Eukaryotic Gene Expression: Basic and Benefits

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Lecture No. # 23

Mechanism of transcriptional activation by nuclear receptors

Welcome to this lecture series on regulation of gene expression in eukaryotes - basics and benefits. This is lecture number 23, the last 2 lectures we focused our attention primarily on how gene expression is regulated by nuclear receptors. We first looked at the regulation of gene expression by steroid hormones, which are called as type-1 receptors, then we came and then looked at the regulation of gene expression by non-steroid hormone receptors or the type-2 receptors, which include vitamin D receptors, thyroid hormone receptor, retinoic receptors, and so on and so forth. And we discussed primarily, how these receptors bind to the DNA.

We had we had discussed that, the steroid receptors primarily bind as homodimers to inverted repeat sequences, whereas the non-steroid receptors or the type-2 receptors were bound to DNA as heterodimers, and the heterodimeric partner is a receptor called as retinoic X receptor. And unlike the steroid receptors, these type 2 receptors bind to DNA as heterodimers with RXR as a common heterodimeric partner, and they bind to direct repeat sequences.

So, having discussed, how these receptors bind to DNA, today we are going to discuss the mechanism by which these receptors activate transcription. So, what is the mechanism of transcription activation by nuclear receptors? So, we are going to focus our attention on the ligand bearing domain, whereas in the last 2 classes, we had focused our attention primarily on the DNA binding domain. So, let us now see, once the receptors bind DNA, how do they activate transcription that is going to be the focus of today's lecture.

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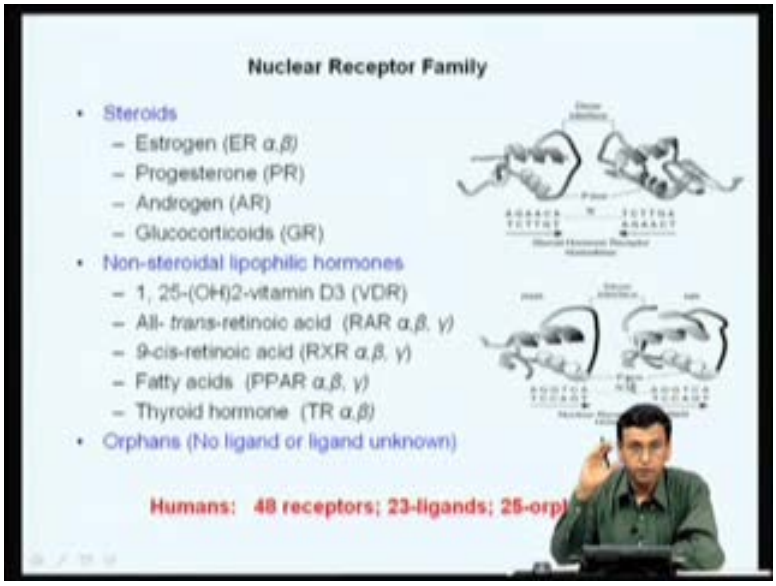

So, the nuclear receptor super family consists of basically steroid receptors, as well as, the non-steroid receptors. Under the steroid receptors, we discussed about the mechanism-wise steroid hormones, such as estrogen, progesterone, androgens and glucocorticoids regulate gene expression. Basically, we had demonstrated that these receptors primarily bind to DNA as dimers and the DNA binding domain contains what is called as a P-box, which is responsible for recognizing this specific DNA sequence. And there is also a dimer interface, which is involved in the dimerization of the nuclear receptors and these steroid hormone receptors are primarily bind to inverted repeat sequences. For example, here, the glucocorticoids receptor binding it, is soon it binds A G A A C A T G T T C T. Whereas, in direct contrast to the steroid hormone receptors, the non-steroid, the lipophilic hormones, like vitamin D, retinoic acid, fatty acids, thyroid hormone, all these receptors bind as heterodimers, and all these receptors, the common heterodimeric partner is a receptor, called as retinoic X receptor or RXR and the RXR nuclear receptor complex, then binds to direct repeat sequences in this example.

For example, A G G T C A A G G T C A, separated by a specific spacer; if these 2 half set are separated by 3 nucleotide spacer, it becomes a vitamin D response element; if the spacer is 4 bases, it becomes a thyroid hormone response element and if it is a 5 base pair, it becomes a retinoic acid response element. So, we discussed about it, what is called 1 2 3 4 5 rule, and so on and so forth. So, let us now see, once they bind DNA, how do they activate transcription?

Now, the nuclear receptor super family is a large family and as we discover more and more new receptors, then the family size is increasing, and as recently as last year, at least 48 nuclear receptors have been identified in humans and at least 23 ligands have been identified for many of these receptors. There are still 25 receptors, which are known as the orphan receptors, that mean, you still do not know, what are the ligands for these nuclear receptors. So, a lot more work needs to be done to understand and characterize all the members of this nuclear receptor super family.

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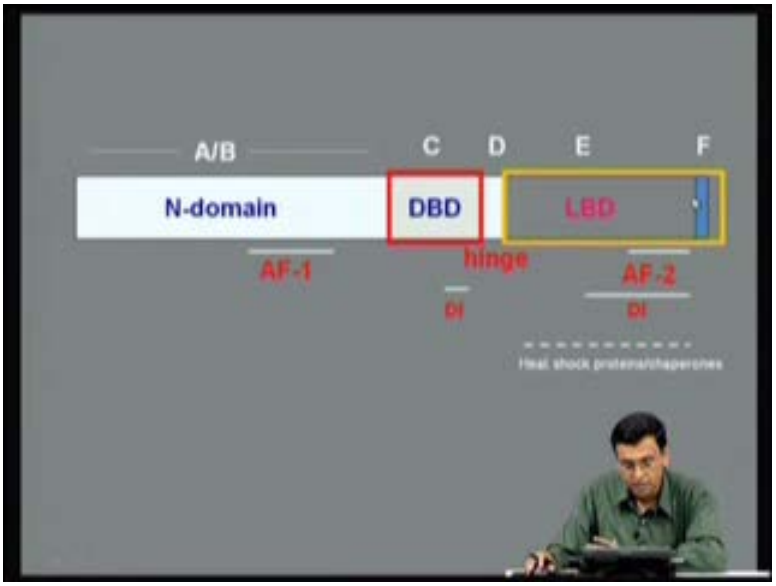

Let us now see, when the receptor binds DNA, how does it activate transcription? And we all know, that I have discussed in detail, that all the members of this nuclear receptor family share common structural features. They contain amino terminal domain, which consist of a transcription activation function, known as the activation function 1, which is ligand independent. It consists of a DNA bind domain, which consists of 2 zinc fingers, which in the case of steroid receptors bind to inverted repeat sequences. In the case of non-steroid receptors, it binds with direct repeated sequences and then it has a ligand binding domain, which is involved in the ligand binding, and it contains a very important transcription activation function, called AF-2 or activation function 2, which is a ligand dependent function. The AF-1 in the N terminal domain is a ligand independent transcription activation domain, whereas the AF-2 in the ligand bearing domain is a ligand dependent transcription activation function.

So, the discussion in the last 2 classes has been primarily on the DNA binding domain. How exactly these receptors bind to DNA? Today we are going to shift our focus to the ligand binding domain and ask the question, once they bind DNA, how does the ligand binding domain interact with the transcription machinery, leading to increase in the rate of transcription or activation of genes in response to these hormones?

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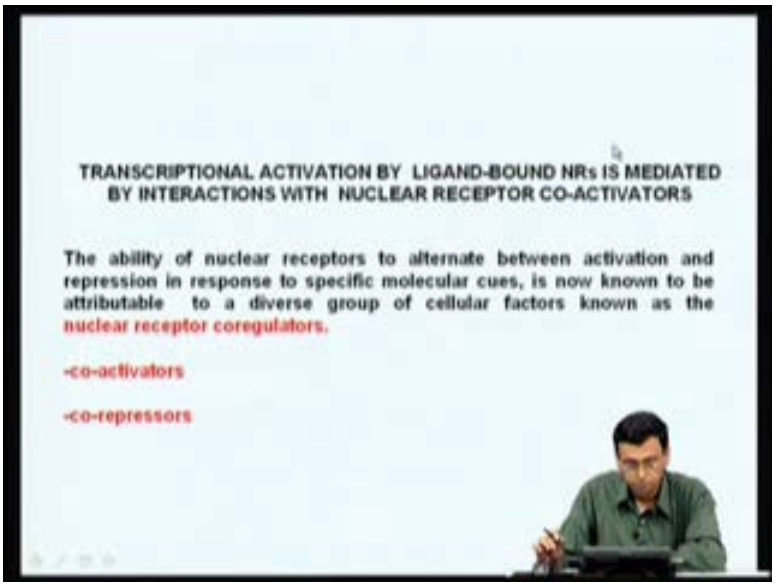

Now, the transcription activation by the ligand bound nuclear receptors is mediated by interactions with nuclear receptor co-activators. We had discussed on our early series of lectures, especially the 1st 15 lectures, how activators bind to enhancer sequences, interact with basal transcription machinery through co-activators, and how histones play a very important role in this transcription activation process? So, whether it is nuclear receptors or whether it is a cyclic amp response element binding protein, or whether it is nuclear factor kappa-B, any of this transcription factor, ultimately, the word mechanism by the activate transcription is by interacting with specific co-activators, and these co-activators, in turn, recruit histone modifying enzymes, like histone acetylases, and so on and so forth, resulting in the removal of the histone in the vicinity of the promoter and recruiting the basal transcription machinery and enhancing the rate of transcription. So, the same mechanism operates for the nuclear receptors as well.

So, the ability of nuclear receptors to alternate between activation and repression in response to specific molecular cues is now known to be attributable to a diverse group of

cellular factors, known as the nuclear receptor co-regulators. So, the nuclear receptors take the help of these co-regulators and by interacting with these specific co-regulators, they can either enhance the transcription of target genes or they can repress the transcription of target genes. If these receptors interact with co-repressors, then results in the repression of transcription, whereas if they interact with co-activators, it results in the activation of transcription. So, what we will discuss in the next few minutes, if you see, what is the mechanism by which these nuclear receptors interact with co activators and co repressors?

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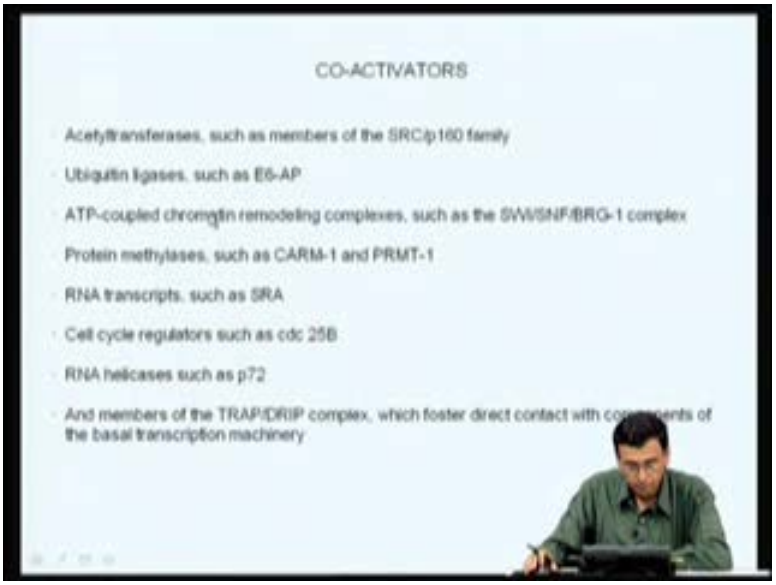

In general, the co-activators for many of these transcription factors have been very well characterized; not only for nuclear receptors, but also for other transcription factors and it followed a number of categories. For example, there are co-activators, which are basically acetyltransferases and these belong to members of, what is called as, steroid receptor co-activators or p160 family. So, their primary job is acetylate histones and this acetylation of histones leads to removal of the histones from the chromatin, resulting the recruitment of general transcription factors in RNA polymerase, resulting in the activation of transcription.

Some of the co-activators can be ubiqutin ligases and one of the examples is what is called, E6-AP. The co-activators can also be chromatin remodeling complexes and the example is SWI SNF BRG-1 complex. Sometimes, the co-activators can be protein

methylases, for example, histone methylases and examples can be CARM-1 and PRMT-1. Again, these are all histone methyl transferees or histone methylases. Sometimes the co-activators need not even be proteins, they can even be RNA and one example is called as the SRA, which is actually involved in a regulation of steroid hormone receptors. So, even RNA molecule can be a co-activator and sometimes proteins involved in cell cycle regulation, like cdc 25B, can also function as co-activators for certain transcription factors. Certain RNA helicases, like p72, have also been shown to be function as co-activators and members of TRAP-DRIP complex, which foster direct contacts with components of basal transcription machinery, can also serve as co-activators.

So, what we will do in next few slides is to see, which of these actually function as co-activators for the steroid thyroid receptor super family or the nuclear receptor super family.

