Eukaryotic Gene Expression: Basics and Benefits

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Lecture No. # 16

Regulation of gene expression by cyclicAMP

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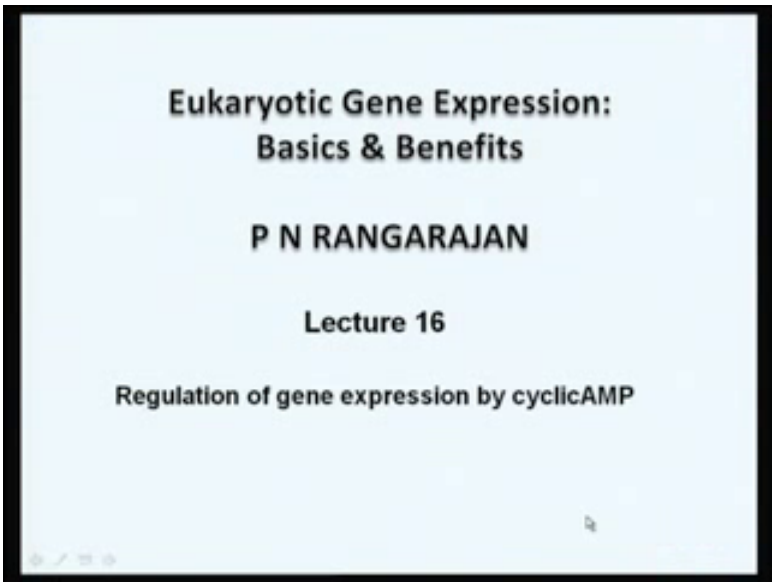

Welcome to this series of lectures on signal transaction pathways. We just began in the last class, where we gave an introduction to signal transaction pathway and then we discussed, how hydrophobic molecules, which can diffuse to the plasma membrane, can enter the cell and then activate gene expression through intracellular receptors. We also discussed about molecules, which are hydrophilic, therefore cannot cross the plasma membrane. Therefore, they interact with specific cell surface receptors and then transduce signals and then activate gene expression programs. So, what I will do in this class is to discuss it with you about the regulation of gene expression by cyclicAMP. Molecules, which interacts with cell surface receptors and this interaction of this ligander cell surface receptors results in the activation of the GPCRs, that is, the G-protein couple receptors, which are the 7 serpentine receptors or the 7 membrane receptors. And this G-protein activation ultimate result in activation of adenylate cyclase and adenylate cyclase now produces cyclicAMP; cyclicAMP now activates protein kinase A; then, protein

kinase A goes inside the nucleus and activates number of genes. So, what we will discuss in this class is to go in more detail, greater detail to understand the mechanisms by which cyclicAMP regulates gene expression.

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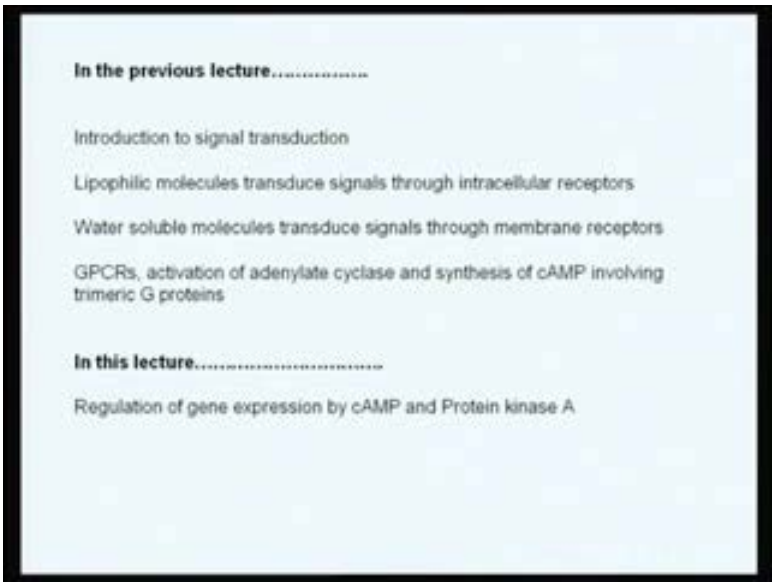

So, this is the summary of what we discussed so far in the previous lecture. We discussed introduction to signal transduction; we discussed about how lipophilic molecules transduce signals through intracellular receptors; then, we also discussed briefly, how water soluble molecules transduce signals by (( )) membrane receptors. Then, we discussed about 1 class of membrane receptors, namely the G-protein couple receptors, and how, when the G-protein couple receptors are activated by specific ligand, it results in the activation of adenylate cyclase leading to synthesis of cyclicAMP involving the trimeric G proteins.

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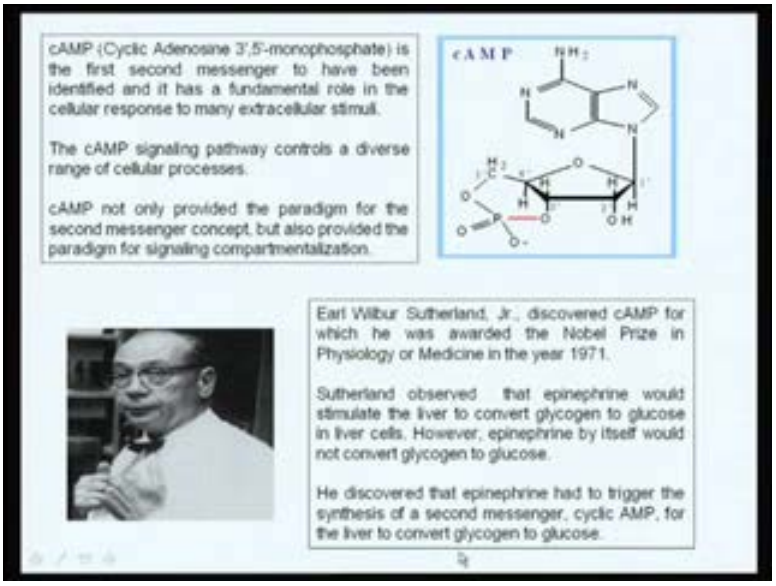

So, let us now discuss and see how the cyclicAMP activates gene expression. Now, cyclicAMP is the first 2nd messenger to have been identified and it has a very fundamental role in cellular response to number of extracellular stimuli. And this is the structure of cyclicAMP, which I have shown here, you can see, how the 3 prime (( )) cyclicAMP and the phosphodiester bond, so that you have the 3.5 from cyclic is monophosphate. Here, the phosphate is attached to the sugar by 3 prime 5 prime phosphodiester bonds.

Now, cyclicAMP signaling pathway controls the number of cellular processes and cyclicAMP not only provided, that the paradigm for 2nd messenger concept, but also provided the paradigm for signaling compartmentalization. As we know, in the cell, everything is compartmentalized and this compartmentalization plays a very important role in the regulation of number of physiological processes.

In fact, the credit for discovery of cyclicAMP as a 2nd messenger goes to Earl Wilbur Sutherland junior. The photograph is shown here, who actually discovered the cyclicAMP and then showed for cyclicAMP is a 2nd messenger for which he was awarded the Nobel prize in 1971.

Now, Sutherland observed that, epinephrine would stimulate the liver to convert glycogen to glucose in the liver cells, but epinephrine by itself would not convert glycogen to glucose, which means, the epinephrine is doing this job indirectly and then,

he discovered the epinephrine has to trigger the synthesis of 2nd messenger cyclicAMP for the liver to convert glycogen to glucose.

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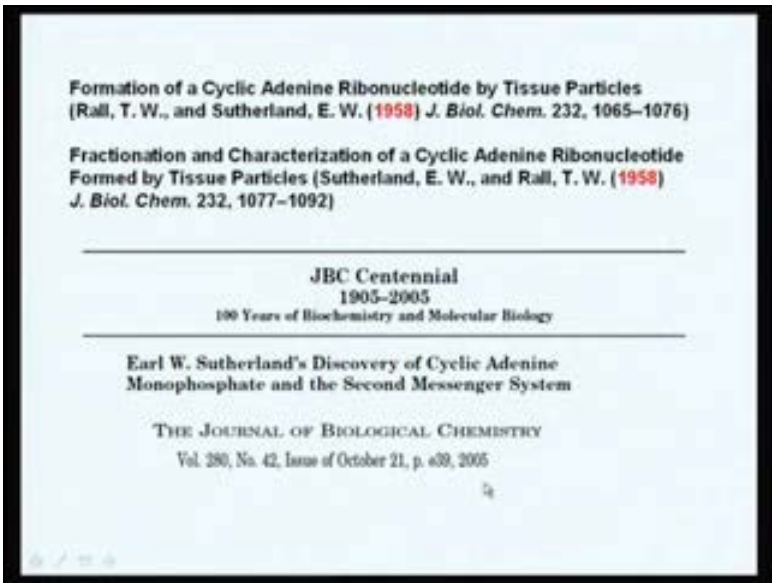

In fact, some of the classic papers published by Donald Sutherland (( )) had just shown here. In 1958, 2 papers, which was published in the (( )) and in fact, these were actually reproduced in the centennial year of journal biological chemistry in the year 2005, where they actually reproduced some of the classical Earl Sutherland discovery of cyclic AMP, cyclic adenine monophosphate and the 2nd messenger system.

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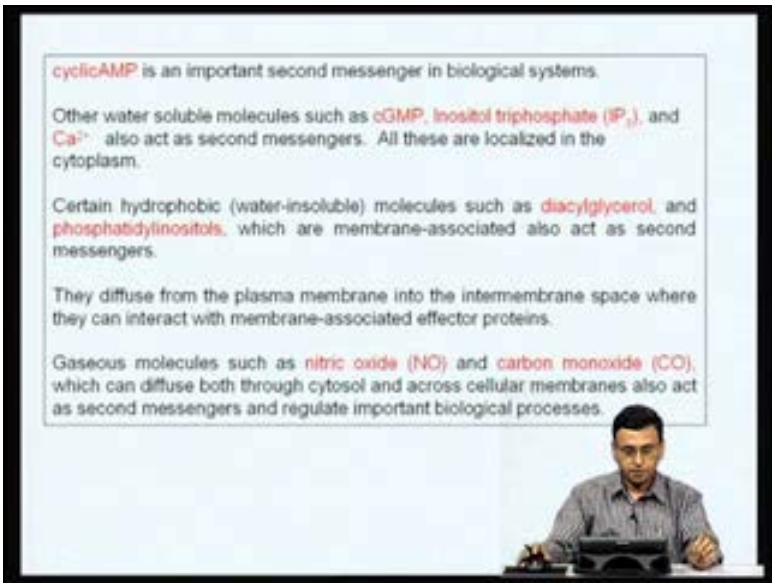

So, the discovery of cyclic AMP as a 2nd messenger is one of the important landmarks in the biological chemistry. Now, cyclic AMP, as I said, is a very important 2nd messenger molecule and in addition to cyclicAMP, there are all some many other 2nd messengers, which are operating in living systems, some of them are shown in red here. For example, cyclicGMP is another cyclical (( )), known as Inositol triphosphate or IP 3, as well as calcium also act as 2nd messenger. In the next class, we will discuss the mechanism in which these molecules function as secondary messenger and some hydrophobic or water insoluble molecules, like diacylglycerol phosphatidylinositols, which are membrane associate; they also act as 2nd messengers.

So, the G proteins, the trimeric G proteins, which are, which are, which are the root cause or which are the anchors, which we were discussing right now and one of the 2nd messengers, which are produced by the G-protein activation is cyclicAMP. The same G–protein signaling can also activate other 2nd messengers, like diacylglycerol, phosphatidylinositols and so on and so forth, this we will discuss in the next class. Now, all these 2nd messengers diffuse from the plasma membrane into the intermembrane space where they can interact with the membrane-associated effector proteins. And many gaseous molecules, like nitric oxide and carbon monoxide, which can diffuse through the cytosol and across the cellular membranes also acts 2nd messengers and regulate very important biological classes. So, today, we will primarily focus on the mechanism (( )) cyclicAMP acts as 2nd messenger, then next class we will try to discuss the other small molecules, like cyclicGMP, inositol triphosphate, diacylglycerol, phosphatidylinositols and other gaseous molecules; how they function as 2nd passengers and how they activate gene expression programs.

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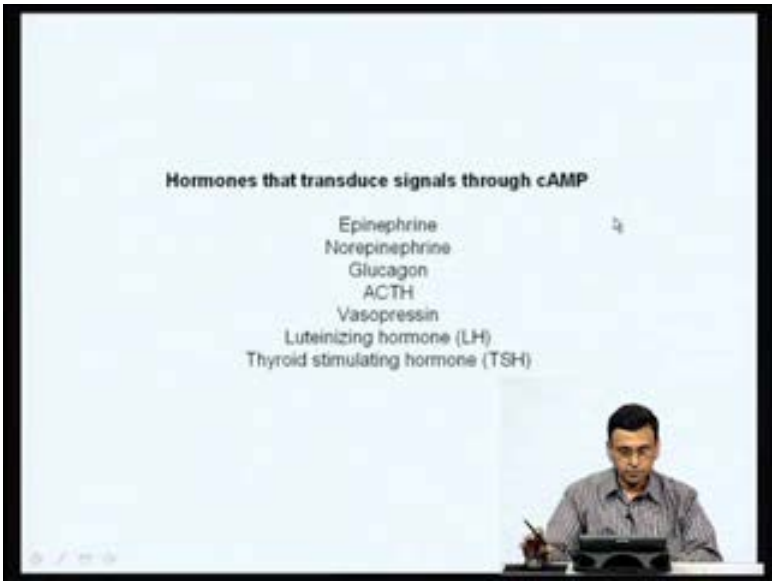

Now, what are the hormones, which can activate the, which one interacts with specific membrane receptors, resulting in the synthesis of cyclicAMP. I have listed here, some of them are: epinephrine, norepinephrine, glucagon, ACTH – adrenocorticotropic hormone, vasopressin, luteinizing hormone, thyroid stimulating hormone; there are many others, but I have just given a few examples; some of them are small molecules and some of them are peptide hormones. So, all these things, by interacting with this specific gene protein, couple receptors can ultimately lead to the synthesis of cyclicAMP, which in turn and goes inside a cell and regulates the number of physiological processes.

