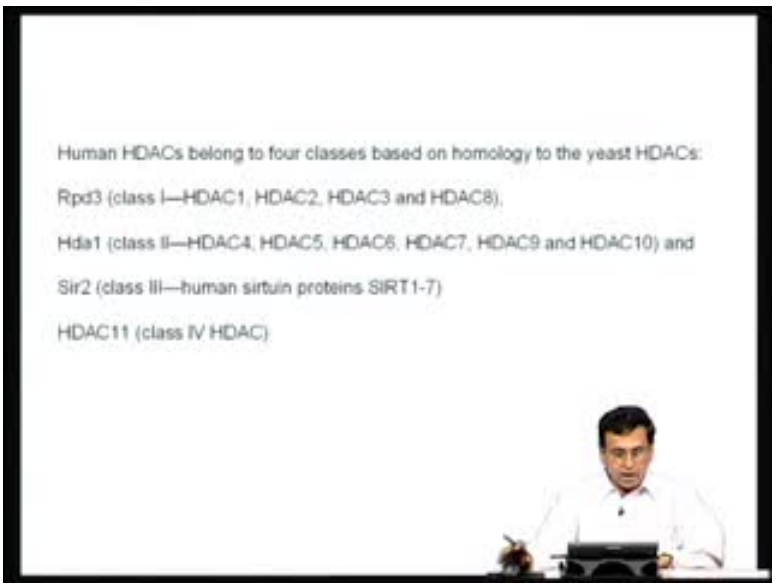

Now, as I mentioned, there are number of HDACs have been discovered in a various number of eukaryotes, and these have been classified into a number of families to for the ease of study. For example, the human HDACs belong to four classes based on their homology, to the ease HDACs, and these are called the Rpd class, HDA1 class, Sir2s and HDAC11 class. Class 1, class 2, class 3, and class 4, and there are various members of these things. We will now talk about the Sir2 class of histone deacetylases. Not… Now. they again play a very important role, and they also have a very important role in aging, and so on and so forth.

We will discuss the role of Sir2s in a in a separate class at a later stage of this course, but today, we will talk about only about other class of HDACs, and what is the relevance for gene expression.

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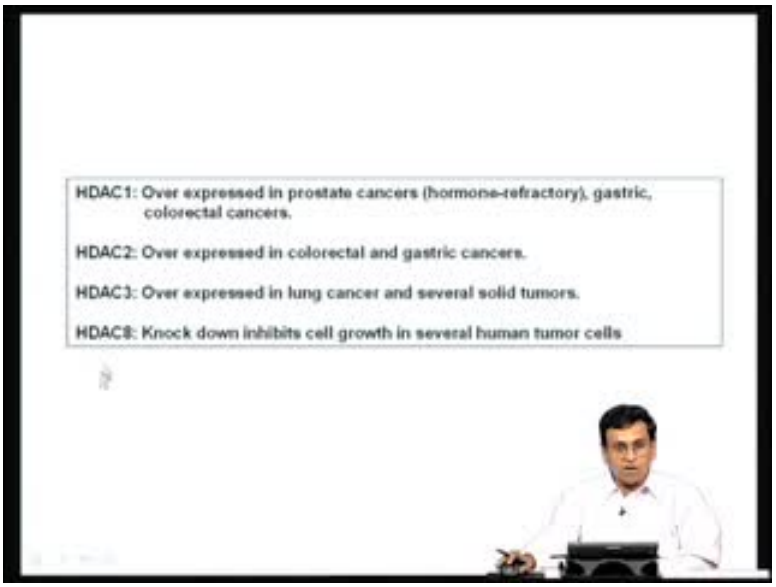

Now, why are these HDACs very important? Now, when people started identifying these HDACs, and then asked the question– where are these HDACS expressed? When you look at the expressions of these various HDACs in normal cells versus tumor cells, and they found, many of this HDACs are actually over expressed in a number of cancer tissues.

For example, the HDAC 1 is actually over expressed in prostate cancers, gastric and colorectal cancers. Now, this is always only correlation that tells me that in many of these cancers, there is a very high HDAC activity.