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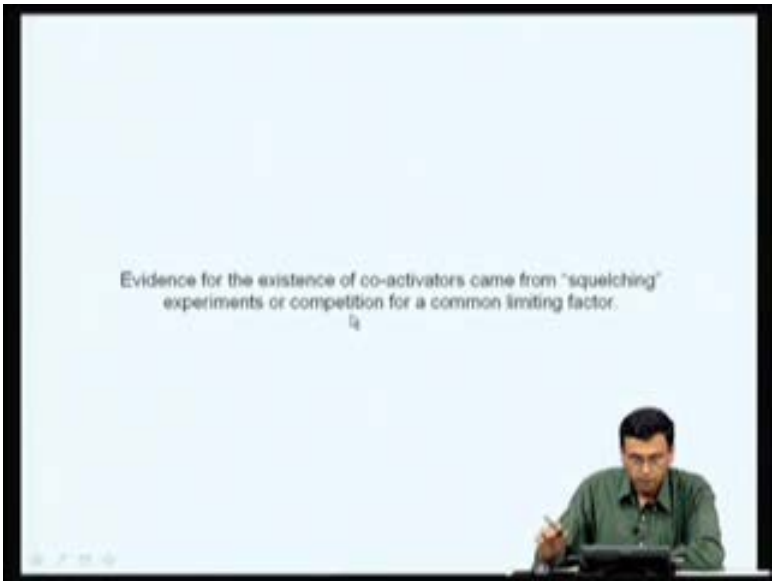

Now, the evidence for the existence of co-activators came actually from what are called as squelching experiments or competition for a common limiting factor. What was observed is that when you take a particular receptor and then, transmit with a response element linked to a reporter gene to which this receptor binds, we normally know, the receptor is expressed from this expression plasmid, which then goes and binds with the response element and activates the expression of the reporter gene. This is what we discussed as cis-trans co-transfection assay in the previous class. Now, along with this

reporter plasmid and this expression plasmid, if you now express another receptor, which does not actually bind to that target element or the cis plasmid, what was actually observed is that this expression of this other receptor, can actually result in the repression of transcription from the receptor A. Although this receptor is not binding to the response element, it is able to repress the transcription of the receptor encoded by the expressed in the other receptor. These kind of experiments, known as the squelching experiments, suggesting, that there are probably certain common proteins with which both these receptor A and receptor B are interacting, and as a result, even though they are not one of this other receptor is not binding to DNA, it is able to bind to this co-activators. And as a result, it is able to repress the transcription from the receptor A, and as a result it is not able to bind a response element to activate transcription. These experiments suggested, that there is some common mediators or common co-activators, which bind to both, receptor A as well as receptor B. Then, the search started, what are these co-activators and how do you identify these co-activators, which interact with these nuclear receptors?

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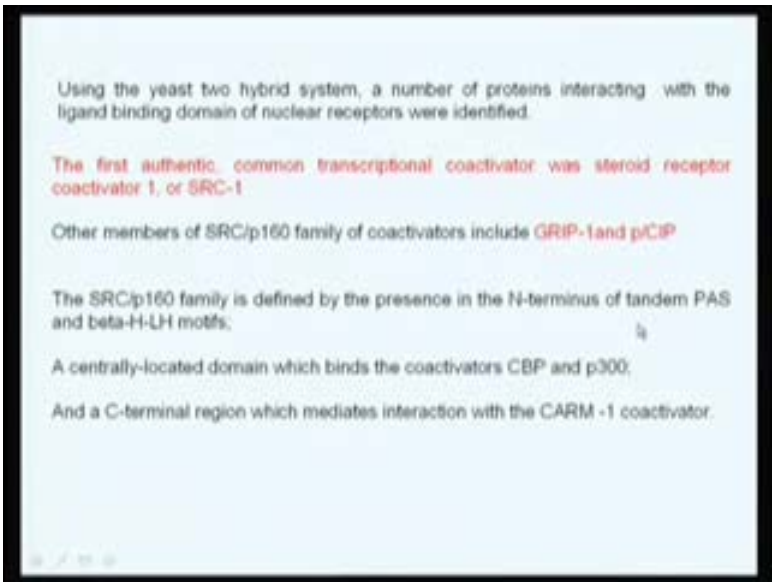

These experiments came from a number of experimental approaches, one of them which was very popular, what is called as the yeast two hybrid system. Again, we discuss this yeast two hybrid system in one of the early lectures, wherein basically, if you want to study protein-protein interaction, you use this yeast two hybrid system, where you have, what is called as the bait vector as well as a target vector, and using the beta gal assay,

you can actually identify, what are the proteins, which are actually interacting with your target protein?

Using that kind of experimental approaches these nuclear, nuclear centric co-activators were identified and 1st authentic, transcription co-activator for steroid receptors was known as the steroid receptor co-activator 1 or SRC-1. Later, many such co-activators have been identified, for example, what are known as GRIP-1 and pCIP. Now, what are these SRC-1 family of protein? These SRC p160 family of proteins basically, contain N-terminus, which contains, what is called as, tandem PAS or beta Helix-Loop-Helix motifs. They also contain a centrally located domain, which binds to certain co-activators, like the CREB binding protein or the p300, which are histone acetyl transferases. They also contain a C-terminal region, which mediates interaction with certain methyl transferase, like the CARM co-activator. So, these are the some of the characteristic features of this SRC family of co-activators.

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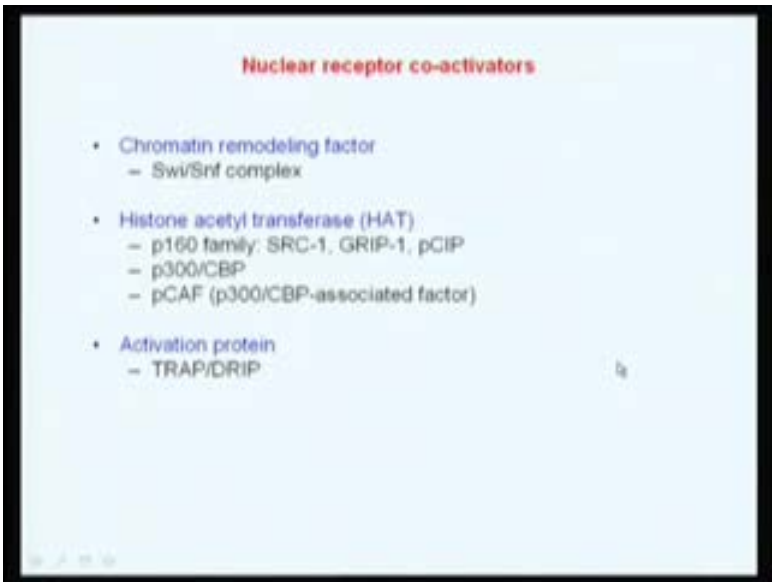

Now, in the case of the nuclear receptors, the co-activators followed a number of categories. There, some of the co-activators for nuclear receptors can be chromatin remodeling factor, like the SWI/SNF complex or in certain cases, they could be histone acetyl transferase, such as SRC-1, GRIP-1, pCIP or it could be p300/CBP, or it could be pCAF or the p300 CBP-associated factor, or in some cases it could be activated protein like TRAP or DRIP, which actually interact with the basal transcription. So, a variety of

proteins, either it can be chromatin remodeling factors or it could be histone acetyl transferases or protein, which directly interact with basal transcription, have all been identified as co-activators, which interact with specifically with nuclear receptors and bring about transcription activation.

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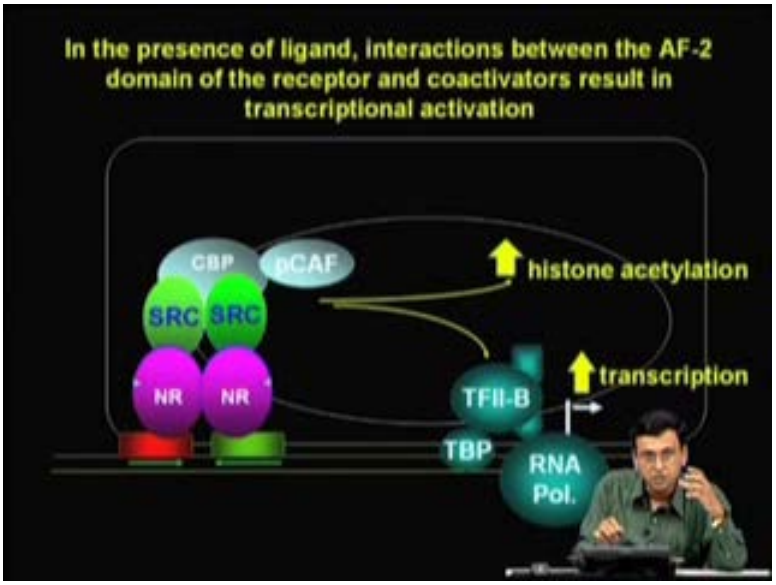

Now, what is the general mechanism by which this transcription activation is brought about? We know that the nuclear receptors bind to DNA as dimmers, either as homodimers in the case of steroid receptors, or heterodimers in the case of the non steroidal lipophilic receptors. And once these receptors bind to the target sequences as a dimer, it results in…, and then, when the ligand is present, the ligand will bind to the ligand binding domain and in the presence of ligand, this receptor, now able to interact this co activators, in this case, for example, the SRC or steroid receptor co-activator and this co-activator in turn interacts with protein, such as the histone acetyl tranferasers, like CBP or p300, which in turn interacts with the CBP associated factor - pCAF and these, then acetylate the histones in the vicinity of the promoter. And this histone acetylation results in the loosening of the histones recruitment of the general transcription factors, DNA polymerase, clearing to activation of transcription. So, this is the general mechanism by which nuclear receptors bring about transcription activation.

So, the ligand bind group receptor recruits or binds specifically this steroid receptor or co-activator molecule, such as the SRC, which in turn binds to specific histone acetyl

transferases, which remove histones, so that the general transcription factors now can bind to the TATA box and then, activate the transcription. So, this is how hormone responsive genes are activated by the hormone bound transcript hormone receptors.

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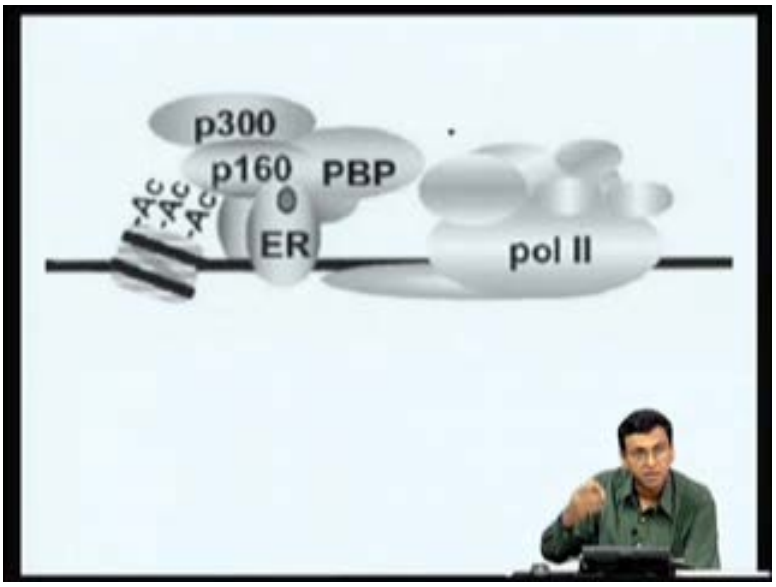

For example, if you take these 2 simple receptors as example, estrogen receptor in the presence of ligand binds to the estrogen receptor as a homodimer and this hormone estrogen receptor now recruits certain proteins, like p160 and PBP. And p160 in turn recruits the p300, which is histone acetyl transferase and p300 or CBP will now acetylate histones in the vicinity of the promoter, and as a result, histones are removed. And now, the RNA polymerase and the general transcription factors can bind and activate transcription. So, this is the mechanism by which estrogen receptor brings about transcription activation in the presence of estrogen hormone.

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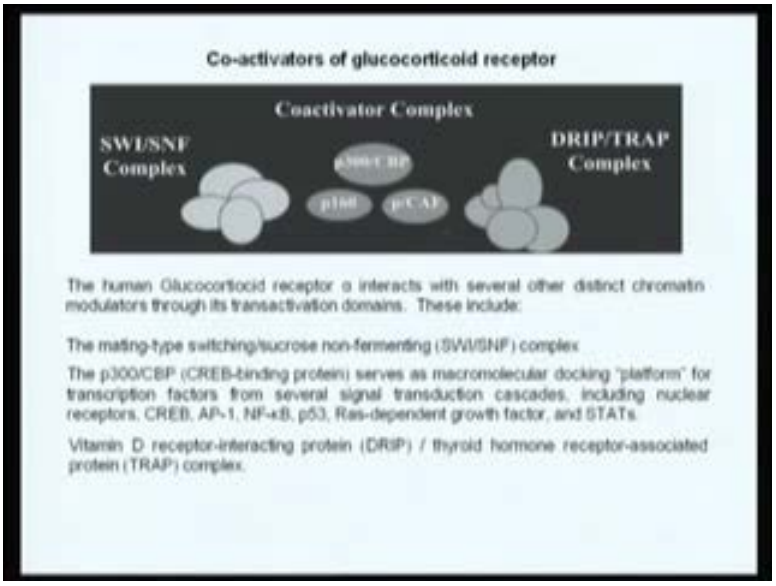

If you come to glucocorticoid receptor, it is, glucocorticoid receptor (( )) activation is one of the most well studied mechanisms of transcription activation by the nucleus receptor's super family and has been shown, a number of co-activators exists for glucocorticoid receptors. It can be SWI/SNF complex, which are chromatin remodeling protein; it could be histone acetyl transferase, like p300, p160 and pCAF; or it could be DRIP/TRAP complex, which actually interacts with the basal transcription machinery. So, the human glucocorticoid receptor alpha interacts with several distinct chromatin modulators through transcription activation domains. These include the mating type switching/sucrose non-fermenting complex or the SWI/SNF complex, which are chromatin remodelers, or it could be p300/CBP, which serve as the macromolecular docking platform for transcription factor from several signal transduction cascades, including nuclear receptors CREB, AP-1, NF-kB, p53 as well as STATs. So, the p300 CBP, which is already involved, already been shown to be involved in transcription activation by number of other transcription factor, is also involved in the transcription activation by glucocorticoid receptor.