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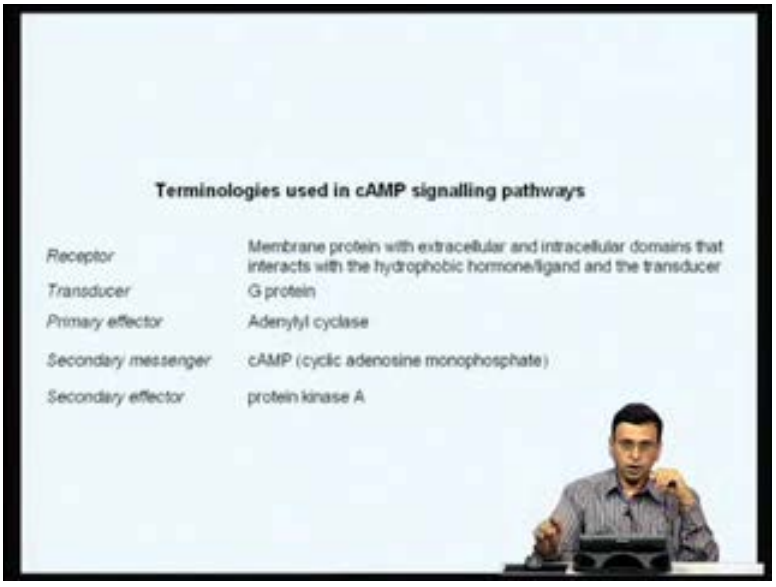

Now, before we start in depth discussion about the cyclicAMP signaling pathways, let us familiarize our self with some of the terminologies, which are used in the, in this discussion. Some of the common terminology, which we will be using again and again in this lecture, is receptor. What do you mean by receptor in this class is, receptor is basically, membrane protein with extracellular and intracellular domains, that interact with the hydrophobic hormones or ligand and therefore, acts as the transducer. So, this is the molecule, which is usually transmembrane protein and it is the one, which is actually going to bind to the extra ligand or the hormone through the extracellular domain.

The transducer is actually G protein here. So, once the ligand binds to the receptor, it then interacts with the G protein and it is a G protein, which transits the signal further and therefore, this is the transducer for the particular receptor.

Now, one, that how does the G protein act as a transducer? It actually goes on, interacts with the primary effect of the molecule and in this case, the primary effect of molecule is adenylate cyclase. Once the ligand interacts the receptor, the trimeric G protein dissociates, the alpha subunit now comes and binds the adenylate cyclase and adenylate cyclase now in the GTP bound form, G alpha subunit now activates adenylate cyclase and therefore, it results in the synthesis of cyclicAMP and cyclicAMP therefore, is the 2nd messenger.

So, the 1st messenger is the hormone, which actually bound to the receptor. Now, what the cyclicAMP do? It now acts on a secondary effector molecule and in this case, it is the protein kinase A. So, cyclicAMP goes and binds to the regular subunits of protein kinase A and therefore, the catalytic subunit is now separated, that now goes and does a number of physiological processes.

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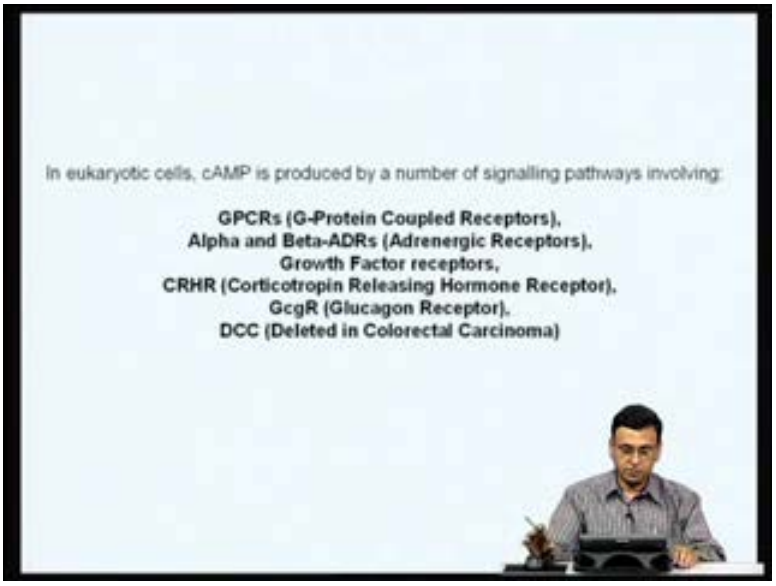

Now, cyclicAMP is produced by number of signaling pathways, some of them I have discussed here. And what we will discuss today is about the GPCR pathways; in addition to that we have alpha beta adrenergic receptors, growth factor receptors, corticotrophin releasing hormone receptor, glucagon receptor, DCC - deleted in colorectal carcinoma, all these receptor pathways ultimately result in the synthesis of cyclicAMP as a 2nd messengers. So, whenever these pathways activated by their cognate ligands or hormones, it results in the synthesis of cyclicAMP, which then acts as a 2nd messenger.

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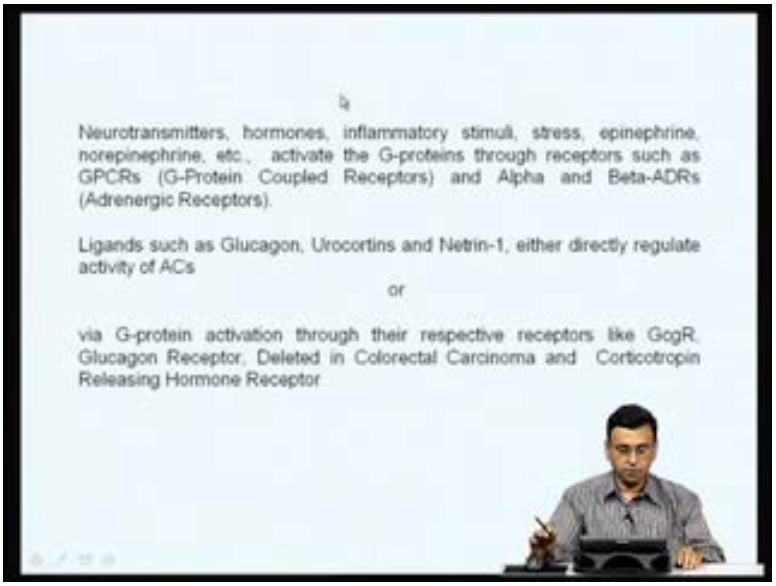

So, neurotransmitters, hormones, inflammatory stimuli, stress, epinephrine, norepinephrine, number of other molecules activate the G-proteins through receptors, such as the GPCRs, alpha beta adrenergic receptors and so on and so forth. Whereas, the ligands, such as glucagon, urocortins and netrin-1, either directly regulate the activity of adenylate cyclases, or they activate via G protein activation.

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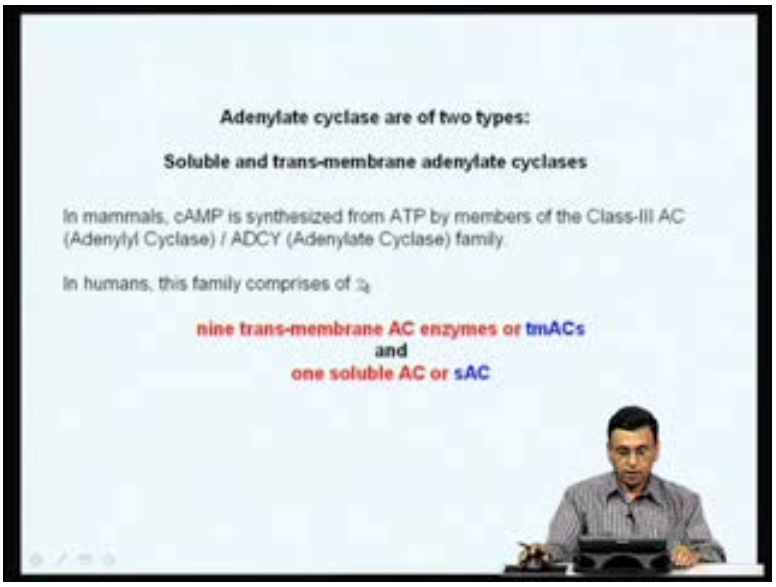

Now, what are these adenylate cyclases? There are basically 2 types of adenylate cyclases. The previous class, we basically discussed about the membrane bound adenylate cyclases or known as the tm-adenylate cyclases. There are also water soluble adenylate cyclases and in the humans there are about 9 trans-membrane adenylate cyclases or tmACs and 1 soluble adenylate cyclases or sAC.

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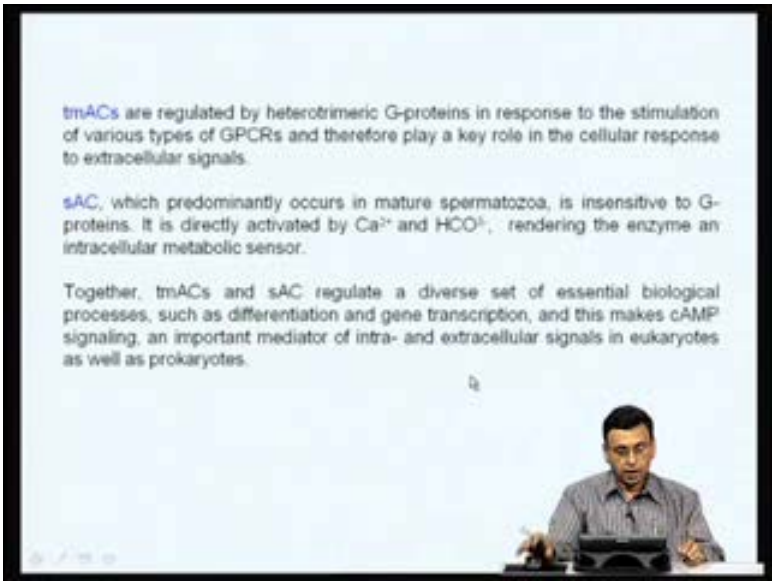

Now, what do we do? The trans-membrane adenylate cyclases of the membrane bound adenylate cyclases are the one, which are regulated by the heterotrimeric G-proteins. Therefore, are very important for the current discussion, which involves the transaction pathways leading to gene activation. Therefore, the trans-node adenylate cyclase are regulated by heterotrimeric G-proteins in response to the stimulation of various types of the G-protein couple receptors and therefore, play a very important role in the cellular response to extracellular signals.

Whereas, the soluble adenylate cyclases, which primarily occurs in mature spermatozoa is insensitive to G-proteins. So, G-proteins signaling takes place primarily through the membrane bound adenylate cyclases. The soluble adenylate cyclases are nothing to the G-protein signaling pathway in the soluble adenylate cyclases, are directly activated by calcium ions and bicarbonate ions, therefore rendering the soluble adenylate cyclases a very important intracellular metabolic sensor. So, these 2 perform totally different functions.

Together, the trans-mode adenylate cyclases and soluble adenylate cyclases regulate the diverse set of essential biological processes, which include differentiation gene transcription and therefore, make cyclicAMP signaling a very important mediator of intra and extracellular signals in eukaryotes, as well as, prokaryotes. In prokaryotes, we

had all described into the 1st class, how cyclicAMP through the (( )) regulates glucose metabolism in prokaryotic cells, like E.Coli.

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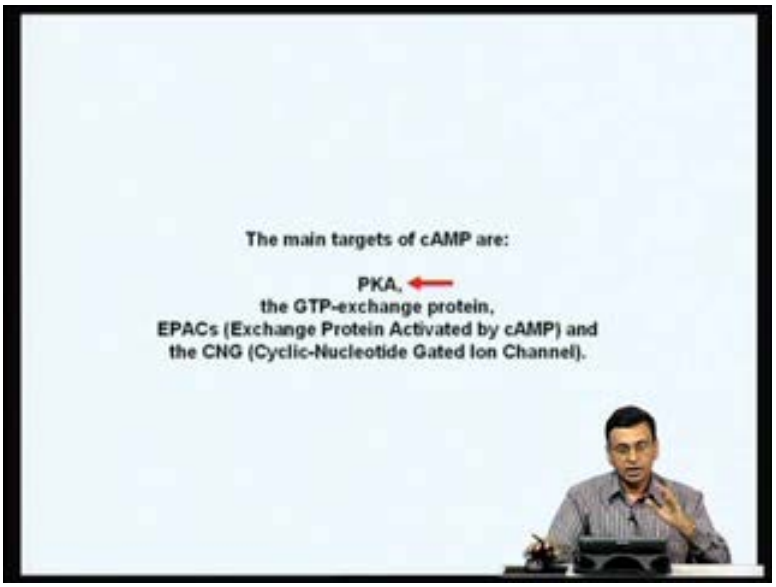

The main targets of cyclicAMP are protein kinase A, GTP exchange protein; exchange protein activated by cyclicAMP or EPACs and the cyclic–nucleotide gated ion channels of the CNG. But for today's discussion we will confine ourselves to how cyclicAMP activated protein kinase A and then, how does protein kinase A activates specific gene expression programs?

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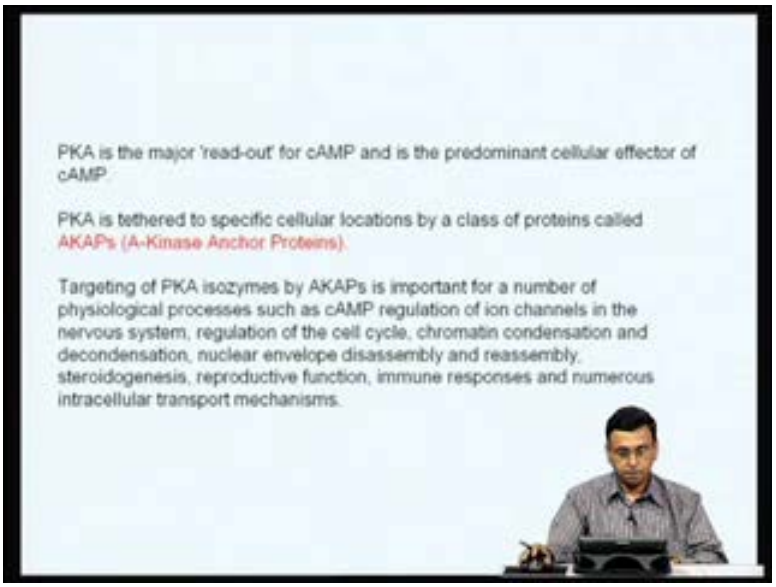

Now, protein kinase-A is the major read-out or the primary mediator of cyclicAMP, biological of a cyclicAMP and therefore, is a predominant cellular effector of cyclicAMP. Now, PKA is not just these present diffuseness cytoplasm. PKA is actually, tethered to specific cellular locations by a class of proteins known as A-Kinase anchor proteins. As I said in the beginning, compartmentalization has played a very, very important role and keeping many of these molecules, like protein kinase A, specific locations has a very, very important part of regulatory cascades.