Similarly, the HDAC2 is over expressed in a number of colorectal and gastric cancers; HDAC3 is over expressed in lung cancer and several solid tumors, whereas HDAC 8, if you knock down, it inhibits the cell growth in several human tumor cells. So, these studies clearly told that, the HDAC, the histone deacetylases, when they are active, they can actually may be involved in cell proliferation.

So, what could be happening is that these HDACs may actually be deacetylating the promoters of many tumor suppressor genes, and therefore, the expression of these tumor suppressors may be blocked, and as the result, the cell has become cancerous, and evidence actually comes from here. In the case of HDAC 8, which is actually expressed in the particular tumor cell line, when we knocked out this HDAC tumor 8, you can

actually inhibit the cell growth of… that means, some of this tumors promoter genes expressions is getting turned down, and therefore, it is resulting in the succession of growth, indicating that these HDACs plays a very important role in cell proliferation and cancer.

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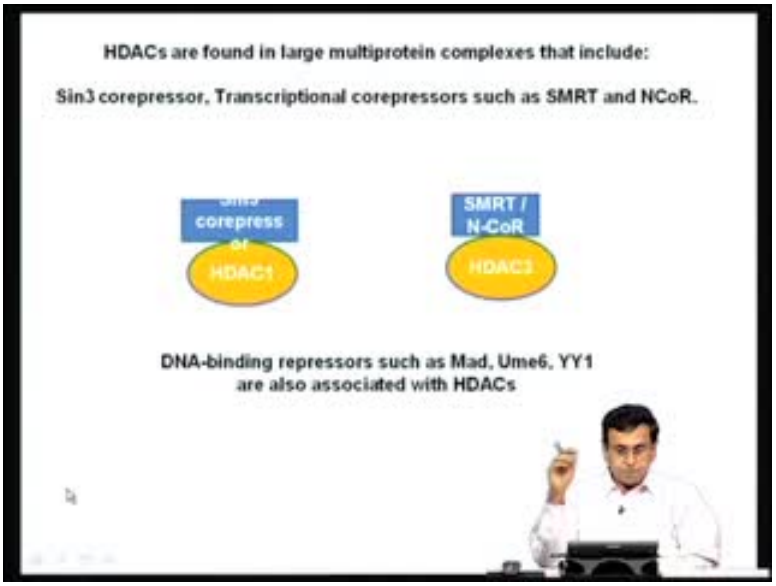

Now, as it is true for many of these HATs, the HDACs are also found in a number of multiprotein complexes, for example, I have given 2 examples here; it is something called as a Sin3 corepressor, and there are transcription repressors such as SMRT and NCoR.

We have discussed all these things in previous classes, and HDAC1 is a very important component of some of these corepressor complexes, and there are also many examples where many corepressors and repressors, which are actually known in negative regulation of transcription, they have been associated with histone deacetylases.

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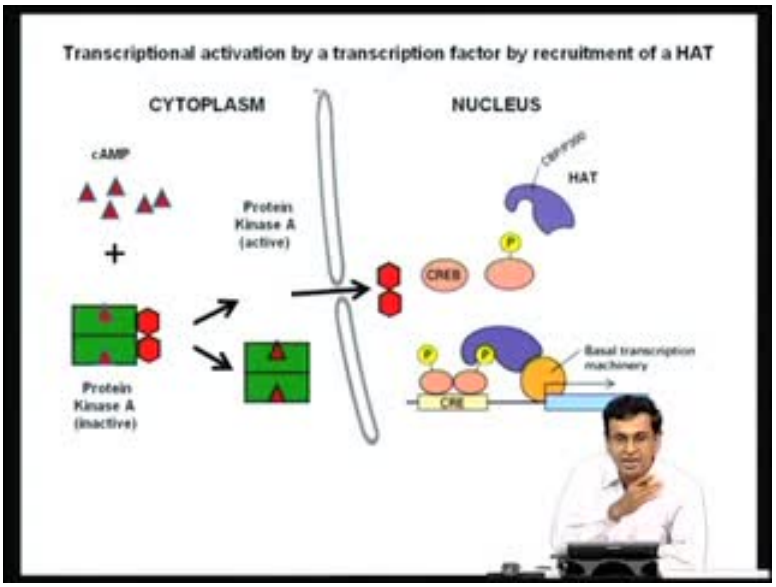

Now, I want to now discuss 2 examples, just to bring in to you get a overall picture of how a particular signal transaction pathway will ultimately result in either activation recruitment of a HAT leading to activation of transcription, or in the presence of HDAC, can result in a repression of transcription.