Similarly, the other groups of co-activators, which are involved in transcription activation glucocorticoid receptor, include the vitamin D receptor-interacting protein or the DRIP, also known as thyroid hormone receptor-associated protein or TRAP. So, you can see, depending upon the cell type, depending on the situation or depending upon the stimulus and depending upon the tissue type or doing this developmental stage, the

glucocorticoid receptor can interact with any one of these co-activators and bring about transcription activation in response to glucocorticoid hormone.

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Now, the question comes, how do the co-activators interact with the glucocorticoid receptor or these steroid hormone receptors? So, how do these AF-2 domains interact with the co-activator receptors? And what are the features of the co-activators or the domains in the co-activators, which is involved in the, as an interaction with these nuclear receptors?

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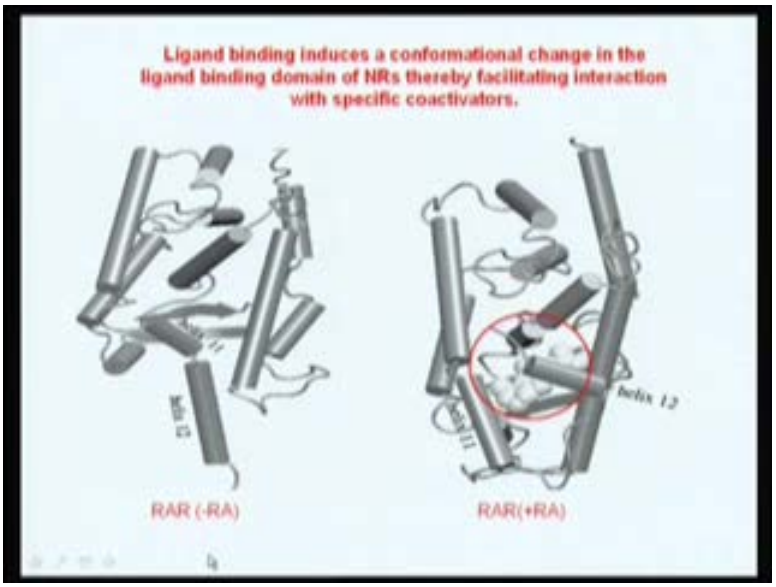

Now, a lot of effort has gone into characterizing the ligand binding domain of the nuclear receptors. You, basically, over express these ligand binding domains in E. coli, purify these proteins and subject it as X-Ray crystallography and based on that you get the crystal structure. Now, the crystal structure of both, unliganded as well as ligand bound receptors have been, is now known for a number of receptors and based on these studies it has been shown, that the ligand binding actually induces a conformational change in the ligand binding domain of the receptor. For example, the ligand binding domain has been shown to have number of alpha helices, which have been named from helix 1 to helix 12 and in the case of a ligand binding domain, the helix 11, helix 12 plays a very important role in the absence of the ligand binding domain. The helix 12 is actually protruding out, but once the ligand binds to this, the ligand binding domain, you can see, the helix 12 is taken in and this, this conformational change or the rearrangement of this alpha helices within the ligand binding domain is what is responsible for, whether the receptor is going to interact with the co-repressor or whether it is going to interact with the co-activator.

So, the conformational change that is induced by the ligand binding domain, now facilitates or now is favorable for interacting with the co-activator or in some cases, when the ligand is not bound, this kind of a confirmation, a co-repressor is able to bind to the ligand binding domain. So, depending upon the conformational change induced by the ligand binding domain in the presence or absence of the receptor, the ligand binding domains receptors are the alpha helices of this receptor, either can interact with the co-repressor or they can interact with the co-activator. These are the structures of retinoic acid receptor and you can see the retinoic acid receptor ligand binding domain, the helical structure in the absence of retinoic acid, whereas when you have retinoic acid, there is a change in the conformation. You can see, now the ligand binding is a conformational change; the helix 12, helix 11 is rearranged and under these conditions, it now can interact with the co-activator.

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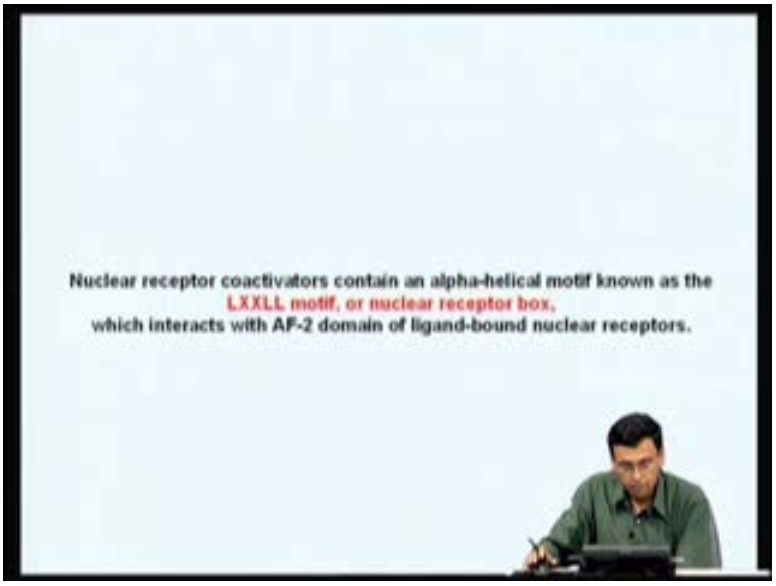

Now, how do these helices - helix 11, helix 12, of these nuclear receptors interact with co-activators? It turns out, the nuclear receptor co activators also contain alpha helical motifs, which contain a specific amino acids sequence, which known as the LXXLL motif, also known as the nuclear receptor box. So, L stands for leucine, X can be any amino acid, again L, L. So, the nuclear receptor co-activators interact with the specific alpha helices of the nuclear receptors through motif, which contain an amino acid sequence, called LXXLL motif, that is, leucine, any amino acid, any amino acid, leucine, leucine - this is the motif that is interacting with the specific amino acid residues of these helices in the nuclear receptor ligand binding domain. So, this LXXLL motif is also known as the nuclear receptor box and through this motif, the co-activators interact with the AF-2 domain of the ligand bound nuclear receptors.

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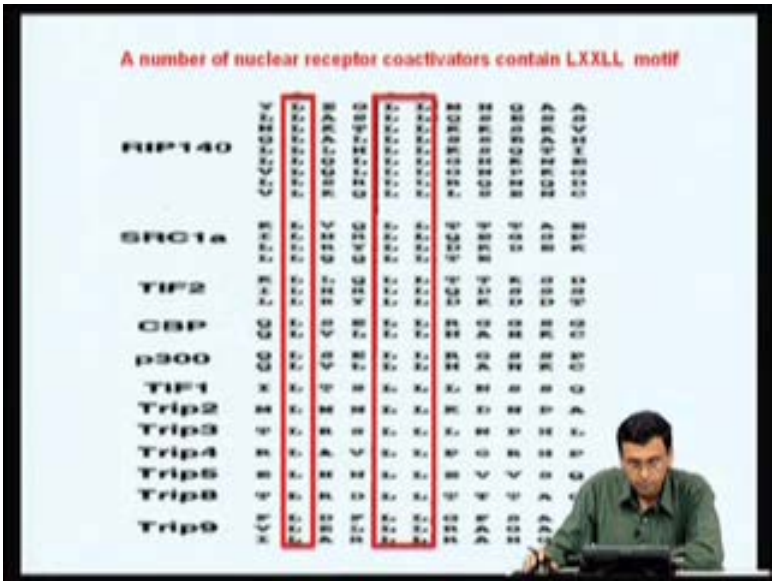

You can see here, these are all the nuclear co-receptor co-activators, which have been identified for a number of receptors and if you look at the amino acids sequence of these receptors, the boxes highlighted in the red, you can see, LXXLL - leucine any amino acids leucine leucine – so, this kind of a LXXLL motif is kind of conserved in a number of co-activators, which are involved and which mediate transcription activation by nuclear receptors. So, by studying the interaction of these various co-activators with a variety of nuclear receptors, people have identified, that all these co-activators interact with these nuclear receptors super family members through this LXXLL motif.

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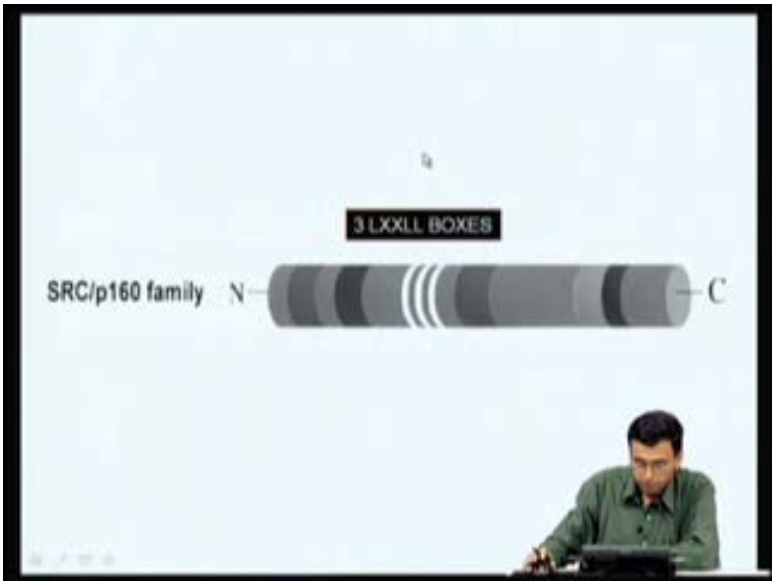

For example, if you now take the SRC p160 family of proteins, they contain 3 such LXXLL box, somewhere close to the amino terminals and it is these motifs, which are actually involved in the interaction with the nuclear receptors.

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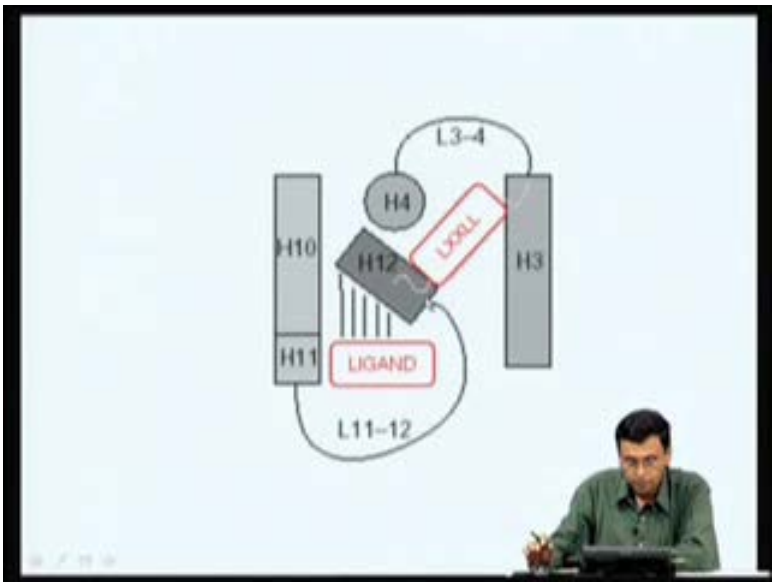

This is just a cartoon to show, that how in the presence of ligand the helix 12, which is, actually is, kind of, exposed outside, is now taken inside and this helix 12 is now able to interact with the LXXLL motif of the co-activators and this is what facilitates transcription activation. And these co-activators, which now interact with the receptors, can now interact with histone acetyl transferases, which in turn acetylate histones leading to recruitment of the general transcription factors and RNA polymerase, and resulting in the activation of transcription.

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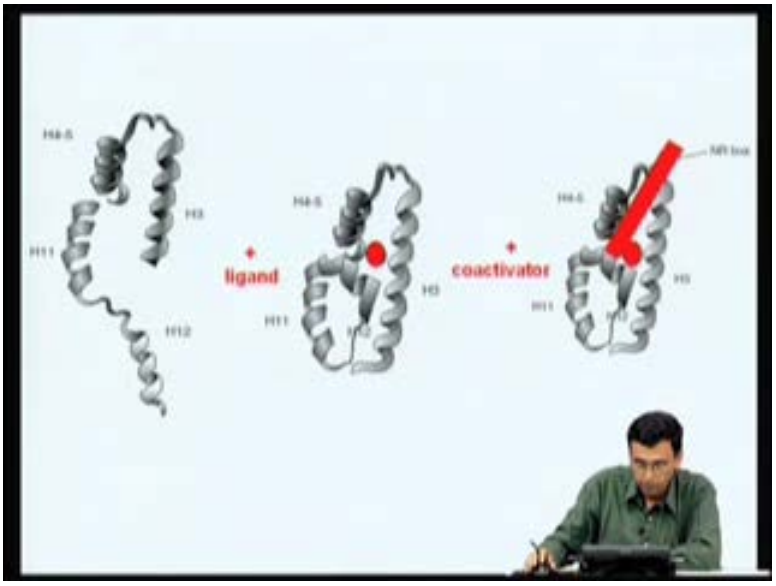

So, the transcription activation by nuclear receptors is, kind of, a 2-step process, so you have a confirmation, which is quite different. The absence of a ligand, some of the key helices in the ligand binding domain, which are very, very important for this transcription activation, are shown here. And this is the arrangement of the helices in the absence of the ligand, and once the ligand binds to the ligand binding pocket of the nuclear receptor in the ligand binding domain. And you can see, there is a rearrangement of the helices, the helix 12 is actually taken inside and under this confirmation, now it can interact with the co-activator, which contains the LXXLL motif or the nuclear receptor box of the co-activator. Now, it can interact with the specific amino acid residues of these helices and this is how co-activators are recruited by the nuclear receptors. So, 1st the ligand binds, induce the conformational change, that thereby facilitating the interaction with the co-activators through the LXXLL motifs.