And one group of protein, called the A-kinase anchor proteins orAKAPs actually keep this PKA bound, in a bound form in specific locations inside the cytoplasm. Targeting the PKA isozymes by AKAPs is very important for a number of physiological processes, such as cyclicAMP regulation of ion channels, nervous system, regulation of cell cycle, chromatin condensation and decondensation, nuclear envelope disassembly and reassembly, steroidogenesis, reproductive function, immune responses and many other intracellular transport mechanisms. So, keeping these PKA in a tethered form by this AKAP plays a very important role in a number of physiological processes.

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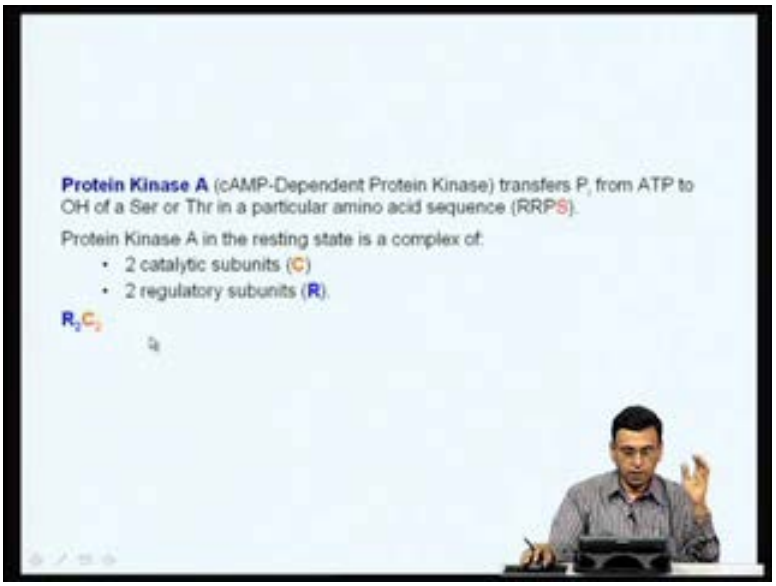

Now, protein kinase A, this we had discussed in the last class itself, also known as the cyclicAMP dependent protein kinase or the CAPK, transfers inorganic phosphate from hydrotriphosphate to the hydroxyl group of a serine or threonine in a particular amino acid sequence. And usually, the sequence, which is recognized by protein kinase A is a

motive called (( )) serine or (( )), can also be other hydrophobic amino acids, like glycine and in this sequence, the serine actually gets phosphorylated. So, the consensus sequence for recognition by protein kinase A is (( )).

If you look at all the proteins, which are targets for proteins kinase A, they invariably contain this consensus (( )), so PKA recognizes this sequence and specifically phosphorylates this particular serine residue. And the protein kinase A in the resting state comprises of 2 catalytic subunits and 2 regulatory subunits, and usually they are designated as R2C2. As long as the catalytic subunits are associated with the regulatory subunits, it is in an inactive form.

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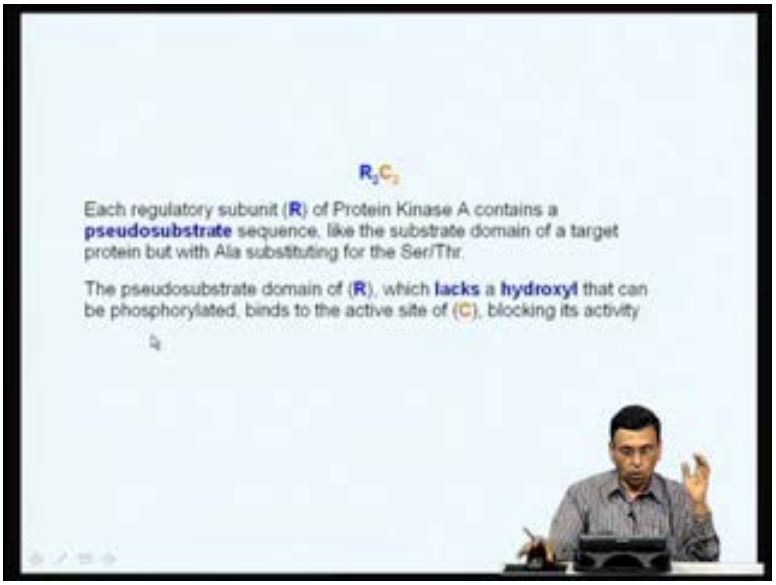

Now, what the cyclicAMP do? The regulatory subunit of protein kinase A contains a pseudosubstrate sequence, like the subset domain of a target protein, but with (( )) substituting serine and threonine. Now, as you can see, (( )) cannot be phosphorylated, therefore it remains inactive. The pseudosubstrate domain of the regulator subunit, which lacks the hydroxyl that can be phosphorylated, binds the active set of the catalytic subunits, thereby blocking its activity. So, this is how one catalytic subunit is (( )) the regulated subunit, the protein kinase A remains in an inactive form.

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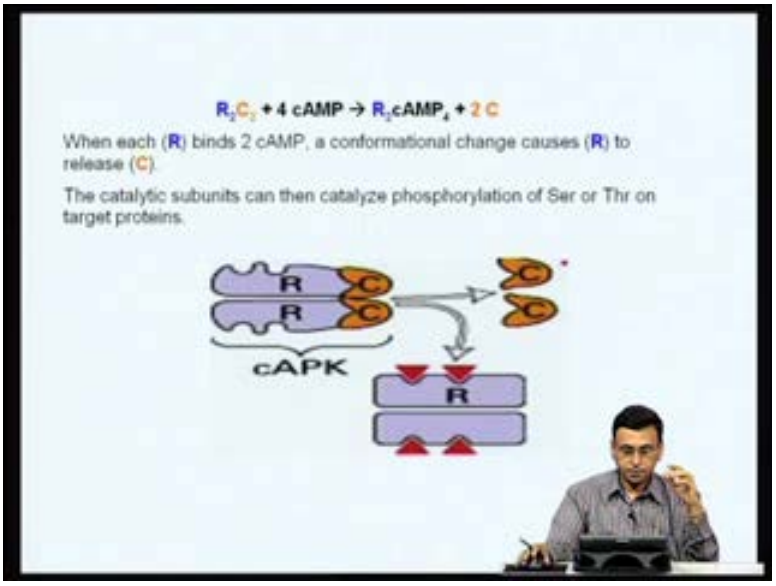

Now, once the 4 molecules of cyclicAMP bind to 1 molecule of the inactive PKA consisting of 2 regulatory subunits and 2 catalytic subunits, and this you can see, two 4 molecules of cAMP can bind to the regulator subunit, and once it happens, a confirmational change is induced into the regulator subunit. As a result, the regulator subunit can no longer interact with the catalytic subunit and the catalytic subunit now, is now free. And it now goes inside the nucleus and recognizes the target sequence (( )) and targets proteins and phosphorylates the serine residue.

So, the inactive form of the cyclicAMP protein kinase are the protein kinase A and 1 cyclicAMP binds the regulatory subunit, undergoes confirmational change and therefore, it no longer interacts with catalytic subunits. Now, the catalytic subunits are free inside and it goes on and does its job.

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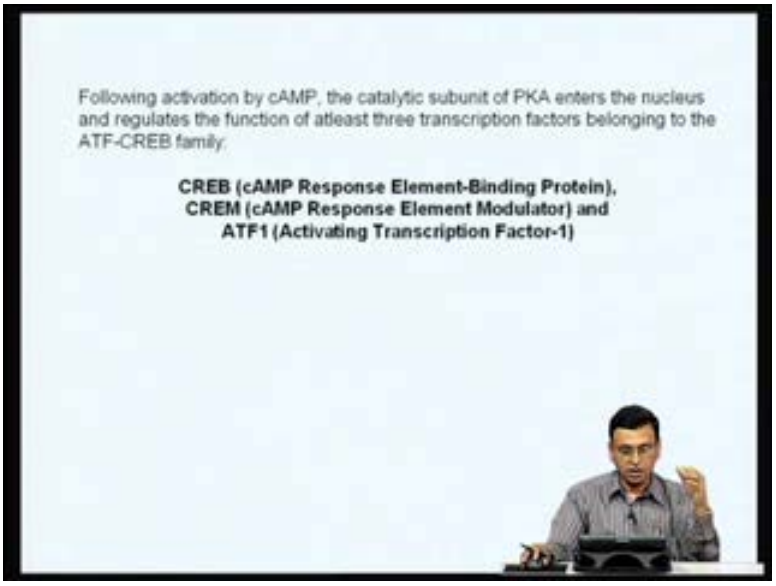

So, following activation by cyclicAMP, the catalytic subunit of protein kinase A enters the nucleus and regulates the function of at least 3 transcription factors belonging to, what is called as, the ATF-CREB family. What are these CREB? As we already discussed in a number of other classes previously, CREB refers to cyclicAMP response element binding protein. There are also many variants of CREB, that is why I, I, I have mentioned here, that it belongs to transcription (( )) belong to ATF-CREB family. There are many members of this CREB family, 1 of them is CREB, the others are, what is called as, the CREM - cyclicAMP response element modulator and activating transcription factor 1 or ATF. Now, what are these? Let us now discuss in detail, what are these transcription factors and what is their biological functions.

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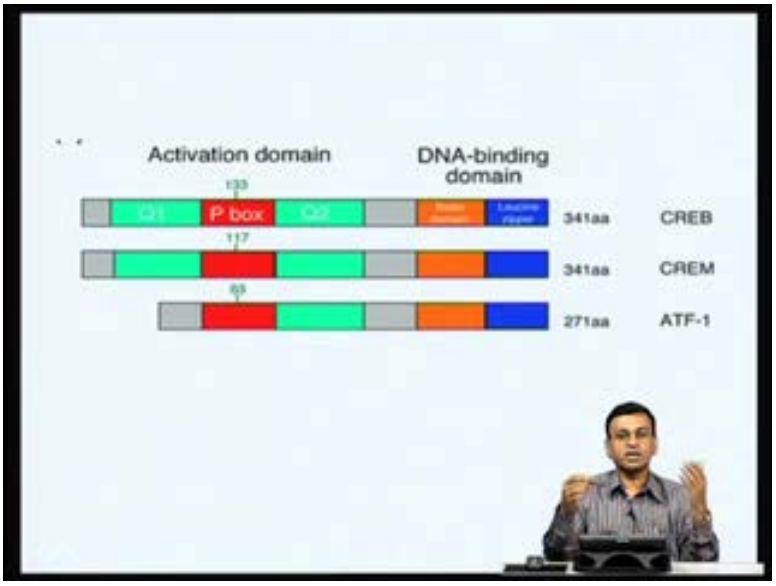

I mentioned some of them here. Here is the CREB, as we had mentioned in the previous class when we discussed in detail about DNA binding domain and transcription activation domains of transcription factors. CREB is a transcription factor, which contains a very interesting DNA binding domain, consisting of a basic region and a leucine zipper. The leucine zipper is actually responsible for bringing 2 monomers of cyclicAMP together. So, it can bind as a dimer and then facilitate the basic regions, so that you can now interact with the DNA.

So, leucine zippers are important for bringing 2 monomers and facilitates the DNA binding domain to interact to the linear sequence and mini proteins, like the CREB (( )). All these things are contained in this leucine zipper and therefore, can bind to the DNA as dimmers. So, the DNA binding domain of the CREB-ATF family of the transcription factors contains a basic region and leucine zipper. The transcription activation domain contains a glutamine-rich region, called, designated as Q1 and Q2 and within this (( )) region lays the target for protein kinase A.

The serine133 in the case of CREB, the serine117 in the case of CREM and serine63 in case of 81 is the, is the target for protein kinase A phosphorylation. So, all these member of, members of the CREB-ATF family of transcription factors contain a DNA binding domain constraint, consisting of a basic region and leucine zipper and contain a transcriptional activation region, which is glutamine-rich residue, and the serine residue

within this transcription activation domain is phosphorylated by protein kinase A, getting to the activation of transcription.

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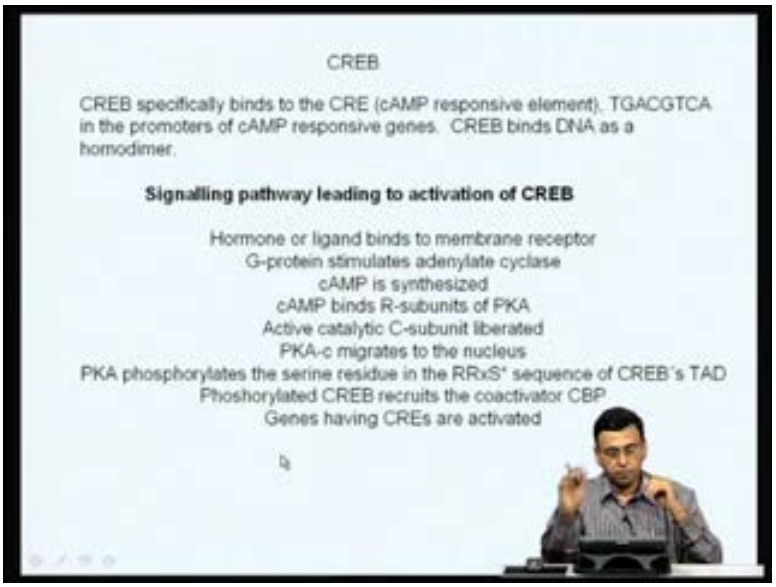

Now, let us discuss in detail, what exactly is the biological function of CREB and how does it act? CREB specifically, binds to an element known as cyclicAMP response element in the promoter regions of its target genes and this cyclicAMP response element primarily, consist of the sequence TGACGTCA, and is very interesting, that instead of TGACGTCA, if this sequence becomes TGACTCA, that is, G is removed, this now becomes a target sequence for a different kind of a transcription factor, called AP1, which consists of C-jun/C-fos.