Now, in this example, I am going to tell you a particular signal transaction pathway, which involves the generation of a second messenger called cyclic AMP. Now, there are certain growth factors; when this growth factor interact with some specific cell surface receptors, this growth factor receptor interaction ultimately results in the activation of an enzyme called adenylate cyclase. This adenylate cyclase converts ATP into a molecule called cyclic AMP.

Now, cyclic AMP is a very important second messenger. I am not going to discuss the all the important activities of cyclic AMP in this class, but remember, cyclic AMP is a very important second messenger and plays a very important role in the regulation of gene expression as well.

Now, when cyclic AMP is not there in the cells, when cyclic AMP is not present, there is an enzyme called protein kinase A, which is present in the cytoplasm of cells, and this enzyme has 2 regulatory subunits, 2 catalytic subunits; the regulatory subunits I have shown it in green, the catalytic subunits I have shown in red.

Now, as long as this regulatory subunit is associated with the catalytic subunit, this protein kinase A is remains in a inactive form. So, it cannot, is not enzymatically active; it cannot add phosphate group to a protein.

Now, when a growth factor binds to a cell surface receptor and activates an adenylate cyclase, and once cyclic AMP is made inside the cells, now this cyclic AMP now comes and binds to the regulatory subunit of protein kinase A, and once the…, once the cyclic AMP binds the regulatory subunit, it causes the dissociation of the regulatory subunit from the catalytic subunit.

Once the catalytic subunit is dissociated from the regulatory subunit, this catalytic subunit now becomes active; it is catalytically active. Now, this protein kinase A, now this translocates from the cytoplasm to the nucleus, and now this catalytic subunit phosphorylates a very important transcription factor called as CREB.

Now, CREB stands for Cyclic AMP Response Element Binding protein. Why is it called? Because this protein actually goes and binds to specific sequences called cyclic AMP response elements, which are in present in the promoters of genes which are activated in response to cyclic AMP.

So, if there are certain genes which are getting activated in response to cyclic AMP, it means, that these genes contains cyclic AMP response elements, and this cyclic AMP response and it should be bound by CREB.

Now, what is important is that the catalytic subunit now goes and phosphorylates a specific serine residue of this CREB, and therefore, the CREB now becomes phosphorylated. Now, this phosphorylated form of CREB now interacts with a histone acetyltransferase called CBP or p300, and as a result, this results in the activation of transcription from genes containing cyclic AMP response elements and their promoters.

So, you can see here, the binding of a growth factor to a growth factor receptor in the cell surface has come all the way, and how the signal is propagated all the way into the nucleus. Ultimately, it has resulted in the recruitment of a histone acetyltransferase leading to the activation of gene expression.

So, genes have to be activated in response to cyclic AMP, recruitment of histone acetyltransferase by phosphorylated CREB is a very important step. So, this is how a signal transduction pathway operates is just one example. Now, I will give you another example to, just to show how a transcription is shut off by another signal transduction pathway, this time involved in a histone deacetylases or a HDAC.

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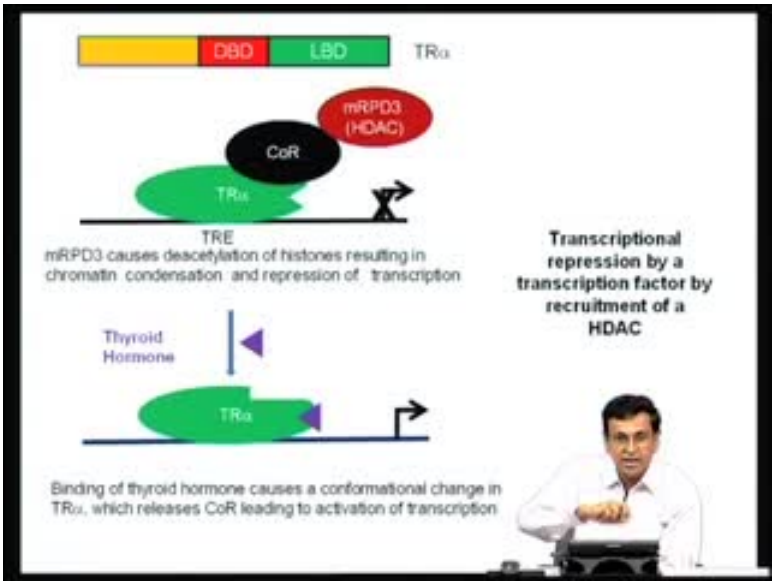