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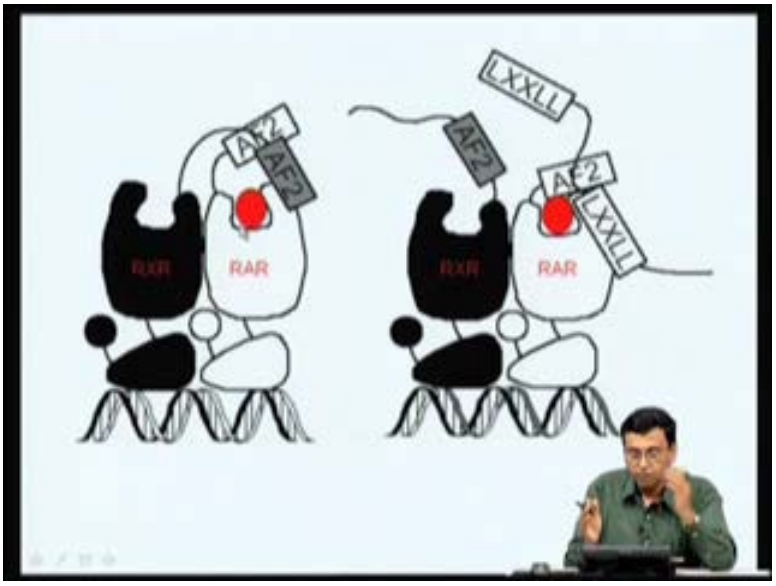

In the case of, for example, this kind of a LXL motif contain co-activators, are involved not only in the transcription activation by steroid hormone receptors, like glucocorticoid receptor, but they are also involved in the transcription activation by the non-steroid hormone receptors or the type 2 receptors. One of the examples shown is the RXR, RAR heterodimers. This is also true, that the partner can be either thyroid hormone receptor or can be vitamin D receptors or peroxisome proliferator-activated receptors or PPAR. So, all these receptors, which bind as heterodimers with RXR, they actually dimerize using the dimerization interface, the AF-2 domain, but once a ligand binds to the retinoic acid receptor of the 3 prime half-side binding partner and you can see, now the interaction between the RXR, RAR ligand binding domain is kind of distanced. And now, a co-activator can now be recruited and LXXLL motif containing co-activators can now interact with the specific amino acids of the heterodimeric partner for RXR resulting in activation of transcription.

So, in this case, it is a retinoic acid receptor, which is intact in the co-activator or if the heterodimer is RXR thyroid hormone receptor, then the thyroid hormone binds to the thyroid hormone receptor here. In the same way, a LXL kind can be recruited by another specific, LXXLL partner can be recruited by that thyroid hormone receptor and the same thing goes for the vitamin D receptor or PPR, and so on and so forth. So, the RXR n r heterodimer can interact with DNA. Once the ligand binds, the, the AF-2 domain can

now go and interact with the LXXLL containing activator, trans co-activators lesser than the activation of transcription.

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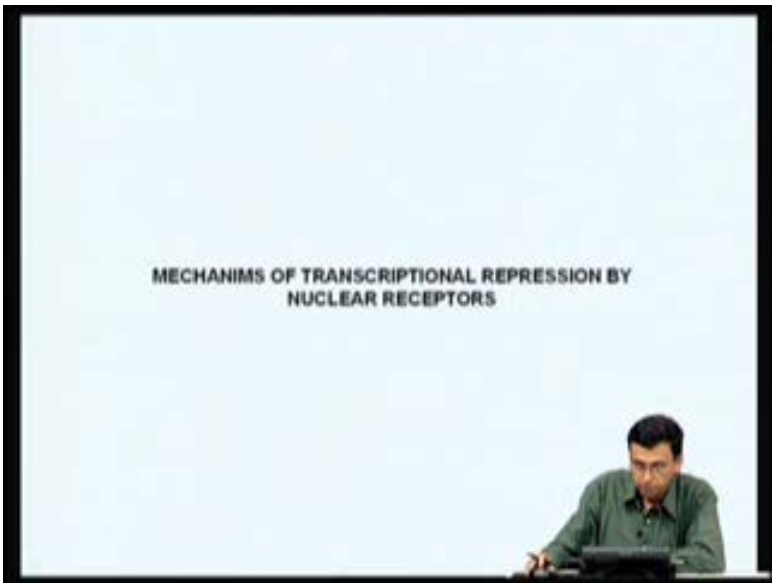

Now, so, what we have discussed so far is the mechanism by which nuclear receptors activate transcription. Nuclear receptors activate transcription primarily, by co-recruiting nuclear receptor co-activator and these co-activators can be wide variety of types, they can be chromatin remodeling protein, they could be histone actyl transferases, they could be protein methylases, and so on and so forth. But the basic mechanism of recruitment of the nuclear receptor co-activators by the nuclear receptors involves interaction of the nuclear co-activators through the LXXLL motif with specific alpha helices of the ligand bound nuclear receptor. This is the general mechanism by which these nuclear receptors are able to recruit these co-activators and brings about transcription activation.

Now, the question is, how about transcription repression? How do the nuclear receptors interact with transcriptional repressors? So, what is the mechanism by which transcription repression is brought about by nuclear receptors? Because when there is no hormone or there is no ligand, many of these nuclear receptors are actually repressed transcription, from the, of the target genes. So, how is this transcription repression brought about?

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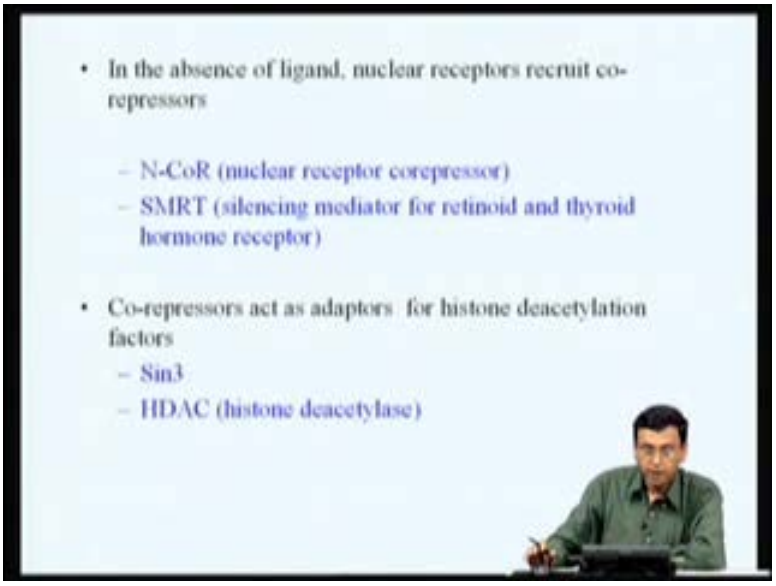

Turns out, in the absence of the ligand nuclear receptors interact with specific co-repressors. So, in the presence of hormone, because of a conformational change induced by the hormone, the ligand binding domain can now interact with the co-activators, whereas in the absence of hormone, the conformation of the ligand bind domain facilitates interaction with specific co-repressors. Some of the co-repressors, which have been identified to be involved in transcription repression by nuclear hormone receptors or, whereas in N-CoR or nuclear receptor co-repressor, SMRT or silencing mediator for retinoic and thyroid hormone receptor and some of the co-activator, co-repressor, and these co co-repressors, which interact with unliganded nuclear receptors, actually serve as adaptors for histone deacetylation factors. So, just like the co-activators of nuclear receptors interact with histone acetylates, bring about histone acetylation and activate transcription, in the absence of a ligand the same nuclear receptors interact with nuclear receptors, nuclear co, nuclear co-repressors and these co-repressors, in turn, recruit molecules such as Sin3 or histone deacetylase, results in deacetylation of histones, therefore tight binding of histones going in to repression of transcription.

So, the general mechanism, which we studied for during the initial stages of this lecture series, where transcription activation primarily involves losing of the histones by recruiting protein, such as histone acetyl transferases, whereas repression of transcription primarily involves recruitment of histone methylases or histone deacetylases, result in the tight binding of the histones during repression of transcription. This mechanism is

true for the nuclear receptors as well. So, a number of such molecules, which serve as transcription repressors for nuclear repressor, have been identified, these include the N-CoR, SMRT, so on and so forth.

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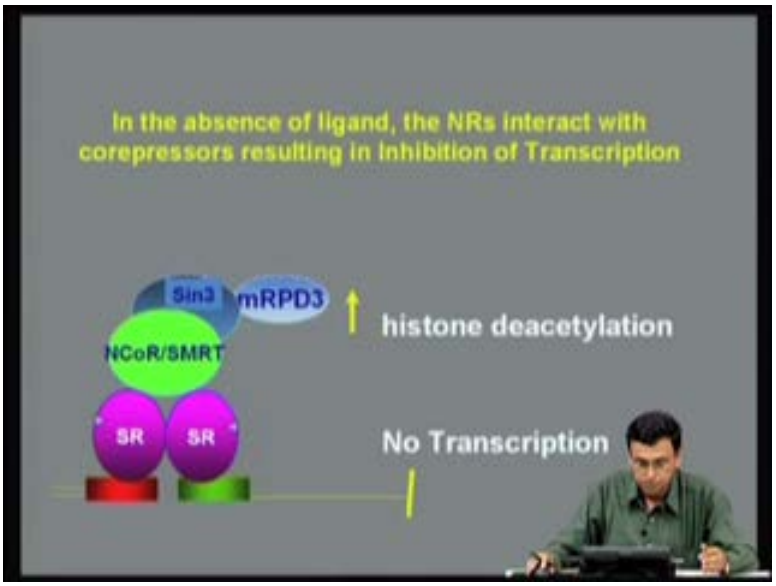

And the general mechanism of transcription repression of steroid receptors or the nuclear receptors, actually involves when the nuclear receptors bind to the target sequences as dimmers. In the absence of the ligand they interact with specific co-repressors, which could be either N-CoR or SMRT, which in turn recruit this. Sin3 are the histone dyacetylases and they actually cause histone deacetylation resulting in the repression of transcription. So, this is the general mechanism by which nuclear receptors bring about transcriptional repression.

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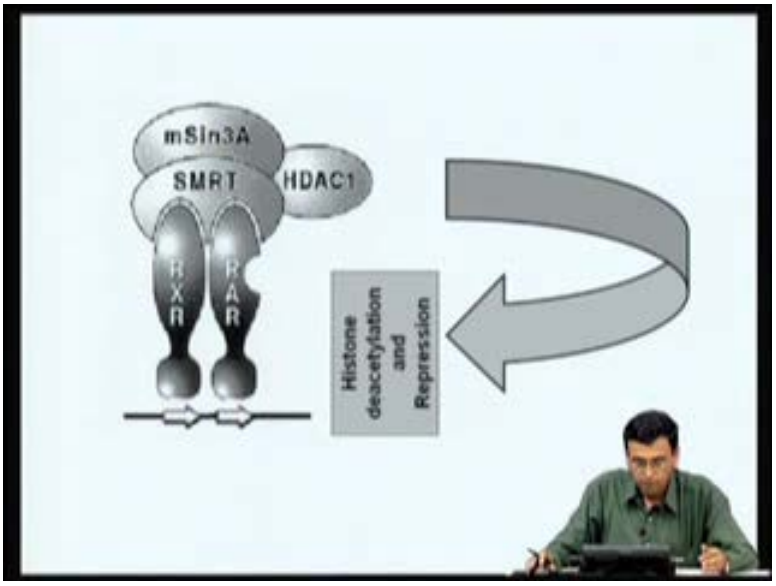

For example, in the case of the retinoic acid receptor, when there is no retinoic acid in the system, the RXR RAR and RAR heterodimer, which bind to the DR5 or the Direct Repeat 5 kind of a sequence, now interacts with the SMRT co-repressor. This is somewhat, in turn recruits, what is called as mSin3A, as well as, the histone dyacetylases of the HDAC1 and as a result, the histones are deacetylated, this resulting in the repression of transcription. So, this is the mechanism.

What kind of co-repressor is recruited depends, varies from one receptor to another, in this case it could be SMRT and other it could be N-CoR and so onm and so forth. So, by recruiting specific co-repressors, which in turn recruit either histone methyl trasferases or histone dyacetylases, transcription repression is brought about by nuclear receptors.