And we will discuss those things when you talked about protein kinase C signaling pathways. So, you can see, 1 nucleotide difference can become, can change the target specificity of a DNA sequence from CREB to that of another transcription factor. So, the CREB actually binds a cyclicAMP response element, which consist of TGACGTCA, but if this sequence now becomes TGACTCA, it becomes a (( )) for some other transcription factor. So, all genes, which are responsive to the cyclicAMP or which need to be activated in the response in the cyclicAMP, must contain this kind of a target sequence, which is now recognized by the cyclicAMP response binding protein.

As I told you, because of the presence of the leucine zipper, the CREB actually binds to this target sequence as a homodimer. Now, what all the various events, that actually can

lead to the activation of CREB? Let us just recapitulate, what all will happen once a hormone or a ligand, whether it can be a epinephrine, whether it can be a glucagon or it may be ACTH, once is ligands binding their cells of surface receptors, it results in the activation of the membrane receptor and activation of the G-proteins and these G-protein trimer. Now, the alpha subunit of a trimeric G-protein now dissociates and then, acts, stimulates the adenylate cyclase.

And GTP hydrolyses results in the synthesis of cyclicAMP from ATP by adenylate cyclase. Now, the cyclicAMP now goes and binds regulator subunits of the protein kinase A, therefore dissociating the regulator subunits from the catalytic subunits; the catalytic subunits, the catalytic subunits is thus liberated. Now, the protein kinase A catalytic subunit migrates the nucleus phosphorylated serine residues of the (( )) in target proteins, like cyclicAMP, response, responsible binding CREB or CREMor ATF and so on and so forth, which is present in the transcription activation domain.

And once CREB is phosphorylated in the phosphorylated form, CREB is now recognized by a histonestle transfers, called the CREB binding protein or CBP. And once CREB binds to the CBP, CBP acetylates the histones in the immediate vicinity and makes the histones fall upon, thereby facilitating the recruitment of TABs and privatization of complex, and therefore, the transcriptionation can takes place and all the genes having cyclic in response element, now will get activated. So, this is the basic scheme of transcription activation by CREB or cyclicAMP response element binding protein.

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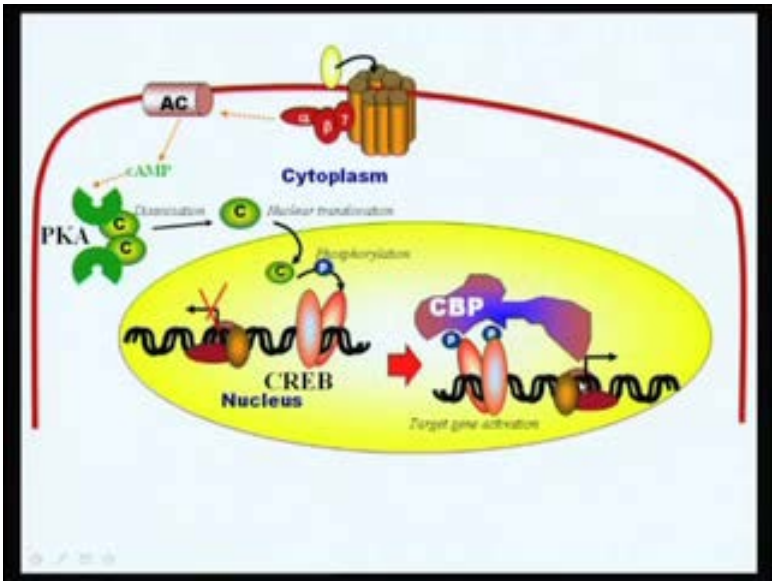

I have designated the same thing in a schematic form. It is very, very important, that is why, I do not mind repeating this signaling pathway. So, once the hormone binds to it, its cells of a receptor, which, what were discussed is, you know, is a GPCR, which contains the 7 trans-membrane domains and once the hormone binds to a GPCR, corresponding GPCRs, it activates the G-protein, the trimeric G-proteins. The beta gamma subunits of G-proteins dissociates from the alpha subunits, alpha subunits now goes and activates adenylate cyclase. Adenylate cyclase now synthesizes cyclicAMP; cyclicAMP now goes and binds to the regulator subunits of phosphorylated protein kinase A, thereby dissociating the catalytic subunit.

The catalytic subunit now goes inside the nucleus and phosphorylated specific (( )) and once CREB is phosphorylated, it now interacts with the CBP or the p300, which is histonestle transferase. Now, CREB now facilitates recruitment of the previous session complex, therefore transcription activation then takes place. So, this is the general scheme, which involves transcription activation by cyclicAMP.

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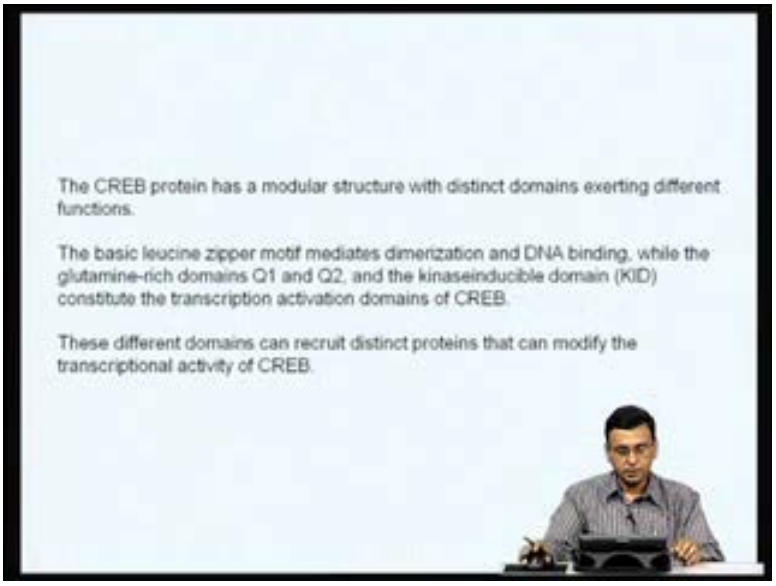

Now, the CREB protein has a modular structure with the distinct domains with, as I discussed earlier, it contains the basic leucine zipper motif, which mediates dimerization and DNA binding. While the glutamine-rich domains Q1 and Q2 and a kinase inducible domain, which is the target for the CBP, constitute the transcription activation domain of cyclicAMP response element binding protein. Now, these are different domains, they can recruit the distinct protein that can modify the transcription activity of CREB.

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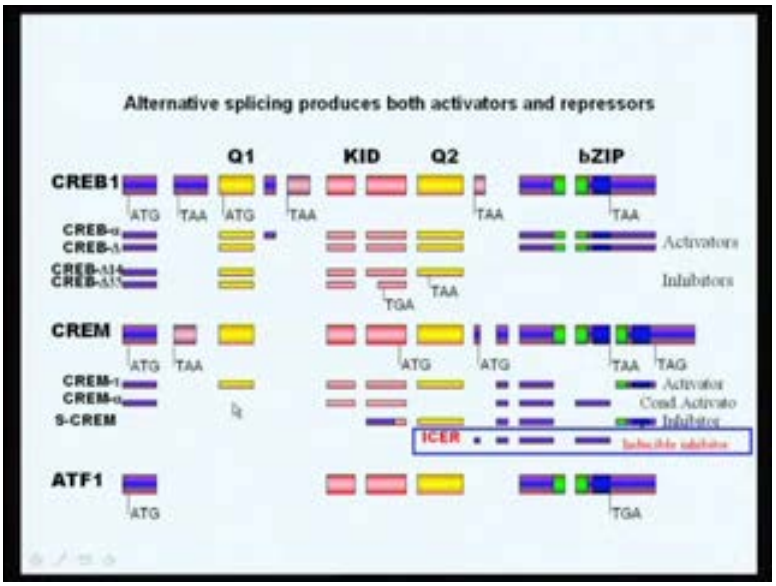

Now, so far we have been saying only CREB, but CREB is not just 1 protein, but there is a family of proteins, which are actually produced by the, belong to the ATF family of protein. And many of variants of the CREB, which can be CREM or ATF1 or molecules called as ICERs, all these things actually arise out of the single gene by alternate spliced.

So, in this schematic, I actually show the various proteins, which can arise from CREB by alternate splicing and some of them contain only the DNA binding domain, some of them contain only the transcription activation domain, and so on and so forth, and all of them are actually, very important regulators of cyclicAMP response. We will not discuss all of them.

But we will briefly discuss some of these variants of CREB, so that you will have some idea, how the cyclicAMP response is governed by a family of proteins belonging to the ATF-CREB family, especially want to pay attention to 1 particular variant of the CREB family, namely the ICER, which actually act as a negative regulator of cyclicAMP response. Therefore, we will discuss it in more detail in the next few slides.

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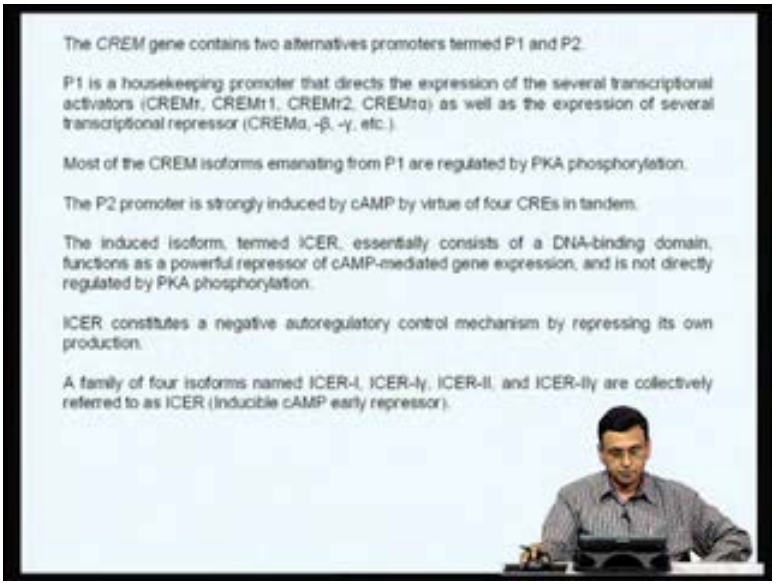

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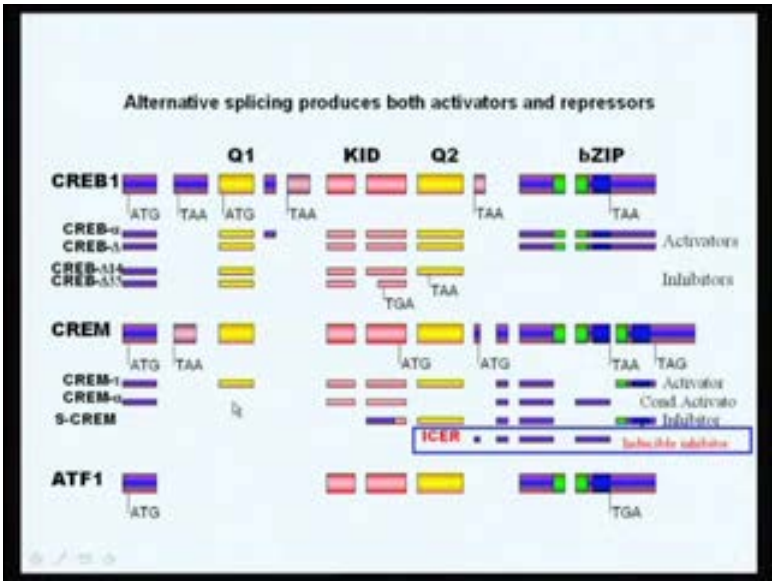

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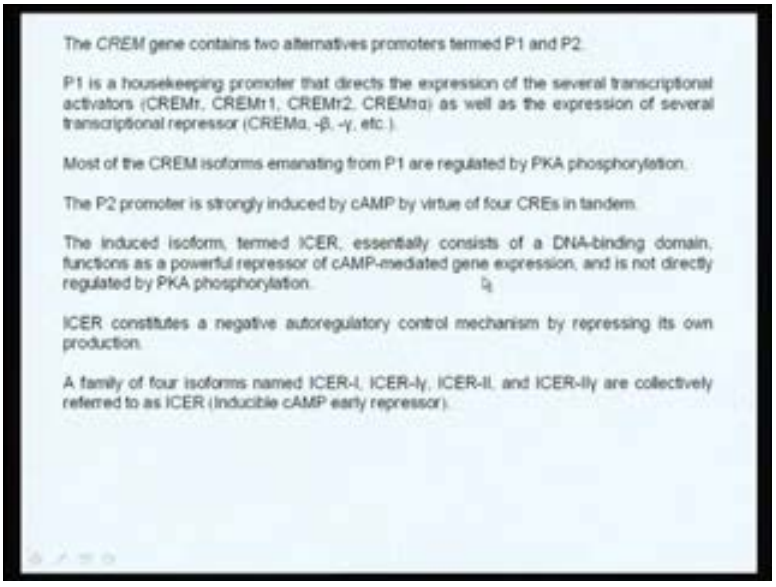

Now, the CREM gene, which is the cyclicAMP response modulator, whose, which is schematically shown in the slide, which is slightly different from the cyclicAMP response, the CREB in terms of homology, because certain domains does not contain, certain domains, which are present in the CREB. Now, CREM actually translates from 2 different promoters, called P1 and P2. Now, P1 is housekeeping promoter, that directs the expression of several transcription activators belonging to the CREM family, namely CREMt, CREMt1, CREMt2 and CREMt alpha, as well as, the expression of several transcription repressors, like CREMalpha, CREMbeta and CREM gamma.

Now, I will not go in to details of what each one them do there, some reviews, which are listed at the end of this lecture and one can go and look up at these reviews and try to understand, what exactly is the function of these different variants of CREM. The most CREM form is emanating from P1 are regulated by PKA phosphorylation because they all contain the target sequence for PKA. Now, the P2 promoter is strongly induced by the cyclicAMP by virtue of 4 cyclicAMP response elements, which are present in the P2 promoter.