Now, histone. We have discussed, for example, steroid hormone receptors. Now, I told you, steroid hormone receptors belong to a family called nuclear receptor super family, and they contain a zinc in that DNA binding domain. They usually contain what is called as a DNA binding domain, through which they go and bind to specific DNA sequences in the promoter regions, and they also contain a ligand binding domain or a transcription activation domain, just like in the case of glucocorticoid receptor, and a glucocorticoid hormone binds to the ligand binding glucocorticoid receptor. It results in the activation of transfer glucocorticoid response genes. In the case of thyroid hormone receptor, the ligand binding domain binds to the thyroid hormone, thyroid hormone.

Now, in thyroid hormone is not present, that is, if you do not treat cells with thyroid hormone, the thyroid hormone receptor which is present in the nucleus interacts with the thyroid hormone response elements in the promoter regions of genes, and this thyroid hormone receptor in the origins of the ligand. This ligand binding domain interacts with a corepressor complex, which contains a histone deacetylases.

As a result these genes are not a transcriptionally active what I am trying to say is that when there is no thyroid hormone inside the cells the thyroid hormone negatively regulates the expression of genes responsible thyroid hormone because it interacts with a histone deacetylases.

Now, the moment you click the cells with thyroid hormone, thyroid hormone enters the cells because thyroid hormone is a very small molecule. It easily enters the cell and it goes and binds to a ligand binding domain or the thyroid hormone receptor, and this binding of the thyroid hormone to the ligand binding domain receptor, which I have shown here in this as a triangle, now cause a conformational change the ligand binding domain, and as a result, the ligand binding domain of thyroid hormone receptor can no longer bind to the corepressor complex, and therefore, the histone deacetylase cannot interact. Therefore, no deacetylation of histone takes place, and therefore, now, genes are activating.

So, the previous example, I described to you how cyclic AMP results the recruitment of histone acetyltransferase leading to activation of transcription. In this case, I told you how the addition of a small molecule– thyroid hormone– results in the dissociation of a histone deacetylase resulting in the activation of transcription.

That is, the positive regulation of gene expression is an example of a negative regulation of a gene expression. In one case, there is a HAT recruitment; in another case, there is a dissociation of HDAC from a transcriptional activator.

So, this is how various signal transduction pathways are integrated. Ultimately, the activation of genes ultimately involves either recruitment of a histone acetylase or a histone kinase, or histone methyl transferase, or histone deacetylase, depending upon what kind of genes we are studying.

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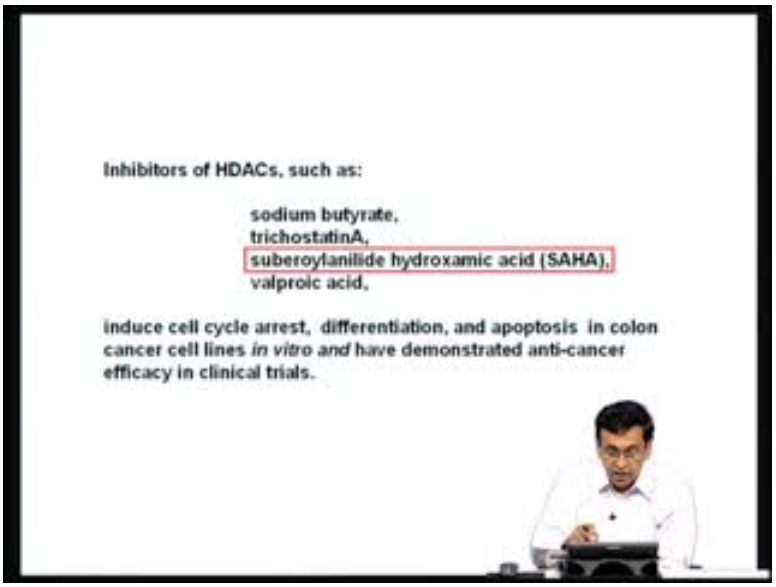

Now, what is very important, I want to now describe is that people are now started identifying a number of small molecules, which have, which can go and inhibit these various HDACs. I have listed some of this compounds here, like sodium butyrate, trichostatin A, suberoylanilide hydroxamic acid or abbreviated as SAHA, valporic acid, and so on.