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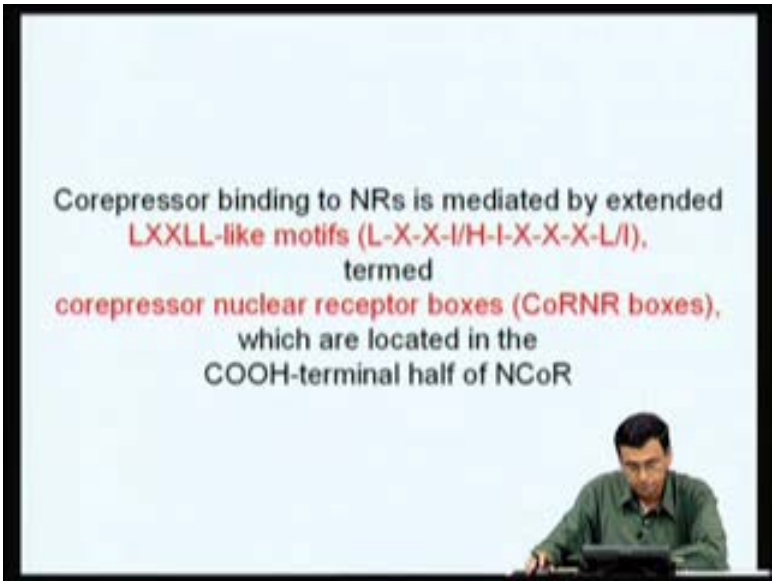

Just as the LXXLL motif is involved in the interaction of co-activators and nuclear receptor ligand binding domain, the same way a motif has been identified in the co-repressor nuclear receptor interactions. This co-repressor binding to the nuclear receptors, for example, many of these co-repressors, which interact with nuclear receptors, contain some LXXLL like motifs, but not exactly LXXLL motif and this could be LXXI or HIXXXL or instead of leucine it can be isoleucine. So, a modified motif of, modified variation of the LXXLL motif has been identified in many of these nuclear receptor co-repressors and this motif is actually involved in the nuclear receptor interaction in the unliganded form.

So, just as the LXXLL box or the NR box or the nuclear receptor box, these are known as the co-repressor nuclear receptor box or CoRNR boxes and these are actually located in the carboxyl terminal of, for example, in the case of nuclear receptor or co-receptor or N-CoR, this kind of a box is present in the carboxyl terminal of the N-CoR, repressor, co-repressor.

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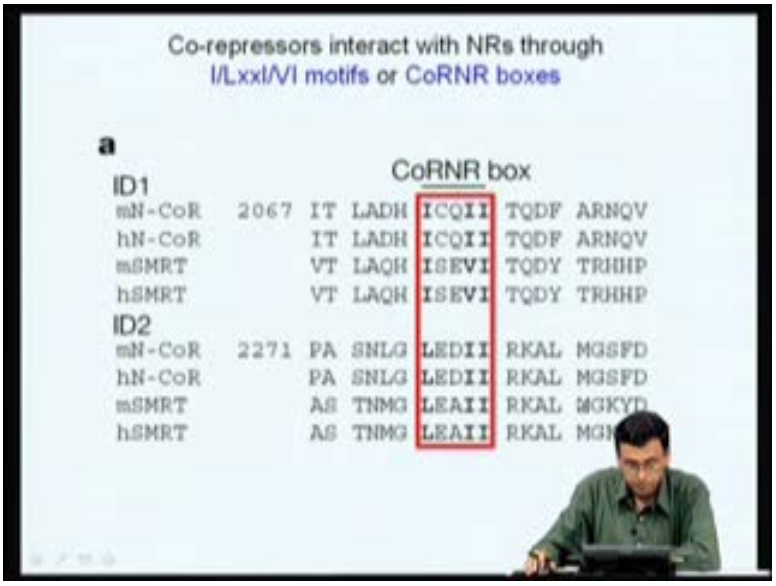

This slide just shows you that, these kinds of CoRNR boxes are the motifs involved in the transcription repression by the nuclear receptor. Co-repressors has been identified in a number of nuclear receptor, nuclear receptor co-repressors and you can see these are the motifs where it can be, it corresponds to consistent sequence shown here, I or L, I LXXCQ, this can be any amino acid, similarly isoleucine or valine or I. So, IRLXXII, so this is the motif that has been identified in the co repressors and through this motif, these co repressors interact with unliganded nuclear receptors and bring about transcriptional repression.

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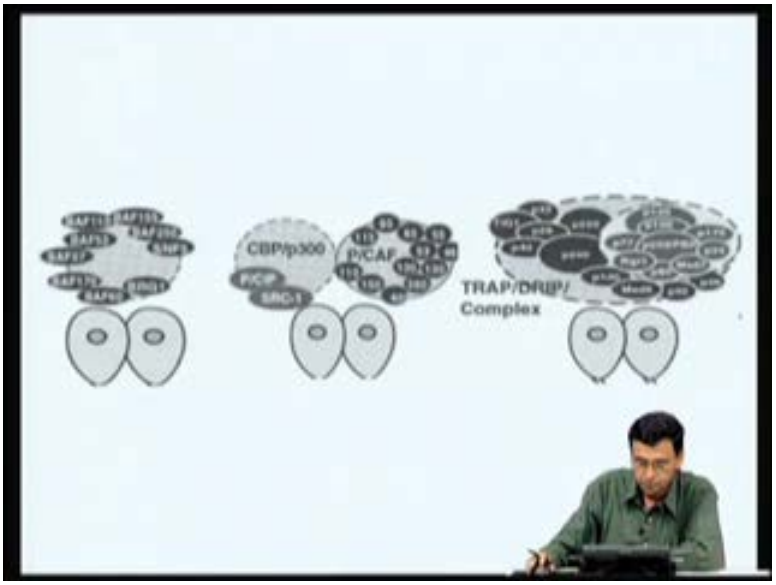

So, we will not go in to the details of the various co-activator co-receptor interactions because there are huge numbers of nuclear receptors. A number of such co-activators and co-activators recruiting proteins have been identified and you can see, this cartoon just shows 3 such examples, where a nuclear receptor dimer is actually interacting with a co-activator complex here, which is quite different. In another case, it could be either SRC-1, which in turn recruits CBP p300 or the other receptor can actually interact with the P/CAF and if these are all multi-protein complexes and these co-activator complexes, for example, is the TRAP/DRIP complex, which is a multi-protein complex and these in turn interact with the general transcription machinery and bring about transcription activation.

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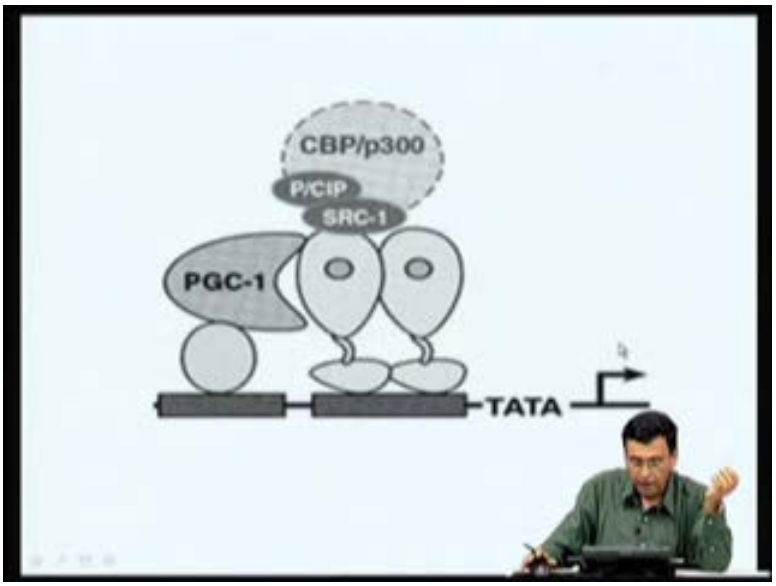

So, what basically happens (( )) another example, where when there are 2 transcription factors binding side by side and one of them can interact with the PGC-1 is called as polycomb group of proteins. Again, we will not go into details of this and we will discuss in later class and whereas the nuclear receptor recruits a histone acetyl transferase, the adjacent transcription factor can interrupt the polycomb, polycomb group of proteins and synergistically they can ultimately result in the activation of transcription. So, a wide variety of variations of these co-activator co-repressor combinations can lead to either activation or repression of transcription in the case of nuclear receptors.

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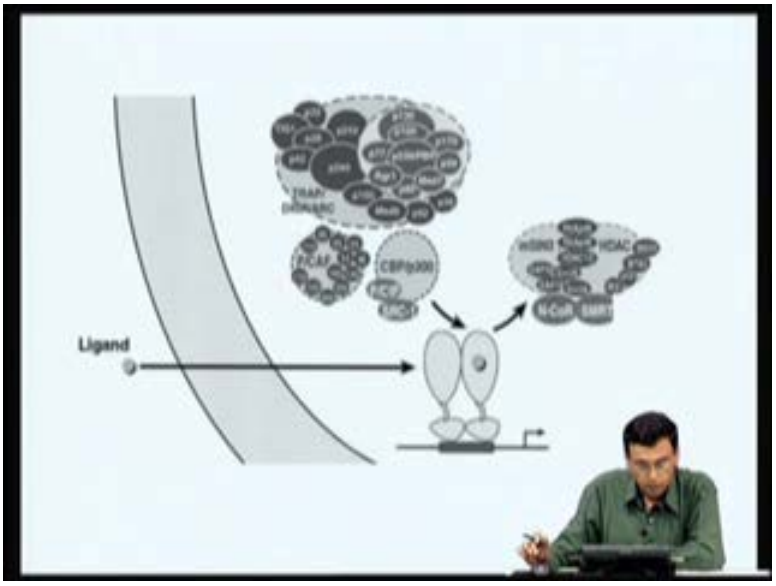

So, what is the take home message? When you have a hormone, that when the hormone enters the cell, it binds to specific nuclear receptors. In the case of steroid hormone receptors, the receptor is present in cytosol, therefore the hormone binds to the cytosolic receptor and then goes to the nucleus. Where, in the case of the type 2 receptors, the receptor is already bound to DNA inside the nucleus and the ligand goes and binds, and when the ligand binds, induce a conformational change and facilitates interaction of the co-activator complexes, which could be either, if it is SRC-1, it can recruit the CBP or it could be P/CAF or it could be the TRAP/DRIP complex, say wide variety of co-activator complex can be recruited by the ligand bound nuclear receptors and these, in turn, interact with general transcription machinery and activate transcription. Whereas, in the unliganded form, the same nucleus receptors actually interact with nuclear receptor co-repressors, which could be either N-CoR, SMRT and so on and so forth. This in turn causes histone deaceytylation and therefore, repressor transcription. So, this is the general mechanism by which nuclear receptors bring about either transcription activation or transcription repression.

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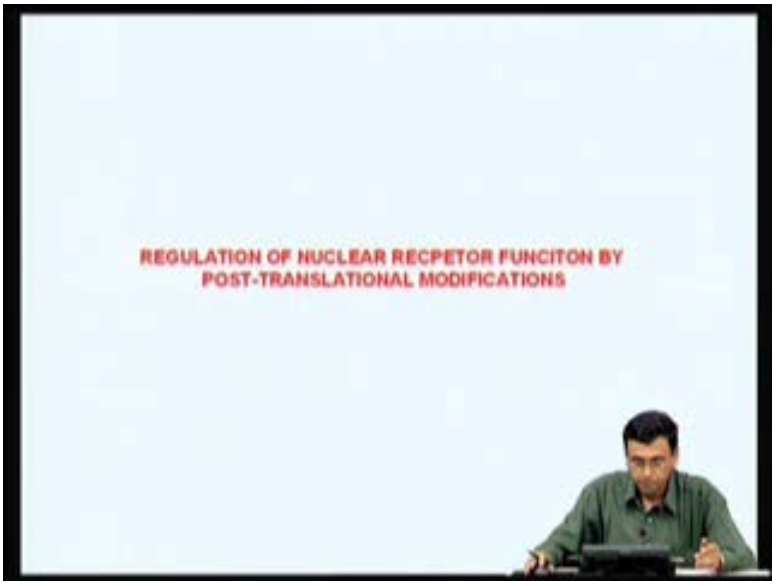

Now, in addition to the recruitment of these co-activators and co-repressors in the presence of ligand, which is a direct mechanism, the co-activator co-repressor interaction of the nucleus receptor can also be affected by specific post-translation modifications of these nuclear receptors.

In the absence of hormone, specific residues of nucleus receptors may be phosphorylated or they can be acetylated or they can be simulated, they can be (( )). A number of post-translation modifications are possible and in the absence of hormone, certain residues undergo post-translation modification and when the same receptor binds to a hormone, different sets of residues undergo post-translation modification and this post-translation modification of receptor can determine, whether the receptor can interact with a co-activator or whether can interact with a co-repressor and ultimately, whether the target genes can be activated or they can be repressed. So, nuclear receptor function can also be regulated by specific post-translation modifications of amino acid residues of nuclear receptors.

Let us now look at and see what kinds of amino acid residues undergo post-translation modifications in the case of nuclear receptors?