So, the CREM gene contains 2 different promoters when transcription activation takes from P1 promoter. This CREM is constituently produced, whereas transcription activation takes from the P2 promoter. It is inducible promoter because it contains cyclicAMP response element, therefore it is a cyclicAMP inducible promoter.

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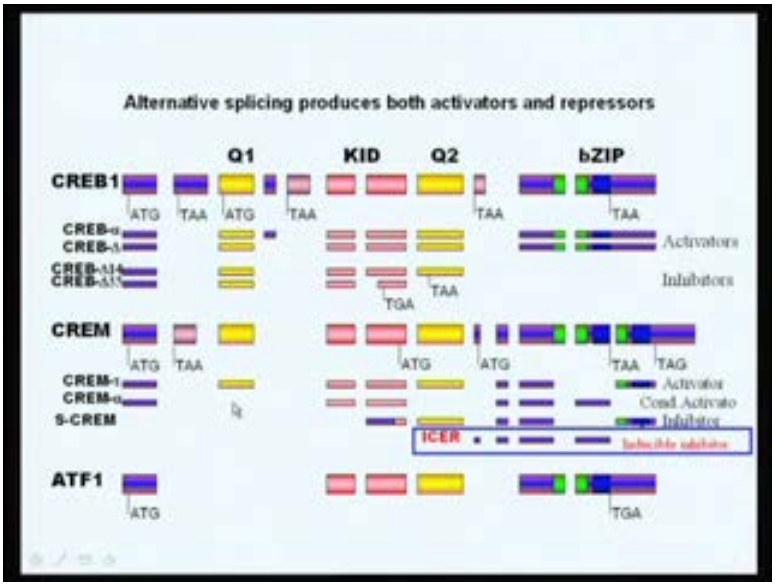

The induced isoform, so when the P2 promoter is activated, a different form of CREM is synthesized, which is actually now called as ICER, which actually refers to inducible cyclicAMP early repressor. It actually consist only a DNA binding domain and therefore, functions as a very powerful repressor of cyclicAMP immediate gene expression and this is not directly regulated by PKA phosphorylation.

If you just go back and look at the would schematic series, you can see, the ICER contains only the DNA binding domain of, DNA binding domain, and therefore it does not contain the transcription activation domain and therefore, you can only bind DNA,

but it cannot be phosphorylated by PKA. Therefore, it cannot interact with the transcription active CBP and therefore, it cannot activate transcription.

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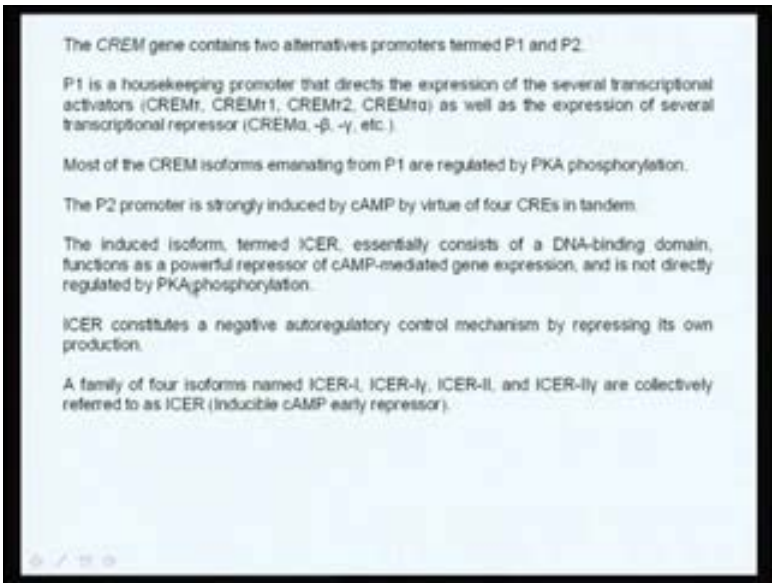

So, the induced isoform, namely the inducible cyclicAMP early repressor, essentially consists of a DNA binding domain. Therefore, functions are powerful repressor of cyclicAMP immediate gene expression. The very interesting aspect about the ICER mechanism of activation is that the ICER constitutes the negative regulatory control mechanism by repressing its shown promoter. Because it is a cyclicAMP inducible promoter, so 1 cyclicAMP produces PKA and PKA binds to this 4 cyclicAMP response elements. In the ICER promoter and synthesizer, the ICER, which is actually made, goes and represses it showing production. And at least, 4 isoforms of ICER, namely ICER 1, ICER 1 gamma, ICER 2 and ICER 2 gamma, correctly referred to the ICERs, now function as negative regulators of cyclicAMP response.

So, remember, when we say cyclicAMP response and transcription, it consists of not only cyclicAMP response binding protein and molecules, like a CREM. It also contains a very, very important negative regulator of cyclicAMP response, namely ICER. The ICER is actually produced from P2 promoter of the, CREM promoter, CREM gene and this is the cyclicAMP inducible promoter. And since ICER contains only the DNA binding domain and (( )) transcription activation domains and ICER functions are negative regulator of cyclicAMP response.

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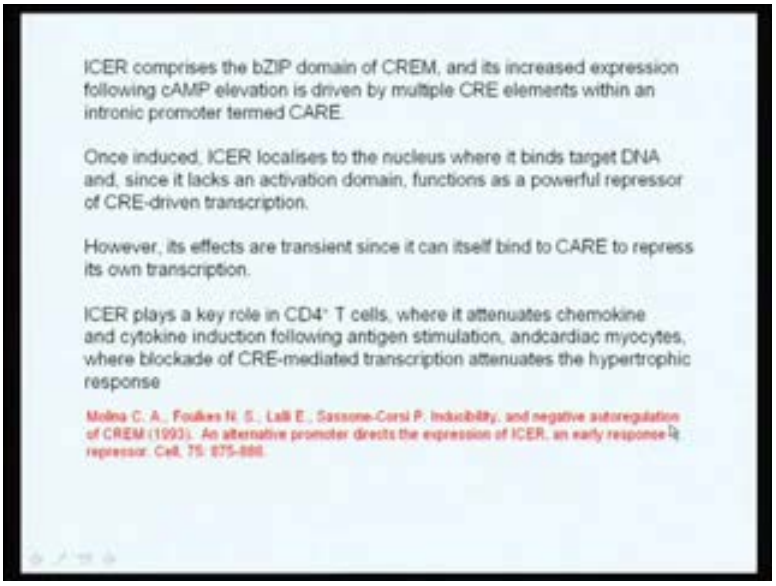

The ICER comprises only the bZIP domain of CREM and its increased expression following cyclicAMP elevation is driven by multiple CRE elements within an intronic promoter, namely CARE. cyclicAMP regulatory element once induced, the ICER localizes the nucleus where it binds to target DNA and therefore, since it lacks the transcription activation domain functions as a very powerful repressor of CRE-driven transcription, so if transcription is activated from the P2 promoter of CREM, it does not do the synthesis of very powerful negative regulator of cyclicAMP response, namely the ICER.

However, effects of ICER are transient since ICER itself can bind to CARE and repress its own transcription. ICER plays a very important role in the CD4 positive T cells, where it attenuates chemokine and cytokine induction following antigen stimulation, as well as, cardiac myocytes, where blockade of cyclic response element, immediate transcription, attenuates the hypertrophic response.

So, the ICER plays a very important role in the regulation of antigen stimulation in the lymphocytes in the T cells, as well as, the cardiac myocytes. A very nice article by Sassone-Corsi group, actually talk about the inducibility and negative order regulation of CREM, first published in the 1993 itself, where it actually demonstrates an alternative promoter directs the expression of ICER, an early response repressor. So, this is the

landmark paper that led to the discovery of an ICER and how ICER actually functions as a negative regulator of cyclicAMP response.

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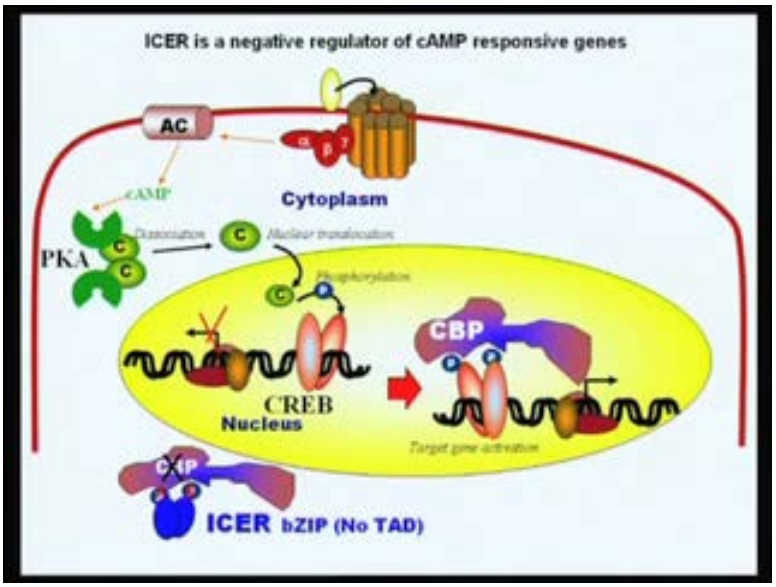

This is just a cartoon, which actually, again tells you, how important is the ICER. We have already discussed this cartoon in the previous slide with only thing, now I am adding here is that when ICER expression is induced, instead of CREB, ICER now goes and binds to the cyclicAMP response. And since ICER contains only the DNA binding domain, it cannot be phosphorylated by protein kinase A and therefore, it cannot interact with CBP and therefore, ICER acts as a negative regulator of cyclicAMP response elements.

So, when CREB or CREM binds to the cyclicAMP response element, it is an activation of transcription; when ICER binds to the (( )) response element, it results in negative regulation of, or it does not result in the activation of the genes involved in cyclicAMP response.

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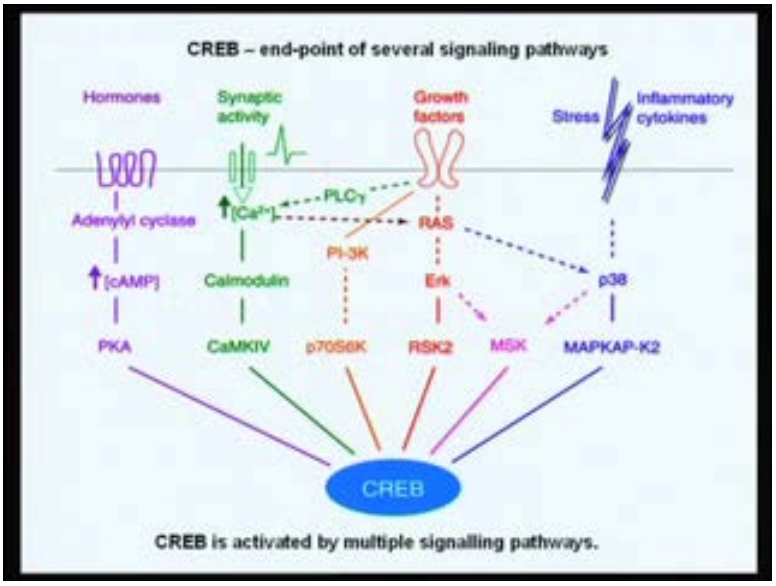

Now, so far I have been telling you, that CREB is activated primarily by protein kinase A. so, cyclicAMP is inside in cells is an cyclic (( )) protein kinase A, protein kinase A phosphorylates and therefore, CREB by now activate all the cyclicAMP responsible genes. But in addition to the PKA pathway involved in the GPCRs, CREB can also be activated by number of other pathways. So, here is an example where multiple signaling pathways can lead to the activation of a single transcription factor. We have not discussed in detail the other signaling pathways, so we will discuss these in the later classes.

But this is a very important aspect, that one has to remember, that (( )) in the biological systems any transcription factor more as a rule than exception are usually activated by a number of signaling pathways. For example, in addition to the hormones through the GPCRs, adenylate cyclase, cyclicAMP, PKA, CREB can also be activated by during in the neuro cells.

Whenever there is a synaptic activity and this synaptic activity, which involves specific neurotransmitters, when neurotransmitters are released near the synapsis, these neurotransmitter cause release of calcium ions and this calcium ions then, activate calmodulin and calmodulin now goes and activates a kinase called calcium domain calmodulin kinase 4, and this calmodulin kinase 4 also (( )) phosphorylates the same serine residue, which is phosphorylated protein kinase A. And therefore, CREB can be

activated not only by cyclicAMP protein kinase A, but also through the calcium calmodulin pathway.

So, you can see, 2 different kinases activated by 2 different signals, can activate the same transcription factor. In addition, growth factors can activate CREB through specific kinases, like the, and specific stress signals can also activate through map kinase pathways CREB. We will discuss in detail what these different growth factors, signaling pathways, map kinase pathways, in the next couple of lecture where we discuss other signaling pathways, that activate transcription factors.

Just remember, it is a very important point, that take-on messages, CREB is can be activated by multiple signaling pathways, like the PKA pathway, the calcium-calmodulin pathway, the receptor tyrosine kinase or the growth factor signaling pathway, as well as, through the map kinase pathway. And different kinases can go and phosphorylate either the same serine residue or different serine residues. They did not do activation of the CREB function.