When you treat cells with various HDAC inhibitors, they induce a number of important processor like, for example, they can induce cell cycle arrest; they can cause differentiation of cells; they induce apoptosis in colon cancer cell lines; and so on and so forth, and many of these compounds, which are HDAC inhibitors have found to have anti-cancer properties.

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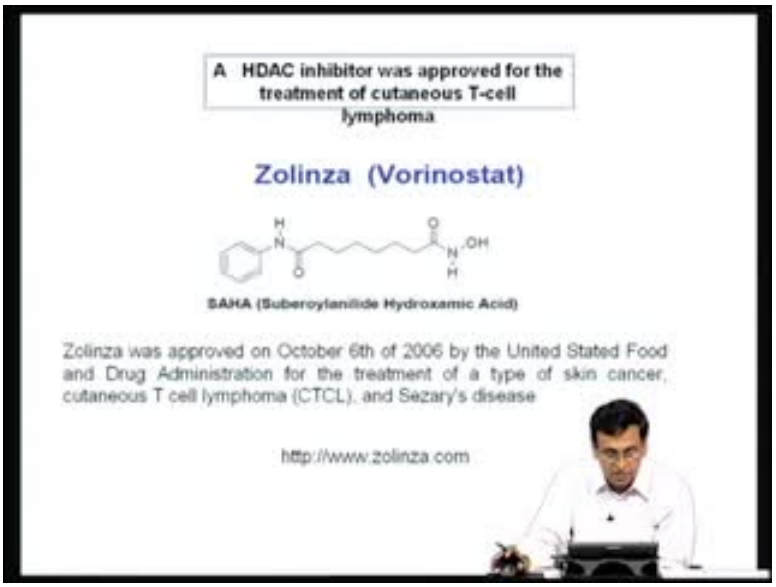

I will only give one example because it has a very…, it has already entered clinical trials. This particular example, called SAHA, which is a very potent inhibitor of HDACs, is actually now commercially available as Zolinza as a trade name, and Zolinza was actually approved on October sixth 2006 by United States Food and Drug Administration. So, for the treatment of a specific type of a skin cancer called cutaneous T cell lymphoma, and Sezary's disease.

You can actually get all the details if you actually click on to this website. So, here is an example where an inhibitor of a histone deacetylase is now being used for treatment of a specific type of cancer, clearly indicating that these HDAC inhibition of the activation of… this inhibition of the HDAC probably is the reason the activation of certain tumors suppressor genes, and that is why it is causing cessation of cell proliferation, and here you can actually see how basic research, understanding gene regulation can translate in the form of benefits.

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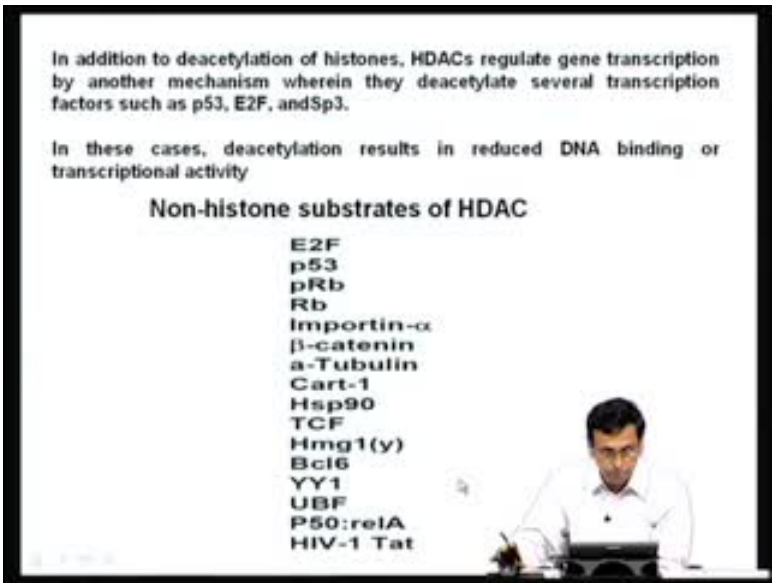