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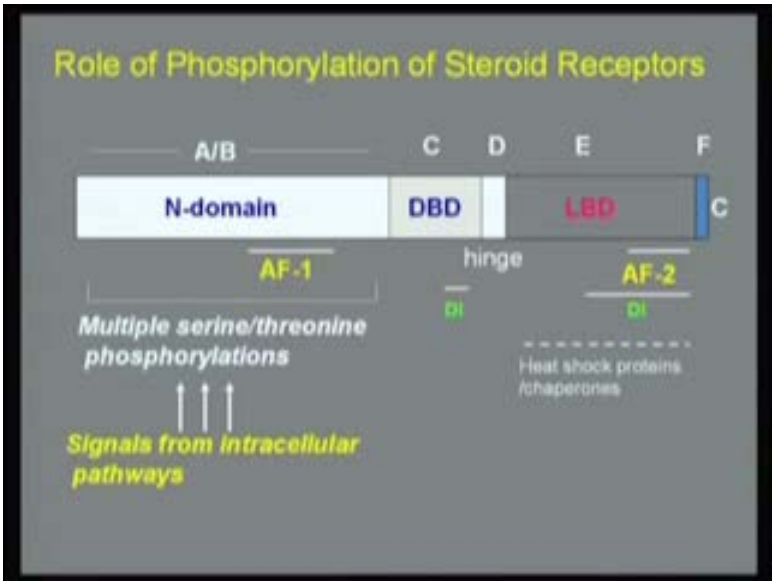

For example, a number of signaling pathways, it could be GPCR signaling or it could be tyrosine kinases receptor signaling or it could be STAT or the cytokine receptor signaling, and in many cases, when signaling molecules interact with the specific receptors the kinases, which are activated by the signaling pathways, sometimes they also go and phosphorylate specific amino acid residues in nuclear receptors and activate transcription of the hormone responsive genes.

So, the genes, which are responsive to hormones, sometimes are (( )) activated or repressed not only in response to the presence or absence of hormone, but they can also be activated or repressed in response to specific membrane receptor signaling pathways. So, when certain molecules binds to specific membrane receptors, the kinases, which are activated by this membrane receptor signaling, may also phosphorylate the nuclear receptors and then, alter the interaction of this receptors with co-repressors co-activators and therefore, target genes, which are normally activated by hormones, can now be activated by membrane receptor signaling through this phosphorylation and cascade. So, the activation function AF-1 domain of the n terminals or the activation functions to the ligand bind domain. The amino acid residues of these 2 domains can serve as targets for a number of protein kinases.

Let us now discuss 1 or 2 examples to see, how phosphorylation modulates the function of nuclear receptor function.

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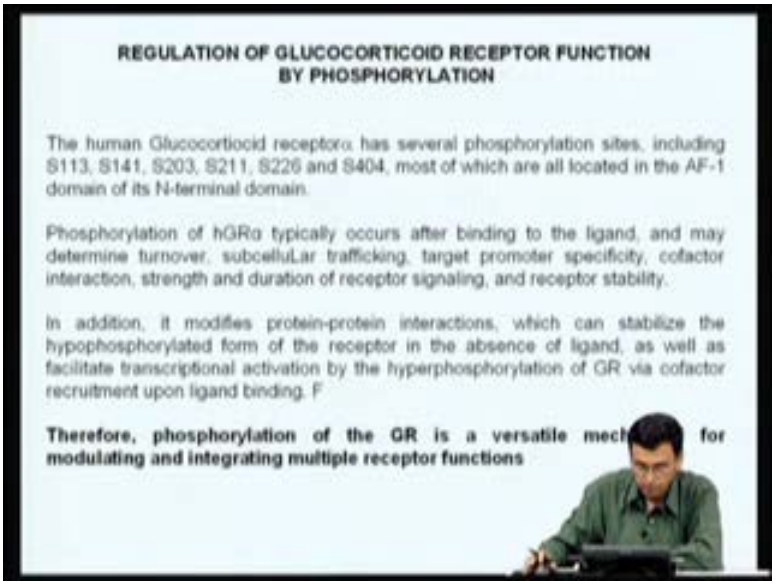

Let us take the example of glucocorticoid receptor. The human glucocorticoid receptor alpha has been shown to have several phosphorylation sites such as serine113, serine141, serine203, serine211, serine225 and serine404. So, all these serine residues in the receptors in the glucocorticodic receptor are targets for a number of protein kinases and many of the serine receptors are located in the activation function-1 or the N-terminal domain of the nuclear receptor.

And phosphorylation of the human glucocorticoid receptor typically occurs after binding to the ligand and may determine, this phosphorylation can determine the turnover of the receptors. It can affect the subcellular trafficking of the receptors or the target promoter's specificity, cofactor interaction, strength and duration of receptor signaling and so as receptor stability.

So, you can see, in addition to the ligand binding phosphorylation, status of the nuclear receptor can affect a number of functions related to the nuclear receptor. It can affect a turnover of their receptor, it can affect the subcellular trafficking, whether it should stay in cytoplasm or go to the nucleus, or it can affect the target genes specificity of the promoter or interaction with cofactors, co-activators or co-repressors. The strength and duration of receptors are how long the receptors have to bind to chromatin and stay there. They sometime determine by phosphorylation status of the receptors and even the stability of the receptor is determined by the phosphorylation sometime, when

phosphorylation of the receptor triggers unique utilization of the receptor leading to degradation of the receptor. So, phosphorylation of these nuclear receptors plays a very important role and number of cells in residues. The amino terminals of glucocorticoid receptor have been shown to be phosphorylated by variety of protein kinases in the cell. Phasphorylation also modifies protein-protein interactions, which can stabilize the hypophosphorylated form of the receptors in the absence of ligand, as well as, facilitate transcription activation by the hypophosphorylation of the glucocorticoid receptor via recruitment of the ligand binding cofactor, recruitment by the ligand binding. So, phosphorylation of the specific amino acid residues can also modulate the ability of the receptor interact with cofactors, such as co-activators and co-repressor.

Therefore, phosphorylation of nuclear receptors in general and here glucocorticoid receptors are permit in particular is a very versatile mechanism for modulating and integrating multiple receptor functions. So, phosphorylation plays a very, very important role in the nuclear receptor signaling.

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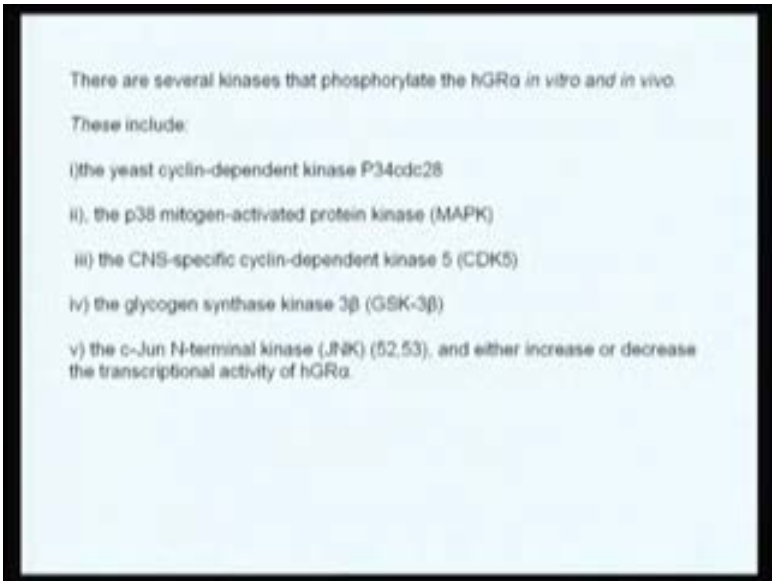

A number of kinases, protein kinases, which actually phosphorylate the glucocorticoid receptors have been identified, some of them are listed here, for example, the yeast cyclin-dependent kinase P34cdc28 has been shown to be one of the protein kinases that can phosphorylate nuclear receptors. Similarly, the p38 mitogen-activated protein kinase or the MAP kinase is another protein kinase that can phosphorylate glucocorticoid

receptor. The central nervous system specific cyclin-dependent kinase 5 or the CDK5 has also been shown to phosphorylate glucocorticoid receptor and similarly, glycogen synthase kinase 3beta or the GSK-3beta, which is actually involved in the glycogen synthesis, also is one of the kinases, that can phosphorylate glucocorticoid receptor. Similarly, the c-JUN N-terminal kinase, which is actually activated by a number of membrane receptors or the growth factor signaling or the JNK, can also either increase or decrease transcription activity of glucocorticoid receptors.

So, this, this, this inclusion of the, the role of protein kinases nuclear receptor signaling has added a new dimension for gene regulation by nuclear receptors. So, the, not only the receptors, can activity, can be influenced by the nuclear receptor hormones, the receptor activity can also be influenced by phosphorylation of the receptors and this phosphorylation, in turn, can be determined by the specific protein kinases, which can be activated by multiple pathways including membrane receptor signaling pathways. So, a new paradigm emerged in the nuclear receptor signaling when it became clear, that nuclear receptor function can be modulated by protein kinases, which in turn can be activated through the membrane receptor signaling pathway.

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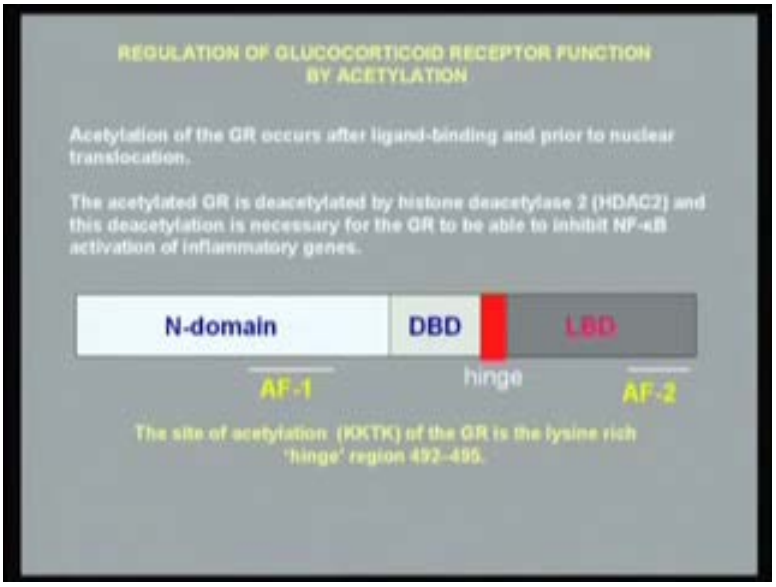

So, in addition to phosphorylation, number of other post-translation modification, such as acetylation can also play a very important role. For example, the acetylation of glucocorticoid receptor has been shown to occur after ligand binding, prior to nuclear

translocation and the acetylated glucocorticoid receptor was acetylated by histone deacetylase, and this deacetylation is necessary for glucocorticoid receptor to be able to inhibit nuclear factor kappa-B activation of inflammatory gene. So, in addition to the nuclear receptors activating transcription by themselves in response to hormone by recruitment of co-activators, they can also, glucocorticoids are very potent anti-inflammatory agents, and the mechanism by which sometimes the anti-inflammatory effects of glucocorticoid receptors are brought about, depends on the acetylation state of the receptor.

So, the deacetylation of the acetylated glucocorticoid receptor is very important for the inhibition of the NF kappa-B activation of the inflammatory genes. So, you can see, the acetylation and deacetylation of nuclear receptor plays a very important role in the anti-inflammatory response of properties of glucocorticoids. And in fact, the exact site of the glucocorticoid receptor, which is acetylated, has been identified. This is called as KKTK motif within the hinged region between the DNA binding domain, ligand binding domain and amino acids (( )) between 492 to 495 in the glucocorticoid receptor in the hinged region has been shown to be acetylated by specific acetylases, and this acetylated receptor cannot activate anti-inflammatory genes and when it is deacetylated by the histone deacetylates, it then can prevent the activation of genes involved in inflammation.

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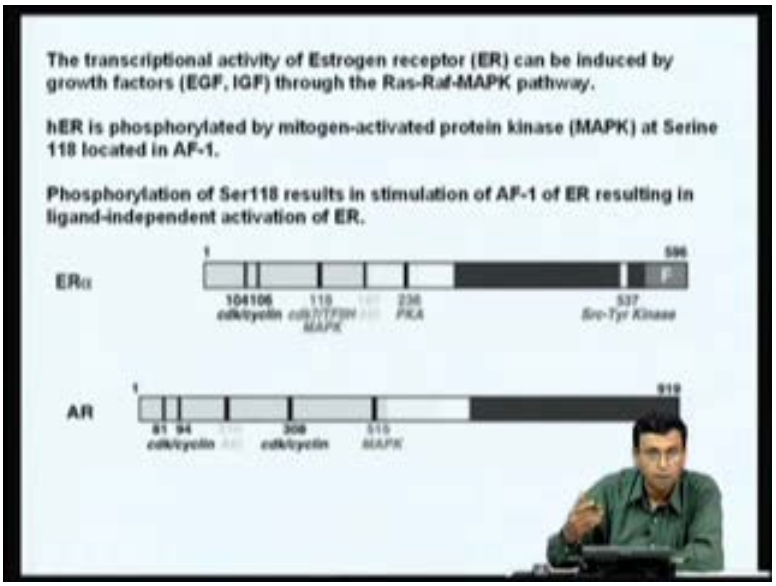

Similarly, in the case of estrogen receptor, the transcription activity of estrogen receptor can be induced by growth factors, such as epidermal growth factor or insulin growth factor through the Ras-Raf-MAP kinase pathway. We have discussed these pathways in our previous lectures, how growth factors actually activate the transcription of genes by activation of the MAP kinase pathways and you can see here, when growth factors bind to these growth factor receptors and the MAP kinase, which are activated, can also phosphorylate glucocorticoiod receptor or Estrogen receptor, and this can also activate Estrogen responsive genes. And human Estrogen receptor has shown to be phosphorylated by mitogen activated protein kinase at Serine118 located in the AF-1 domain here. So, see, some of the amino acids of the nuclear receptors are target for MAP kinases and these MAP kinases inter-activated by membrane receptor signaling.