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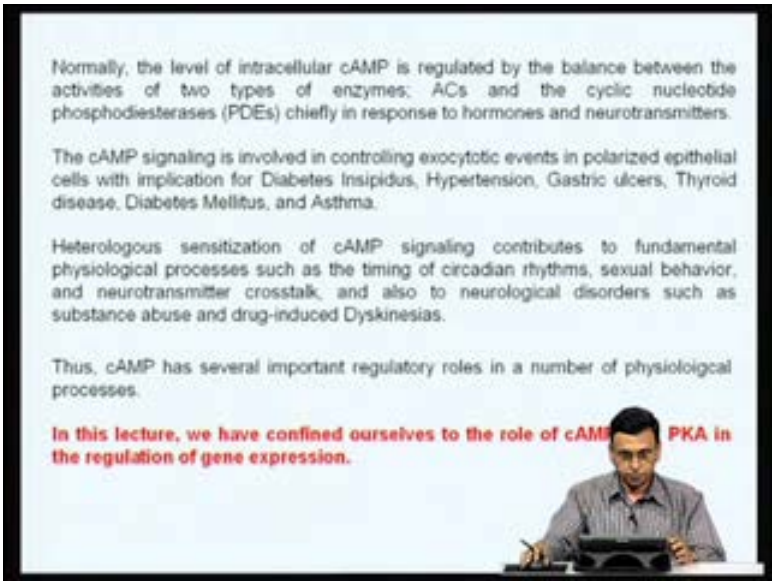

Normally, the level of intracellular cyclicAMP is regulated by the balance between the activity of 2 types of enzymes, namely admirate cyclizers and cyclic phosphodiesterases, chiefly in response to hormones and neurotransmitters. So far, you have been talking only about the positive regulation, that is, activation of adenylyl cyclase, leading to synthesis of cyclicAMP, activation of protein kinase A and so on and so forth, but since

cyclicAMP has very potent cyclic messenger, as soon as cyclicAMP is synthesized, the cyclicAMP are subjected to or cleaved by phosphodiesterases and therefore, get immediately inactivated.

So, the cyclicAMP signaling is involved in controlling a number of cellular processes, especially exocytotic events involving release of molecules outside the cell in polarized epithelial cells. And, this has very important implication for diseases, like diabetes, like insulin secretion for example, is regulated by cyclicAMP, hypertension, gastric ulcers, thyroid disease, diabetes mellitus and asthma. So, a number of these diseases, which involves exocytosis and these exocytotic events, are controlled by cyclicAMP.

Heterologous sensitization of cyclicAMP signaling contributes to fundamental physiological processes, like timing of circadian rhythms, sexual behavior, neurotransmitter crosstalk and also number of neurological disorders, such as drug abuse and drug-induced Dyskinesias, so on and so forth. So, what I am trying to tell you from these things is that, cyclicAMP plays a very important regulatory role in a number of physiological processes, which primarily involve not only transcription, but also a number of other cellular processes. It can be released of secretary granules; it can be activation of metabolic enzymes, and so on and so forth.

But since this lecture series is primarily about regulation of eukaryotic gene expression, we are going to confine ourselves to primarily the mechanisms by which cyclicAMP activates gene expression. So, we are not going to talk about other cellular processes, which involve activation of the metabolic enzymes or activation of calcium release and so on and so forth, we will focus only about the regulation of gene expression.

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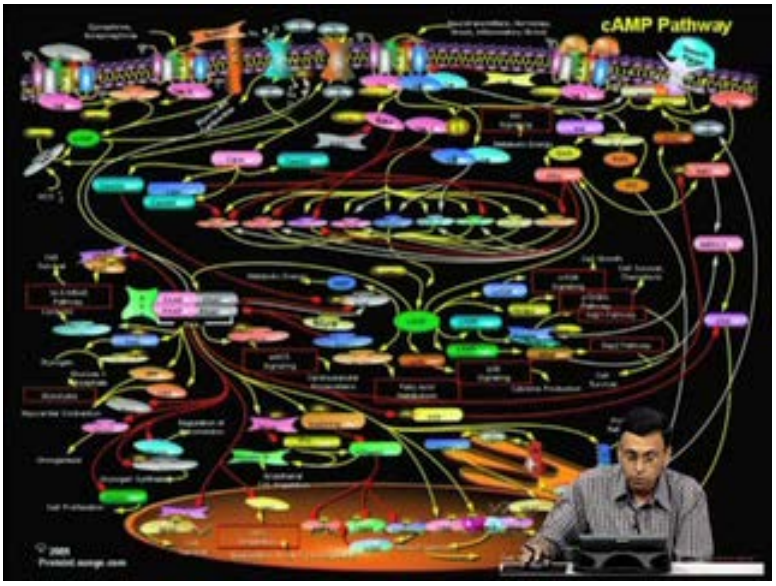

There is a complicated style, which actually purchased from the assay bio sciences here, which actually tells you, how complicated is the cyclicAMP signaling pathway. So far I have told you, that a ligand binds to a GPCR, results in the activation of (( )) G-protein adenylate cyclase is activated and CMP is synthesized. It activates protein kinase A, protein kinase phosphorylates CREB family of transcription factors. But if you now see the various physiological effects that cyclicAMP can bring about, you can see how complicated is this cyclicAMP signaling and its entire pathway, we are only discussing 1 particular aspect in this lecture series.

So, there are a lot of things, that cyclicAMP does in the signaling. So, those who have time can freeze this slide and they may go through and try to understand, what are the various things, that cyclicAMP does.

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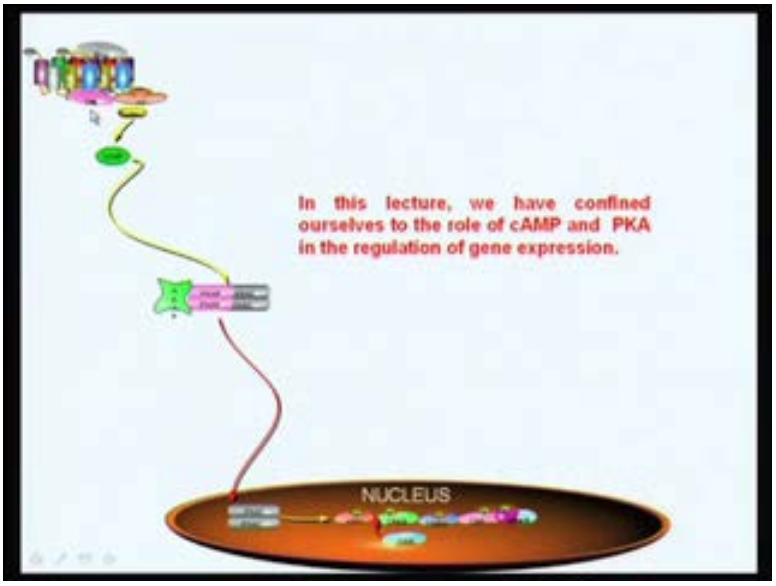

I will not go through the complicated signaling pathway, but I am going to confine myself, as I showed here, just 1 part of this complicated signaling pathway, namely once a hormone binds to the GPCRs, it is in the activation of the G-protein, is in the synthesis of (( )) cyclase. cyclicAMP now comes and phosphorylates, binds the inactive protein kinase A, catalytic subunit of protein kinase A now comes inside the nucleus and phosphorylates a number of transcription factors.

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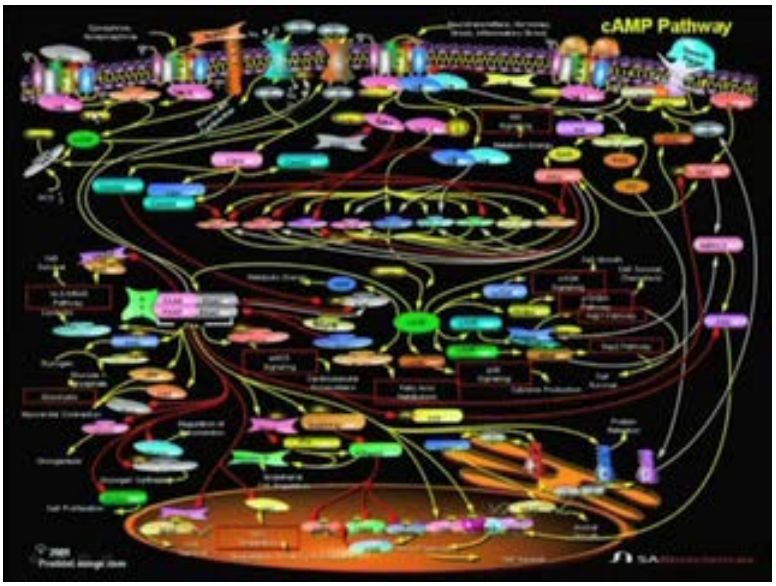

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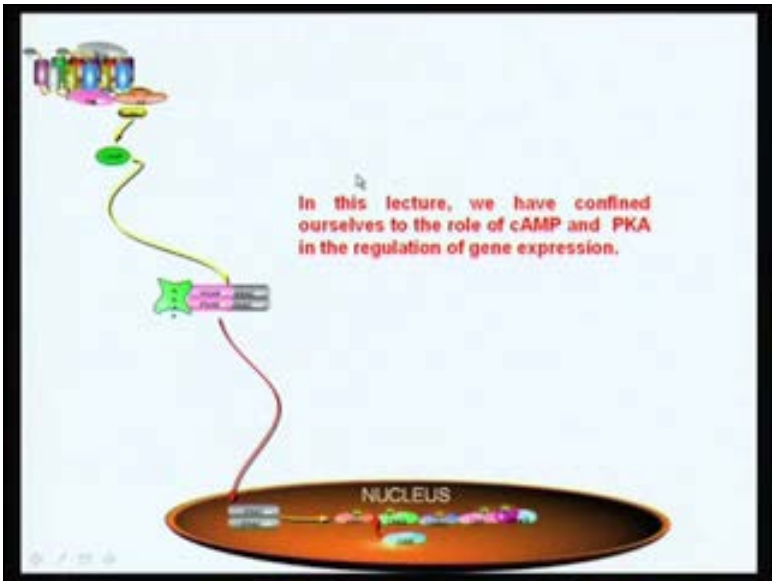

So, this particular pathway is just one aspect of what is shown in the others. So, in this entire complex events, that cyclicAMP mediates inside cells, we are not going to talk about, we are talking only about 1 particular pathway, which is highlighted in the slide. So, I am emphasizing time and again, do not think, that all the physiological effects of cyclicAMP are primarily due to activation of gene expression, there are also many regulatory events, that take place; many events, that are activated by cyclicAMP involved in cyclicAMP, involves, does not involve activation of gene expression, but involves a number of other cascades and cyclicAMP pathway is also. There is a crosstalk with other signaling processes; as showed in the previous slide, there is a lot of crosstalk between the other signaling pathways. So, in the (( )), the cyclicAMP responds the very, very complicated network of events.

Since we are going to be focused primarily on gene expression mechanisms, we are confining our cells to 1 cyclicAMP synthesis in the cell, how the cyclicAMP activates gene expressions. So, we are only focusing on 1 aspect of this entire series of events that take place by that can be regulated by cyclicAMP. So, in this lecture, we have confined ourselves to the role of cyclicAMP and PKA in the regulationof the gene expression and have skipped a lot of other things, that cyclicAMP does.

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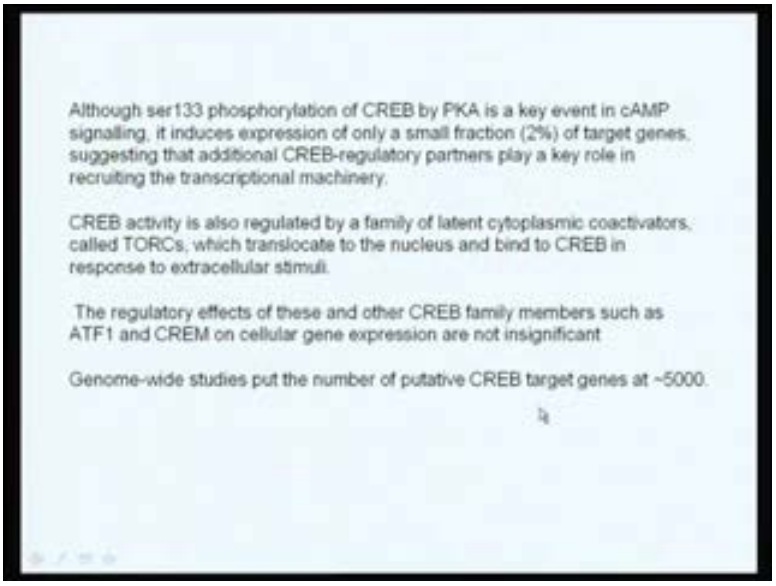

Although serine133 is phosphorylated by CREB, the key event in cyclicAMP signaling is the phosphorylation of serine133, so that the CREB can interact with the CBP, resulting in activation of gene expression, but turns out, only a small fraction of target genes, less than, this is a 2 percent of the gene this event happens, suggesting, that in addition to the phosphorylation of the serine133, interaction of CREB with other regulatory partners play a very important role in the actual activation of transcription of cyclicAMP response (( )), cyclicAMP responsive genes.

In other words, all the events that take place in response to cyclicAMP cannot be explained by the serine133 phosphorylation alone. Phosphorylation of serine133 is just one aspect of the cyclicAMP response, there are number of other things, that actually are essential in order for this cyclicAMP response to a (( )) gene activation. For example, CREB activity is regulated by a family of latent cytoplasmic coactivators, called as TORCs, which translocate a nucleus bind to CREB in response to specific extracellular stimuli. The regulatory effects of these and other CREB family members of CREB, such as ATF and CREM on cellular gene expression are not, are not, are not insignificant.

So, remember, although having focusing time and again, that phosphorylation of the serine133 by PKA is a primary event in the cyclicAMP signaling pathway, leading to gene activation, there are many other events, which are associated with these gene activation, which also play a very important role in the gene activation by cyclicAMP

response of genes. In fact, genome-wide studies put that number of target genes, which are activated in response to cyclicAMP and CREB binding is approximated by 5000 genes.

So, when cyclicAMP levels go up inside the cells, approximately 5000 genes can be activated, but this number can vary from cell type to tissue type and so on and so forth, this is just an approximation. So, assuming, there are about 30 to 50000 genes in a mammalian cell, 5000 is a reasonably high number, so significant number of genes are activated by, are targets for cyclicAMP response to binding protein.