Here is a compound which inhibits the histone deacetylases, and this has a potential as use as an anti-cancer agent. Now, the other important point I would like to discuss is that, in addition to the deacetylation of histones, these many of these HDACs, they also go and deacetylate many other transcription factors, such as p53, E2F and many other factors, and in these cases, just as deacetylation of histones results in tighter binding to the DNA, deacetylation some of these factors results in either reduced DNA binding or reduced transcription activities.

I have just given here a list of a number of proteins which are not histones, but they are all substrate for HDACs. The point I am trying to make is that, in addition to histones, there are many non-histone proteins, which are also substrates for HDACs, and they also have a very important function because it either results in the reduced DNA binding or reduced transcription activation.

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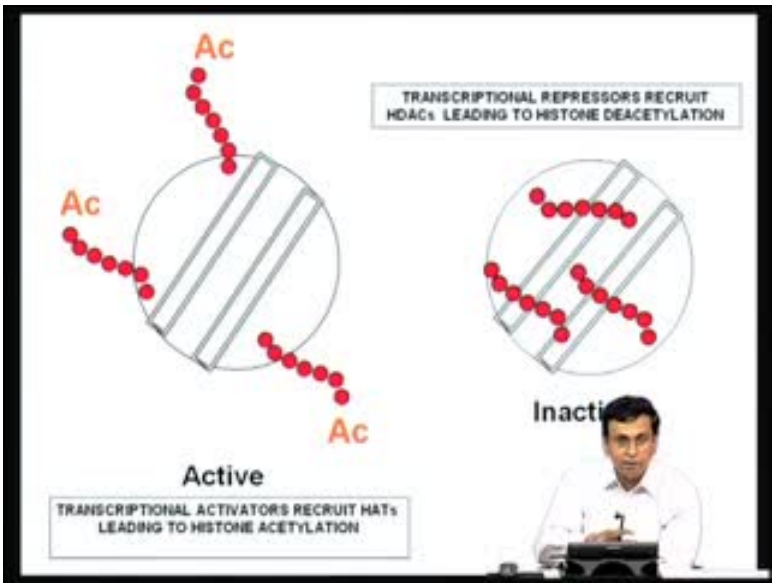

So, the take home message I am giving you from all the studies is that, here is a cartoon which tells you when transcriptional activators recruit histone acetyltransferase or HATs, it results in histone acetylation and transcriptional activation; this is a general principle we have talked so far.

Whereas, when transcription repressors recruits HDACs which remove the acetylated groups from the histones, it results in tighter binding of the histones, therefore, results in inactive chromatin, and therefore, transcription repression. This is the take home message from the discussion we have had so far.

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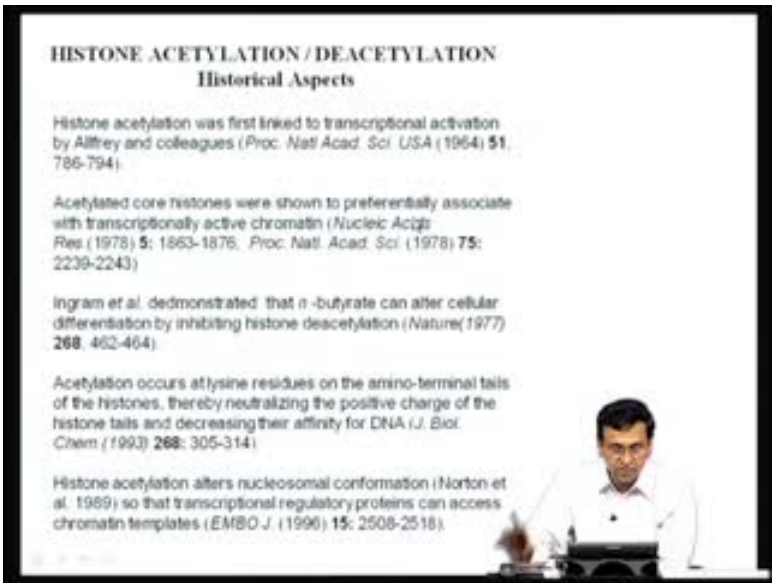

HATs involved means transcription activation, recruitments of HDAC means transcriptional repression. This is the take home message. So, what I have given in these next few slides is that I have actually discussed or listed a number of important papers, which have played a very important role in understanding the role of histone acetylation or histone deacetylation, the regulation noise in gene expression.