So, phosphorylation of Serine118 results in stimulation of AF-1 of Estrogen receptors, resulting in ligand independent activation of Estrogen receptor. So, many a times, genes, target genes, which are actually activated in response to Estrogen hormone, were found to be activated by growth factors also. People did not know how an Estrogen responsive gene could be activated by growth factors. By deciphering this mechanism, it has now become very clear, that the AF-1 domain of the Estrogen receptor can be phosphorylated by MAP kinases, which in turn are activated by binding to growth factor receptors and when these MAP kinases phosphorylate specific residues in the AF-1 domain, since AF-1 is a ligand independent activation function, they can now activate target genes even in the absence of hormone.

So, we can see the dogma, that target genes, which are Estrogen responsive, can be activated only in the presence of Estrogen hormone, is no longer true. These Estrogen response genes can also be activated in Estrogen independent manner through the growth factor signaling involving specific phosphorylation of amino acid residues in the activation function 1 or the N-Terminal domain, which encodes a ligand independent activation function. So, there is a crosstalk between nuclear receptor signaling and growth factor receptor signaling. So, growth factors can also activate nuclear receptors through the MAP kinase pathway.

Similarly, amino acid residues, which are targets for specific kinase, have also been identified in androgen receptors. These are all very, very important because you know, after androgen receptor signaling, our activation plays very important role in prostate

cancer. So, in fact, many of the antagonize for androgen receptor have been developed based on this particular properties. So, activation of androgen receptor can actually lead to prostate cancer and regulation of the activity of androgen receptor by developing specific antagonize and by identifying how antagonize is activated in the prostate cancer, is a very, very important area of biomedical research.

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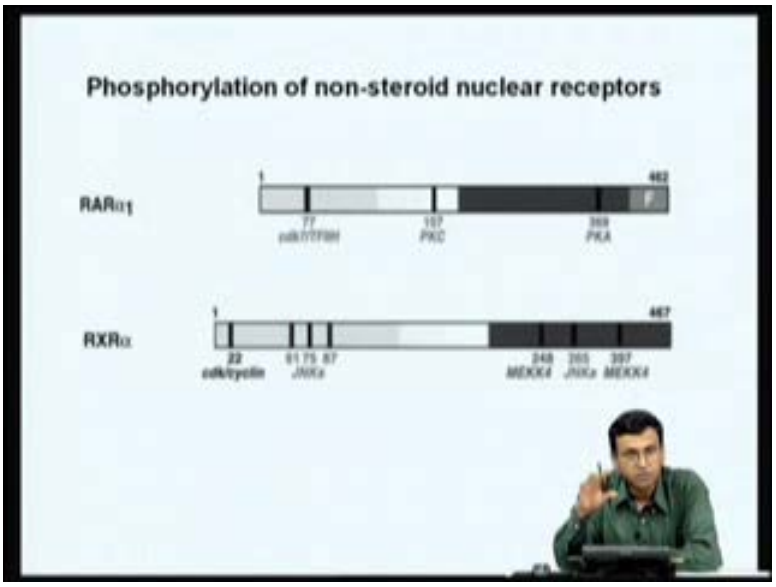

Not only the steroid receptor, like Estrogen receptor, glucocorticoid receptor, androgen receptor or targets for phosphorylation or targets for protein kinases, even non-steroid nuclear receptors, like retinoic acid receptor, retinoic X receptor or vitamin receptor, they also undergo phosphorylation. For example, in the case of retinoic acid receptor specific sites, which are phosphorylated, have been identified in the N-terminal domain as well as in the ligand binding domain and actual kinases, which phosphorylate have also been identified. The same is true in the case of the RXR alpha. So, a number of residues in the amino terminal domain, as well as, in the ligand binding domain are phosphorylated by a number of protein kinases and these can also modulate the activity of these non-steroid receptors.

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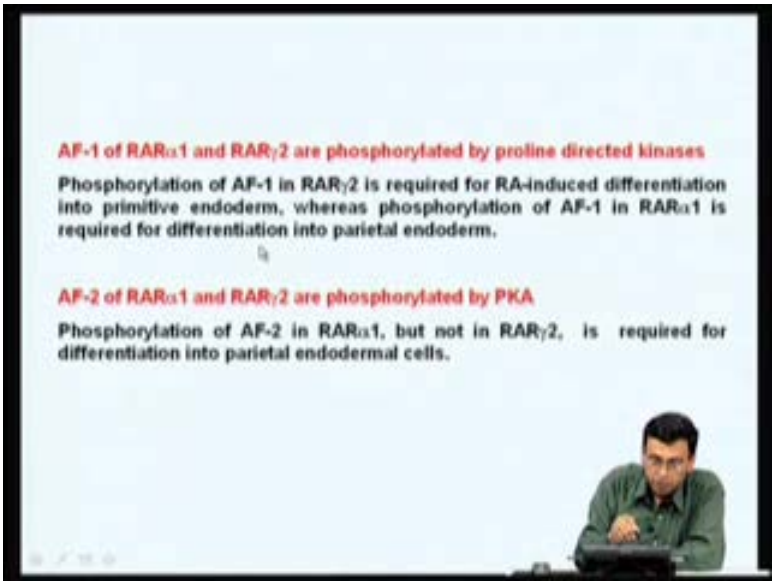

And how this phosphorylation nuclear receptors also plays a very important role in biological processes, for example, the activation function of retinoic acid receptor-alpha-1 and gamma-2 are phosphorylated by proline directed protein kinases, and this phosphorylation is required for retinoic acid induced differentiation into primitive endoderm, whereas phosphorylation of AF-1 by RAR-alpha-1 is required for differentiation into the parietal endoderm.

So, during development, retinoic acid plays a very, very important role in embryonic development and you can see, this retinoic acid induced phosphorylation of specific amino acid residues in the AF-1 function is very, very essential for the proper differentiation during embryonic development. And if you imitate these amino acids, which are targets for retinoic acids phosphorylation in the AF-1 domain, embryonic development is affected indicating, that these, phosphorylation of these nuclear receptors play a very important, very, very important physiological functions.

Similarly, in the case of RAR-alpha-1 alpha-2, the AF-2 domain is also phosphorylated by protein kinase-A and phosphorylation of the AF-2 domain of the retinoic acid receptor-alpha-1 is required for differentiation into parietal endodermal cells. So, not only the phosphorylation of the amino acid is in the AF-1 domain, but the phosphorylation in the AF-2 domain also has very, very important physiological functions, especially during embryonic development.

So, the regulation of nuclear receptor function by phosphorylation has had an entirely new dimension to the nuclear receptor signaling. This phosphorylation can be either ligand independent, or ligand dependent and it can happen either in the activation function 1, or it can be activation function 2, and all these modifications play a very, very important role in the physiological processes.

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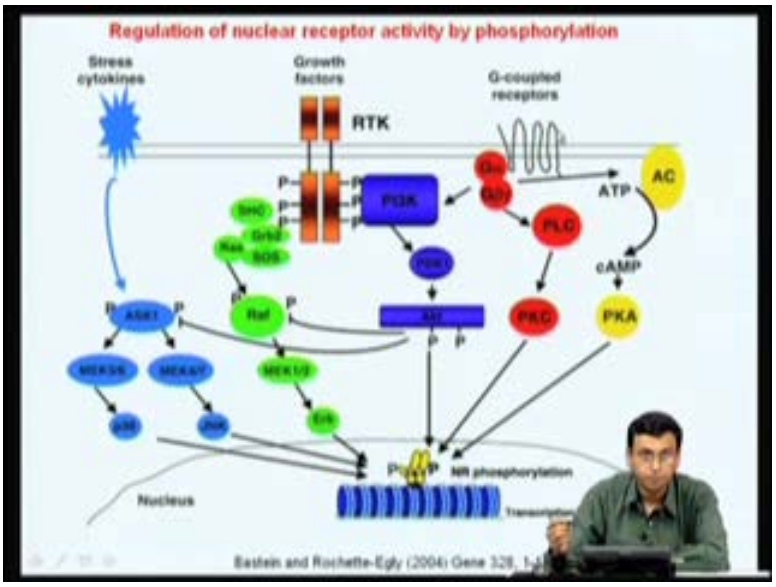

So, this is the gist of what I told you so far. In the previous classes, before we discussed nuclear receptors, we have discussed extensively, how molecules, which interact with membrane receptors, activate gene expression through specific signal transduction pathways. We have studied, how cytokines can activate transcription through the specific MAP kinase pathways; we have studied, how growth factors interact with receptor tyrosine kinases and how, through MAP kinase activation, we can activate transcription of target genes; we also studied, how G protein-coupled receptor or GPCS can activate phospholipase C and lead into activate, either PKC, or cyclic epidemic protein kinase, say activation of protein kinase A, and how they can go and phosphorylate target genes and activate transcription.

What is different in this slide is that these pathways, either the growth factor pathway or the G protein-couple receptor pathways, or the cytokines pathways, can also activate protein kinase pathways and these protein kinase also can go and phosphorylate nuclear receptors and modulate their function.

So, there is an extensive crosstalk between membrane receptors signaling and nuclear receptor signaling. Molecules, which interact with specific membrane receptors, can also activate nuclear receptors by protein phosphorylation cascades. So, it is not necessary, that nuclear receptor need to be activated only by hormones, which defuse to the cell membrane and bind to the ligand binding domain of receptors and activate transcription. There are ligand independent mechanisms of activation of nuclear receptors and which involves specific protein kinases, which in turn are activated by specific membrane receptor signaling pathways.

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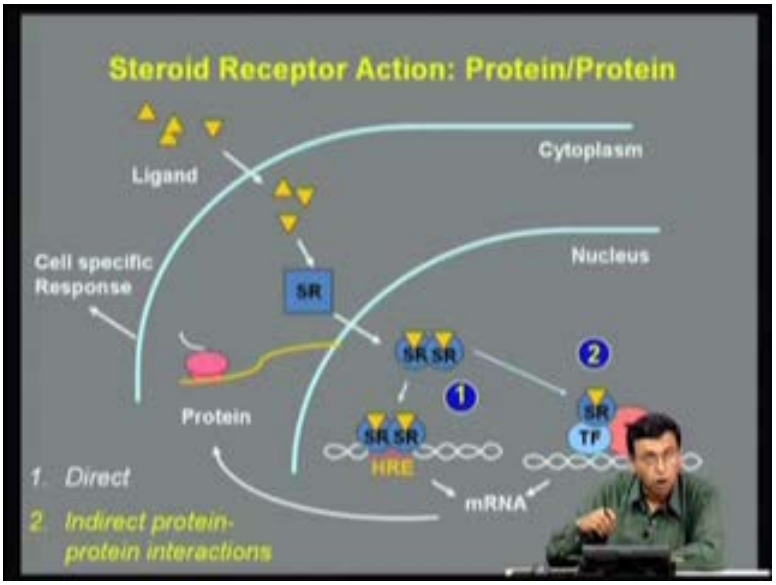

There is also another mechanism by which these nuclear receptors play a very important role in regulation of gene expression. What we have discussed so far is when the ligand defuses through the cell membrane it interacts the steroid receptors and these steroid receptors, then homodimerize and bind to hormone response elements and activate transcription. This is what is called the direct mechanism of transcription activation by nuclear receptors, but there is also an indirect mechanism, where the ligand bind steroid receptor can also interact with other transcription factors and modulate their function. For example, this transcription factor can be AP-1, it can be C-JUN or it can be CREB.

So, some of these transcription factors, which are actually involved in growth proliferation responses or proliferation of growth and when they act, their activity can be inhibited by protein-protein interactions, whereas ligand binding steroid receptors can

interact with these transcription factors and modulate these functions. In fact, many anti-proliferative properties of retinoic acid or glucocorticoid has been shown to be modulated through this pathway, where in the presence of this ligand, these hormone receptors can interact with specific transcription factors and prevent their function and therefore, genes, which are in cell proliferation can be inhibited and in fact, retinoic acid glucocorticoid have shown to have anti-proliferative properties. And by inhibiting the function of transcription factors, like c-JUN and AP-1, the glucocorticoids can actually prevent cell proliferation. So, this is another novel mechanism by which nuclear receptors can modulate gene expression, that is, by interacting with specific transcription factors through protein-protein interactions.

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So, now, come to the last part of this talk. We have discussed so far in the last 3 classes, how nuclear receptors bind to DNA, either as homodimers or as heterodimers, and how they interact with co-activators or co-represent the active transcription of target genes. Now, what are the benefits of understanding this nuclear receptor signaling? Has all this knowledge, which you have amassed in the last 2 decades, has it really gone to the benefit of the mankind?

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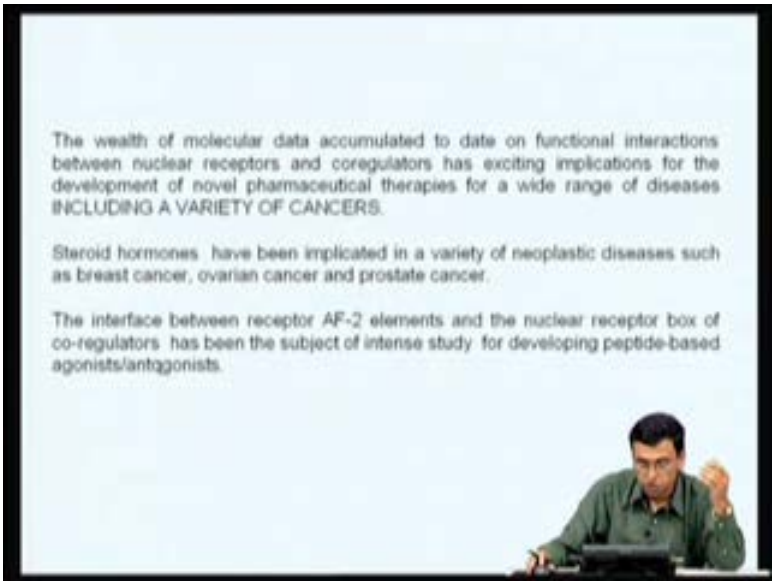

The answer for this question comes, yes, the wealth of information, that has accumulated on the functional interactions between nuclear receptors and co-regulators has exciting implications for the development of novel pharmaceutical therapies for a wide range of diseases, including a variety of cancers.