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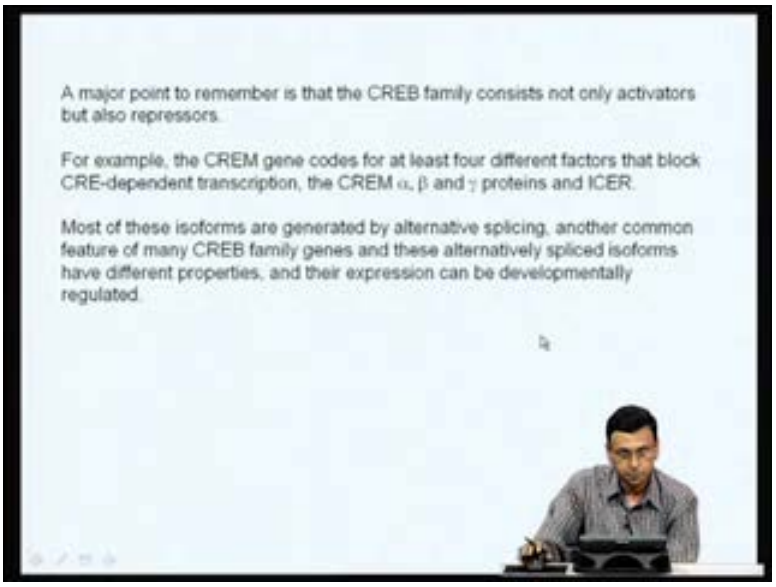

A major point to remember is that CREB family contains not only activators, but also repressors, like I told you, the molecules like ICER, CREB, CREM alpha, beta and gamma, they actually function as repressors. So, do not assume, that whenever there is an increase in cyclicAMP levels inside the cells, it results only in the activation of transcription, it also can result in the repressor of transcription, especially if the increase in the cyclicAMP leads to the expression of proteins like CREM alpha, beta, gamma or ICER. It can actually something negative regulation of cyclicAMP response remains changed.

As I mentioned earlier, CREM gene codes for at least 4 different factors, that blocks CRE–dependent transcription, namely CREM alpha, beta and gamma proteins, as well as, ICER. So, among the various members of the ATF CREB family of transcription

factors, CREM alpha, beta, gamma and ICER, actually act as negative regulator transcription and if you remember, what I told you earlier, the P2 promoter of the CREM actually contains a cyclical response element.

Therefore, when cyclicAMP is induced, CREB can actually go and bind to these cyclicAMP response element, induce the expression of ICER, and therefore, ICER can now compete with CREB. And therefore, when ICER binds, it can actually function as a negative regulator of, there is a competition between CREB and ICER leading to down regulation of these cyclicAMP response. Most of these ICER forms are generated by alternate splicing, I showed in a schematic, how these are all generated. Another common feature of many CREB family genes is that these alternative spliced isoforms have different properties and therefore, expression can be developmentally regulated.

So, here is a very wonderful example of how from one single gene, a number of different proteins can be synthesized and these proteins has different physiological functions. So, from a single gene, by alternate splicing, many variants of a transcription factor is synthesized and some of these variant functions has positive regulators, some of these variant function has negative regulators.

So, you can see, 1 gene 1 protein is not the rule anymore; by mechanisms like alternate splicing, 1 gene can give us too many proteins and these proteins can have in fact, quite opposite functions, some of them can function as activators of gene expression, some of them can function as negative regulators of the gene expression. There are, there are 2, in fact, not only alternative splicing, I also told you, by utilizing 2 different promoters, the P1 or P2, you can generate the different kinds of variants of the CREM proteins.

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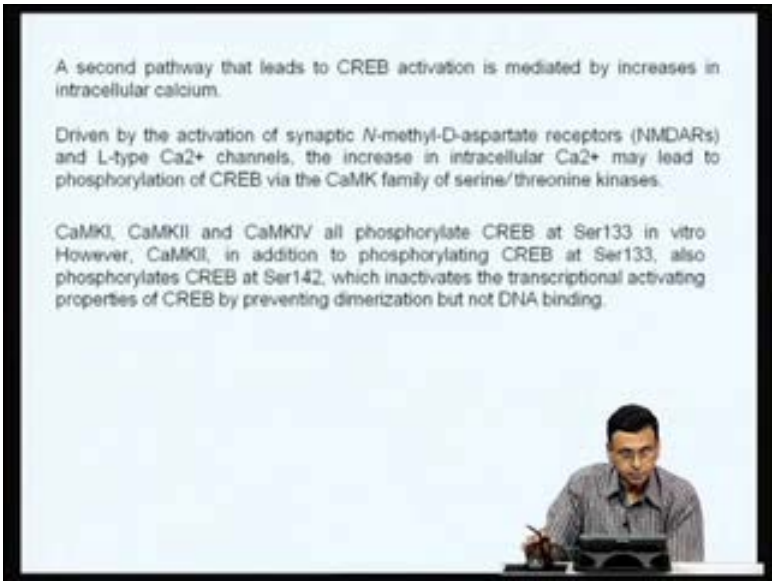

So far, we have been discussing the mechanism by which the CREB transcription factor is activated by cyclicAMP and protein kinase A. In addition, like I also showed a schematic saying, that CREB can be activated by many other mechanisms and a 2nd pathway that leads to CREB activation is by increasing intracellular calcium. I showed you in the schematic, how, especially in the neuro (( )) systems, whenever there is an activation of a synapse by molecules like serotonin for example, which is a very important neuro transmitter, when these neurotransmitters are released in the synaptic junction, it can actually result into the release of calcium.

And as I discussed here, synaptic in the, neurons in the synapses by NMD receptors or N-methyl-D-aspartate receptors, as well as the L-type calcium channels, there is an increase in intracellular calcium and these intracellular calcium may lead to phosphorylation of CREB via the calcium calmodulin kinase family of serine or threonine kinases.

This CAM kinase family is another huge family of protein kinases, which play very important role in the regulation of number of enzymes as well as genes and one of them is also a CREB. So, CREB can be phosphorylated not only by cyclicAMP, immediate protein kinase A, CREB can also be phosphorylated by calcium mediated CAM kinases or calmodulin kinases.

CAM kinase1 CAM kinase2 and CAM kinase4, all of them are capable of phosphorylating CREB at same residue as that of protein kinase A, in vitro. However, CAM kinase2, in addition to phosphorylating CREB at Seronin133 also phosphorylates CREB at Serine 142 and phosphorylation of serine 142 inactivates the transcription activating properties of CREB. So, you can see, how, how beautifully the regulation takes place.

And when serine133, phosphorylated by either CAM kinase 2 or by protein kinase A, it results in interaction of CREB with P300 resulting in the activation of transcription, but when CAM kinase4 phosphorylated serine142 of the CREB, it actually results in repression of transcription, because the serine142 prevents dimerization of the 2 CREB molecules. Therefore, CREB cannot dimerize and therefore, it cannot activate transcription.

So, you can see, how phosphorylation by 2 different kinases can regulate the activity of a transcription factor when PKA and CAM kinases phosphorylated of 133 of CREB, which facilitates interaction with CBP resulting in activation of transcription. But when serine142 is phosphorylated by specific CAM kinase, it can actually prevent dimerization of CREB and therefore it results in repression of transcription. So, phosphorylation or transcription factor, depending upon which residue is getting phosphorylated, can either function as an activator or can function as a repressor.

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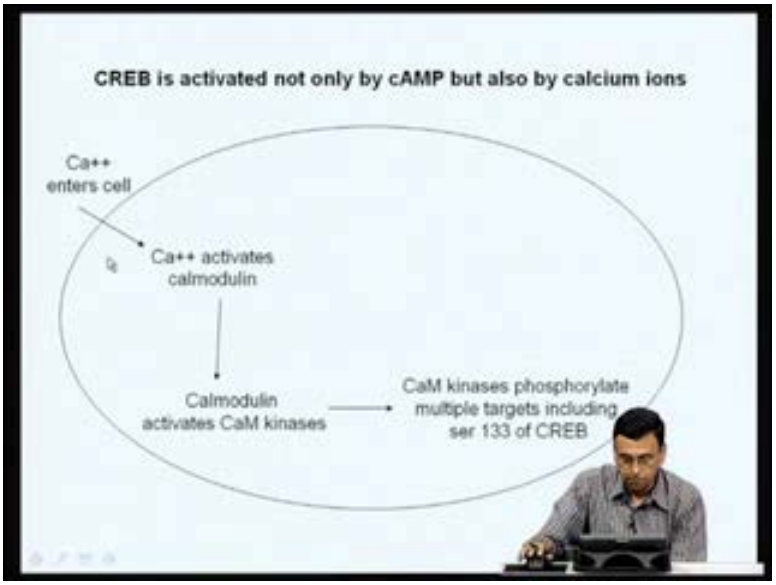

So, this is schematic, that actually shows, that how calcium can activate CREB. When calcium is released, there is an intracellular increase in calcium levels, calcium activates calmodulin, calmodulin now activates the calmodulin kinases or CAM kinases, CAM kinase now go and phosphorylate multiple targets including serine133 of the CREB. So, in addition to cyclicAMP and protein kinase A, calcium through calmodulin kinases can also activate CREB or cyclicAMP is responsive genes.

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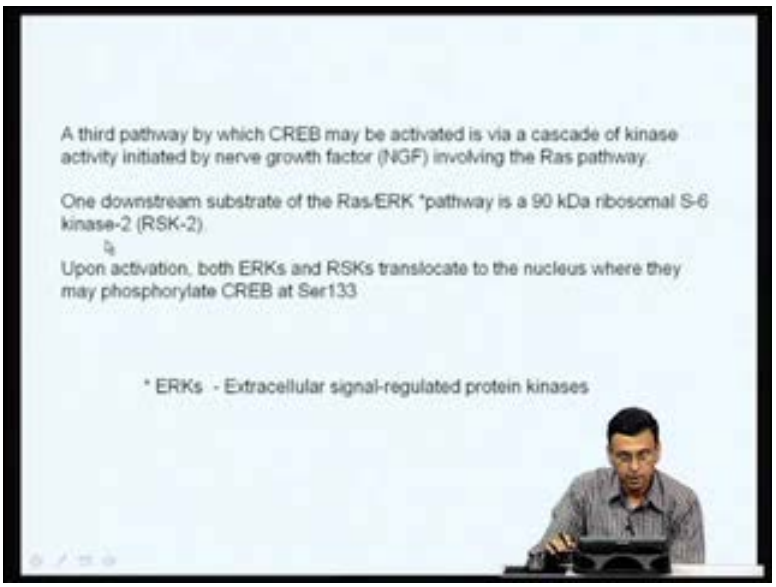

A 3rd pathway by which CREB may be activated involves a cascade of kinase activity initiated by nerve growth factor involving the Ras pathway. In the schematic I have showed in the earlier slides, the 3rd pathway involves the Ras pathway, which involves growth factor signaling. We will discuss the growth factor signaling in the later series of this lectures and one of these downstream substrate of this growth factor form is a specific kinase called a 90 (( )) ribosomal S-6 kinase2 or RSK-2.

And when nerve growth factor binds to nerve factor receptor, it initiates the phosphorylation cascade and as a result, a kinase called RSK-2 is activated. Upon activation this RSK, as well as, other kinases called as extracellular signal regulator protein kinases, ERKs, or ERKs, they translocate at the nucleus where they phosphorylate CREB at serine133.

So, the serine 133, which is the major, I mean, (( )) transcription activation of CREB is a target, not only for cyclicAMP depend kinase A, it is also a target for CAM kinases, it is

also can be targeted by the extracellular signal-regulated protein kinases or ERKs as soon as the ribosomal S-6 kinase2. So, you can see, CREB can be activated by several different mechanisms at the serine133 of CREB can be phosphorylated, not only by a PKA, but also by CAM kinase, but also by kinases, which are activated by specific growth factors signaling pathways.

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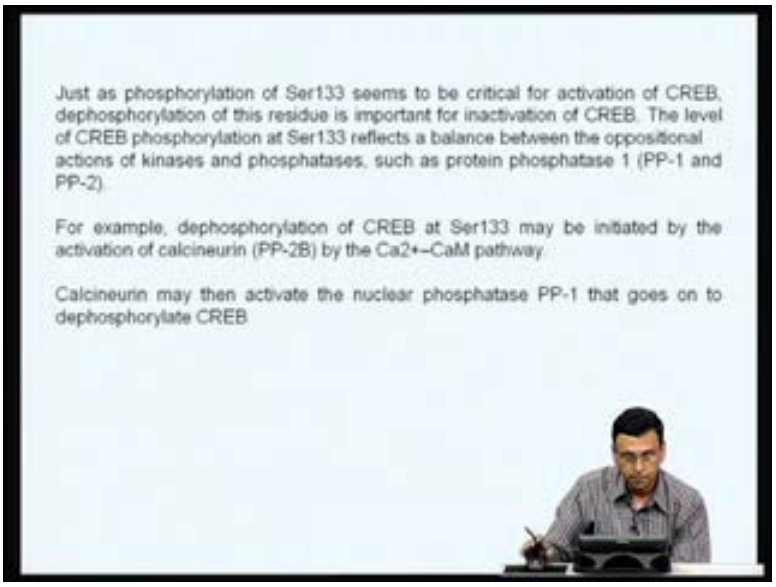

So, just as phosphorylated serine133 seems to be critical for the activation of CREB, dephosphorylation of this residue is as important because once CREB is (( )) has to be inactivated. The level of CREB phosphorylation serine133 reflects a balance between the oppositional action of kinases and phosphatases by protein kinase, is phosphorylated for serine133. There are protein phosphatases, especially protein phosphatase 1 and 2, which actually remove the phosphate, thereby inactivating the CREB.

So, at any given situation, there is an equilibrium between the kinases and phosphatases as rapidly, the serine133 is phosphorylated by this kinase, it is also rapidly dephosphorylated and the equilibrium between this kinase and phosphatases defines how long the signal can be active, how long a cyclicAMP response gene can be active depends on the balance between the activation of the phosphatase and kinases. So, dephosphorylation of CREB at 133 may be initiated by the activation of calcineurin by the calcium-calmodulin pathway. So, there are certain pathways, which activate kinases; there are certain signaling pathways, which activate phosphatases.

And therefore, then these pathways are activated, like for example, the calcineurin pathway. It actually stimulates the activity of phosphatases and therefore, inhibits the CREB activations. The calcineurin may activate the nuclear phosphatase PP-1, that goes onto dephosphorylate CREB. So, there is a dynamic equilibrium between signaling pathways, which activates kinases, that phosphorylated CREB and there are pathways, which activate phosphatases, which dephosphorylated CREB and this equilibrium between this 2 opposite pathways are ultimately depends on how long the CREB signal, how long the cyclicAMP signal and the positive signal can remain active.