So, you can go through some of this papers, which are I would say a original research articles, and have played a very important role in understanding the role of histone acetylation and histone deacetylation in the regulation of gene expression.

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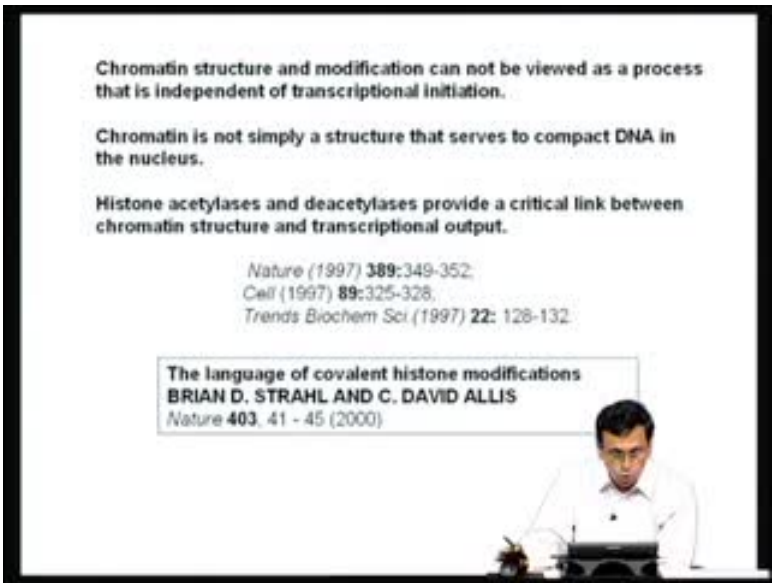

You can just go through; there are also very nice review article in Nature, which talks about the language of covalent histone modifications, and very briefly describes how the covalent modification of histones affect regulation of gene expressions. And also, I have listed 3 papers which discuss very nicely about 3 important points, namely, chromatin structure and modification cannot be viewed as a process that is independent of transcription initiation.

Remember, in the beginning, I told you, before the discovery of relationship between histone modification and transcription regulation, people who were studying chromatin structure were different, people who were studying gene regulation were different. But, once people have realized that chromatin structure plays a very important role in gene regulation, these 2 people started coming together, and people started understanding how the chromatin structure is influencing gene regulation.

These papers very nicely discuss some of these aspects, for example, chromatin structure. Chromatin is not simply structure that serves to compact DNA in the nucleus, but it also has a very important role in transcription activation, and histone acetylase and histone deacetylatses provide a critical link between chromatin structure and transcription output.

So, in addition to chromatin structure, histones also play a very important role in the regulation of gene expression, and today we have discussed 2 important modifications of histones, namely, histone acetylation and histone deacetylation, and now histone acetylation by HATs, it is also an activation of transcription, and deacetylation of histone by histone deacetylases results in inhibition of transcription.

Also, give a very one very important example, where a compound called SAHA, which is a small molecule inhibitor of HDAC has already been approved as an anti-cancer drug for treatment of a specific type of cancer.

So, we clearly indicating that understanding the mechanism of gene regulation, understanding a mechanism by which histones either activate or repress gene expression, and how acetylation or deacetylation of histones can result in activation or repression of gene expression, has very profound implications in a number of disease processors, including cancer, and, at this time, I am going to talk mostly about the basic aspects of gene regulation. We will come back later, at the later stages of our course, and discuss in more detail how some of the basic knowledge that we have gained from understanding the basic aspects of gene regulation has been translated, or is being translated in the form of specific therapies for a number of disorders, including cancer, and so on and so forth.

I think I will stop.