Steroid hormones have been implicated in a variety of neoplastic diseases, such as breast cancer, ovarian cancer and prostate cancer. The interface between the receptor activation function-2 elements and the nuclear receptor box of co-regulators has been the subject of intense study for developing peptide based agonists and antagonists. We have studied so far, that for a nuclear receptor to activate transcription, their interaction with either co-repressor or a co-activator is very essential and we have studied, that these co-activators interact with specific helices of the nuclear receptors, through this LXXLL motifs.

Now, people are now asking the question, suppose if I design peptides containing LXXLL motifs and had to make them enter the cell, these peptides can go and bind to the AF-2 domains of the ligand binding receptor and therefore, compete for binding to the actual co-activator and therefore, can act as antagonist of nuclear receptors. So, can we develop peptide based therapeutics using this kind of a protein-protein interaction?

So, if you now add, for example, peptides contains LXXLL motifs, they can actually compete with the co-activators and therefore, when these LXL containing peptides go and bind to the AF-2 domain, they prevent binding of the co-activators and therefore, the

nuclear receptor will not be able to activate transcription. So, based on this knowledge, people are trying to develop peptide-based pharmacological agents for a number of diseases, which involve nuclear receptor activation.

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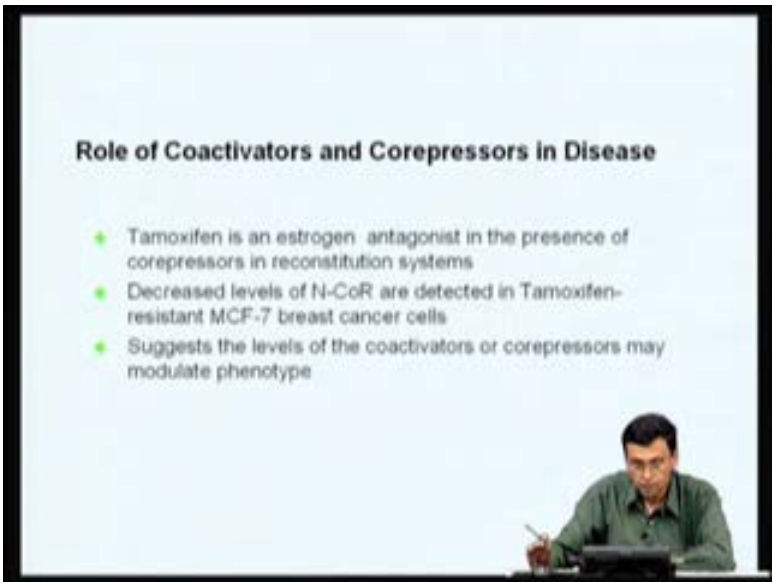

Similarly, the levels of co-activators and co-repressors itself can contribute to disease. For example, Tamoxifen, which is an Estrogen antagonist, has been shown to interact specific co-repressors and decreased levels of nuclear receptor co-repressors have been detected in Tamoxifen-resistant MCF-7 breast cancer cells. Now, one of the major mechanism by which the breast cancer happens is through the Estrogen or Estrogen receptor plays a very important role in the breast cancer, and Tamoxifen often is used as an antagonist of Estrogen to prevent this proliferation of this breast cancer cells. And in some cell lines, which have become Tamoxifen resistant, that is, they no longer respond to Tamoxifen and you look at what is the mechanism by which these cell lines have become resistant to Tamoxifen. They actually found, that in these cell lines, the level of expression of certain nuclear receptive co-repressor is very, very low and therefore, because there is low level of this expression of this co-repressors, Tamoxifen is not able to bring about transcription repression and as a result, these cell lines are now become highly proliferative and resistant to the Tamoxifen therapy.

So, you can see how the level of expression of the co-repressors can also have a specific disease phenotype or contribute to disease phenotype. So, the levels of co-activators or co-repressors can also modulate the phenotype and contribute to a disease process.

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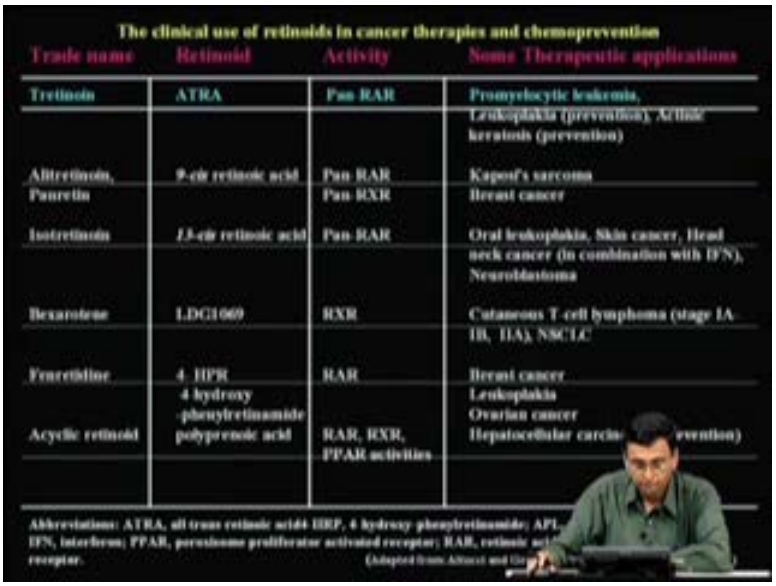

This is just an example to tell you, understanding the molecular mechanism by which retinoic acid activates transcription in which retinoic is a vitamin derivative has opened up a new area of research and this table just shows you, it is actually taken from nature Review Cancer, that a number of synthetic retinoid have been now developed for treatment of a variety of cancers.

For example an All Trans Retinoic Acid, which is now being sold under the trade name Tretinoin, is actually being using for treatment of Premyelocytic Leukemia; certain derivatives of 9-cis retinoic acid, known commercially as Alitretinoin and Panretin or Isotretinoin are being trusted for treatment of Kaposi's sarcoma, breast cancer and skin cancer, and so on so forth.

So, like this, a number of variants of retinoic acid molecules are being clustered for a variety of cancers kindling, that the knowledge, that variants of retinoid or retinoic isomers bind to specific receptor cell types, like RAR, RXRs has now opened up new area of research to see, can we develop specific retinoid analogs or retinoid isomers, which can active selective a retinoic acid receptor phenotypes and therefore, can be used as therapeutic treatment in the case of various cancers.

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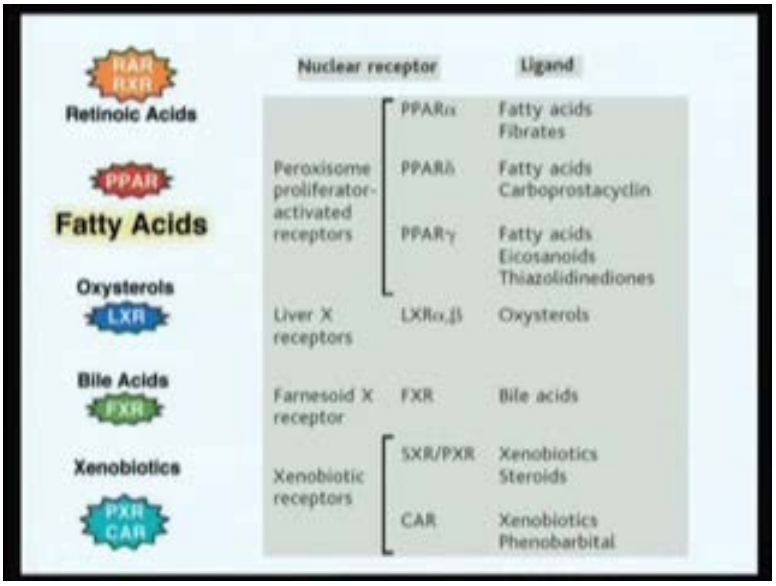

The field has now opened up, what started with steroid hormones. After steroid hormones, we have now shown, that the vitamin, such as vitamin D or vitamin A or even thyroid hormones, activate regulation through this nuclear receptor family. Now, in addition to retinoic acid, we now have shown, even fatty acids can serve as ligands for nuclear receptors. Receptors like peroxisome proliferator-activated receptors can be activated by the fatty acids; oxysterols, derived from cholesterol, can act as ligands for these nuclear receptors, such as LXR; nuclear receptor such as FXR, can be activated by bile acids, so the bile acids in the gastrointestinal tract can actually serve ligands for these FXRs. All these have tremendous therapeutic implications. Similarly, in certain xenobiotics or drug molecules can interact with specific nuclear receptors and can play a very important role in drug metabolism and all these things have a tremendous implication. So, ligands for these nuclear receptors can not only the steroid hormones or vitamins, they can also be fatty acids, oxysterols, bile acids, and so on and so forth.

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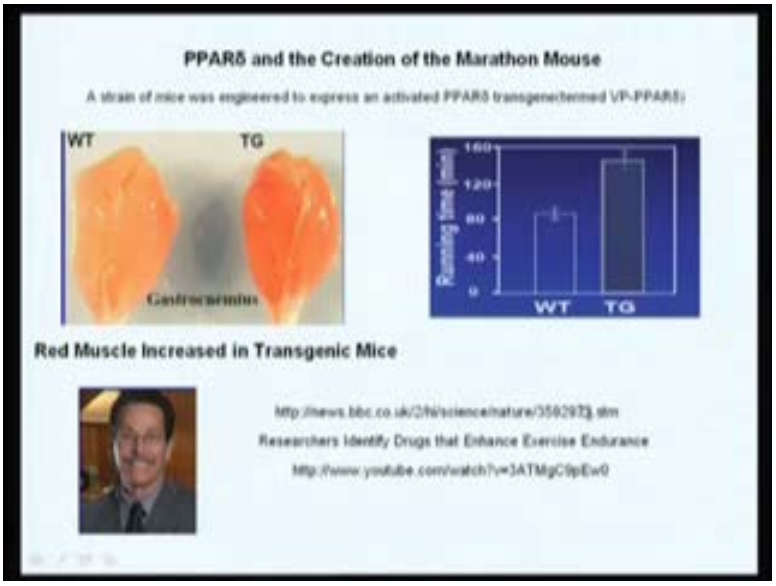

Last, but not the least, very exciting developments have taken place. In this slide, I have actually shown, when you express a particular nuclear receptor called peroxisome proliferator activator receptor delta, you can develop a transgenic mice, which has a very high increase in the red muscle. This is a non-transgenic mice or a wild type mouse muscle and it is the muscle of the (( )), and it has a very red muscle, the red muscle is (( )).

Now, what is the implication? This increase in red muscle contributes to a higher level of exercise activity by these mice. These mice can perform on a treadmill much better than the normal mice. What is the implication? Now, if you want to be an athlete, now if you can now take a drug, which can activate peroxisome proliferator activator receptor delta, you can run much faster and much longer than a normal athlete, so you can actually develop anabolic compounds that can have very important implications in winning races by athletes.

So, a very nice video of how exactly these transgenic mice perform is there in the YouTube and there are a number of web links, you can actually go ahead, link and then see, how these have tremendous implications.

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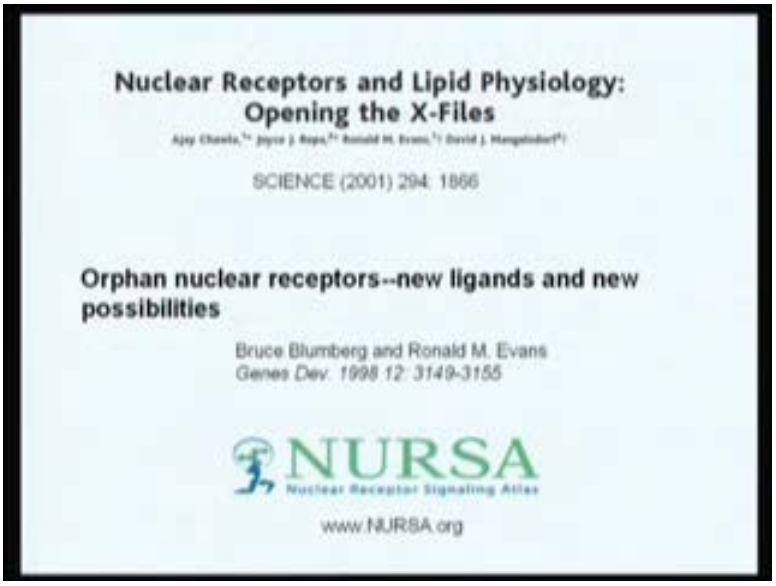

So, these are some of the reference I have listed here, which you can go through and then see, how we can understand some of the exciting developments, that are taking in the area of nuclear receptors and I think I will stop here.