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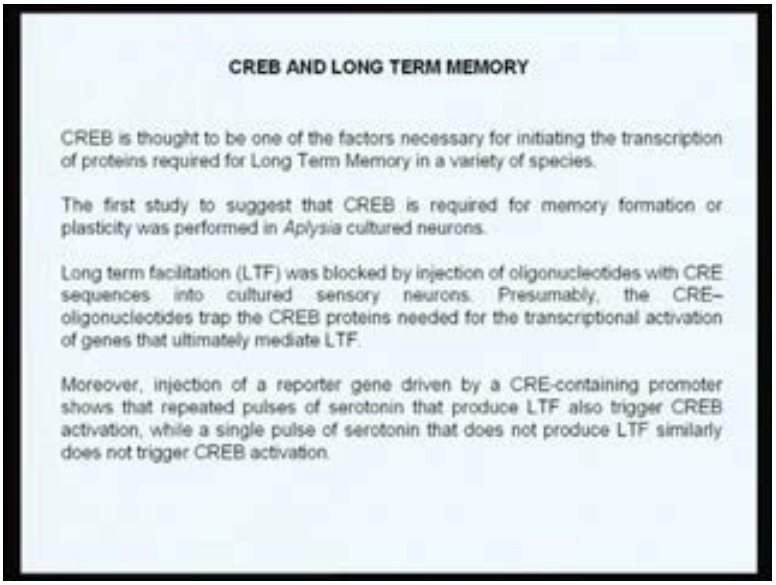

We are now going to discuss a very important aspect of cyclicAMP response, especially the role of CREB in the nervous system, especially in long term memory. Now, CREB is thought to be one of the factors necessary for initiating transcription of proteins required for long term memory in a variety of species. Someone (( )) about 5 minutes time to explain, how CREB plays an important role in the nervous system, especially the long term memory. The first study to suggest, that CREB is required for memory formation of plasticity was performed in Aplysia cultured neurons.

It turns out very elegant experiments were actually done to demonstrate the role of CREB in the long term memory. Long term facilitation was blocked by injection of oligonucleotides; contain the cyclicAMP response elementary sequences into cultured neurons. So, if you know cultured neurons from the Aplysia and into the neurons, if you

simply inject oligonucleotides, which contain the cyclicAMP response elements, you can prevent long term memory in this organism.

It turns out, when injected this oligonucleotides, what is happening? The CREB now goes and (( )) these, like what we discussed about skelting in some of the earlier lectures, these oligonucleotides contains a cyclicAMP response elements. Now, sequesters all the CREB protein, therefore CREB cannot go and activate genes, is not available for activation of the genes of the cyclicAMP response of genes. So, by flooding the system with ordinary oligonucleotides containing CREB binding sides, you are titrating out the CREB and making less CREB available for activation of the target genes. As a result, there is a loss of long term memory in this organism.

Similarly, when injecting a reporter gene driven by cyclicAMP response containing promoter, for example, you take a promoter containing the cyclicAMP response element look it on to the either (( )) or GFPR so on and so forth, and if you inject these cyclicAMP driven reporter gene into these cultured neurons. Now, if you treat these neurons with serotonin and if you treat pulses of serotonin, and serotonin produces long term facilitation, it also triggers CREB activation.

So, whenever the serotonin pulse results in the activation of the long term facilitation, it also, you can see reporter gene expression happen. So, give a pulse of serotonin and you see, there is an activation of CREB clearly indicating, that the serotonin somehow results in a chain of events that ultimately results in the activation of the CREB transcription factor. So, single pulse of serotonin does not produce LTF and does not trigger CREB activation, but repeated pulses of serotonin can result in LTF and also trigger CREB activation.

So, experiments such as this described here clearly indicate, that neurotransmitters, which are actually involved in memory can act, probably act to a functioning through the CREB pathway. So, by injecting oligonucleotides containing CREB binding sides, you can actually lower the memory and also, by, you can activate CREB responsive genes (( )) reporter genes by giving the pulses of serotonin, clearly indicating, that there is a connection between neurotransmitter, CREB activation and memory.

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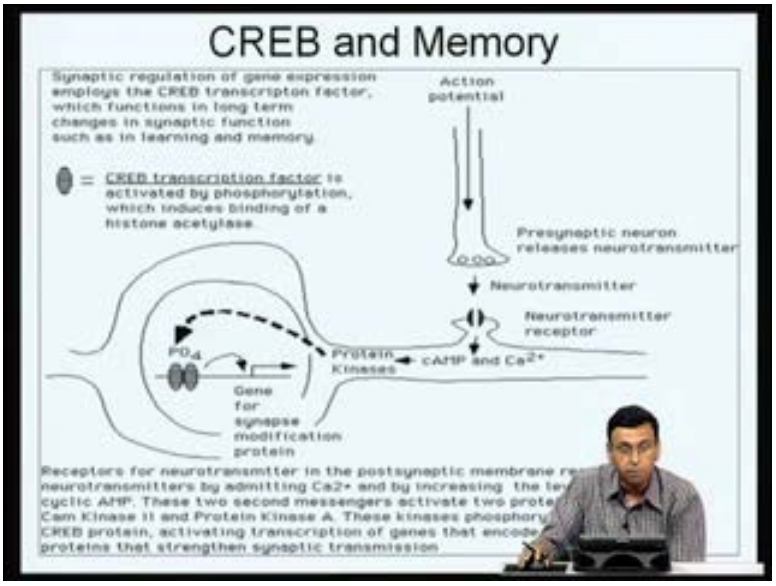

Very elegant experiments have been done to demonstrate the relationship between the CREB and memory; I will just list out the summary of all these things here. For example, whenever there is an action potential and results in the release of certain specific neurotransmitter like serotonin at this junctions, these neurotransmitters bind to the neurotransmitter receptors, and when these neurotransmitter bind the neurotransmitter receptor in the synapsis, it results in the release of calcium and cyclicAMP, and these activate protein kinases, like for example, cyclicAMP activates adenylate cyclase or calcium activates calmodulin kinases and they, in turn, goes and phosphorylated serine133 of the CREB. There is some activation of protein genes, whose proteins are actually involved in synapsis formation and therefore, strength in the synapses translates, leading to long term memory.

So, here is an example, where signaling molecules, neurotransmitters can actually bring about a physical or physiological response in the long term memory through the activation of the specific transcription factor. So, is, this is a very elegant experiment, of demonstrated, to demonstrate CREB plays a very important role in long term memory.

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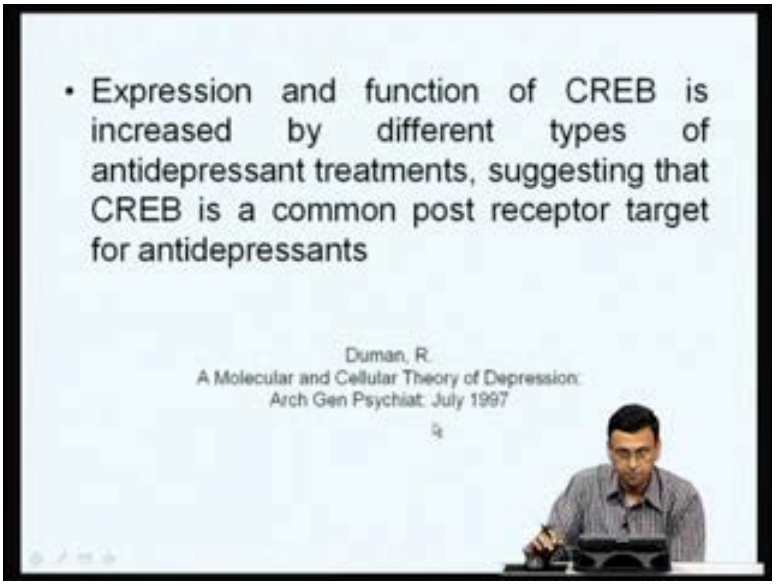

There are number of interesting papers, which actually discuss how important is CREB being in various aspects of nervous system. Here is a paper, which actually demonstrates expression and function of CREB is increased by different types of antidepressant treatment suggesting, that CREB is a common post receptor target for number of antidepressants. So, there is lot of interest among neurobiologist to understand the role of CREB in a number of neurological processes, like long term memory, in this case for example, depression and even drug addiction, and so on and so forth.

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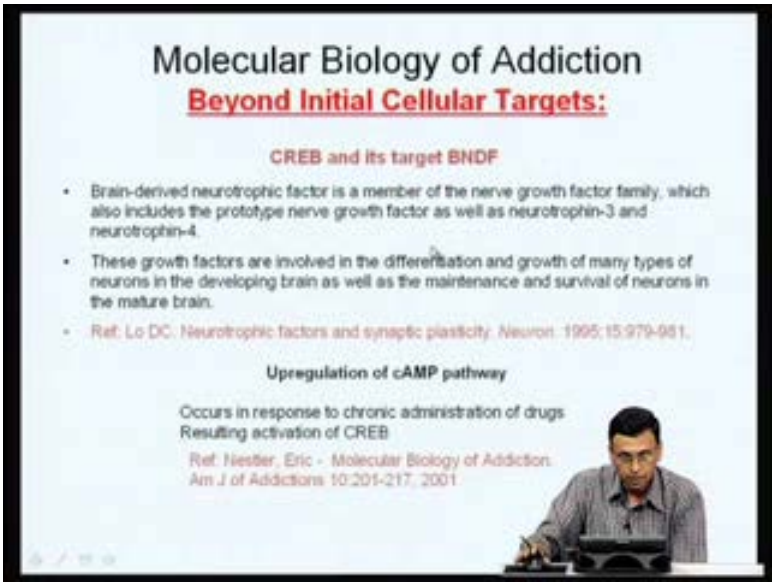

Here is a another very interesting paper, which says, a protein called brain-derived neurotrophic factor is a member of the nerve growth factor family and this growth factor actually targets CREB, saying that the neurotrophic factors synaptic plasticity involves actually CREB. Similarly, upregulation of cyclicAMP pathway as actually being shown to administer results in the chronic administration of certain drugs, which actually involved in certain addiction, so on and so drug addiction, for example.

When you get addicted to drugs, this drug addiction seems to be primarily involved in the activation of cyclicAMP pathway and CREB and this paper actually, very interestingly, describes how drug addiction actually involves activation of CREB and increasing intracellular of cyclicAMP. So, the cyclicAMP response or the calcium increase coupled to the CREB activation seems to be involved in the number of neurological processes inside the brain.

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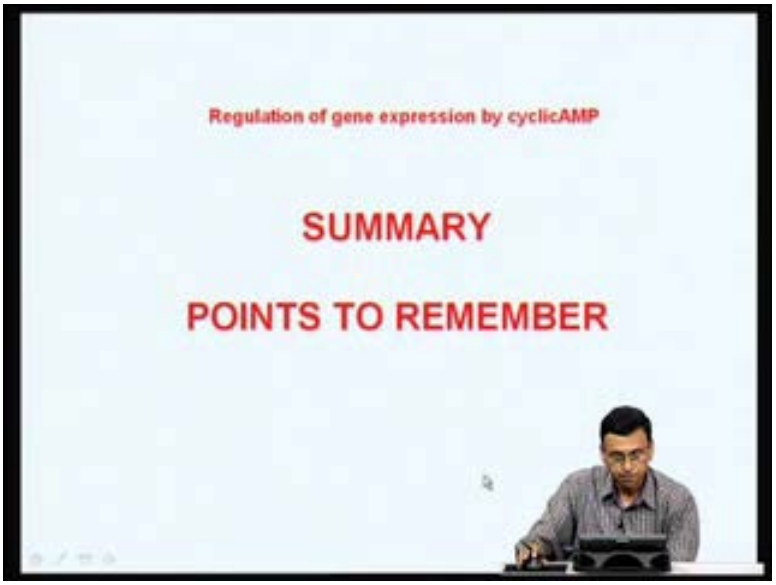

So, what I am going to discuss in the next few slides, I have just highlighted some of the important aspects of cyclicAMP regulation and how cyclicAMP is a very important 2nd messenger and by activating a family of transcription factor belonging to ATF-CREB super family, regulates a number of physiological processes, both in the nervous system as well as outside.

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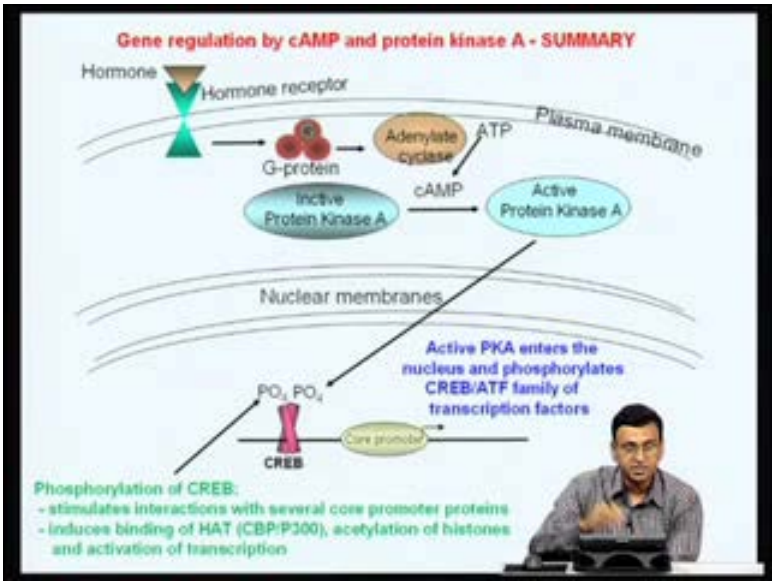

So, some of the important points that you have to remember, whenever you talk about cyclicAMP response and gene expression, I just listed in a next few slides. For example, the summary of what I told you is that whenever a hormone binds to the hormone receptor, this result in the activation of the primary G-protein and this result in the activation of an adenylate cyclase. Now, adenylate cyclase, now converts inactive protein kinase A, active protein kinase A, now active protein kinase A goes inside the nucleus, phosphorylates the serine133 of CREB and this results in the activation of the target genes, leading to through the binding of, for the activation of the histonestle transferase or CBP P300 is acetylation of histones of the activation of transcription. So, this is the summary by which molecules, which activates adenylate cyclase pathway or the PKA pathway (( )) the activation of the CREB.

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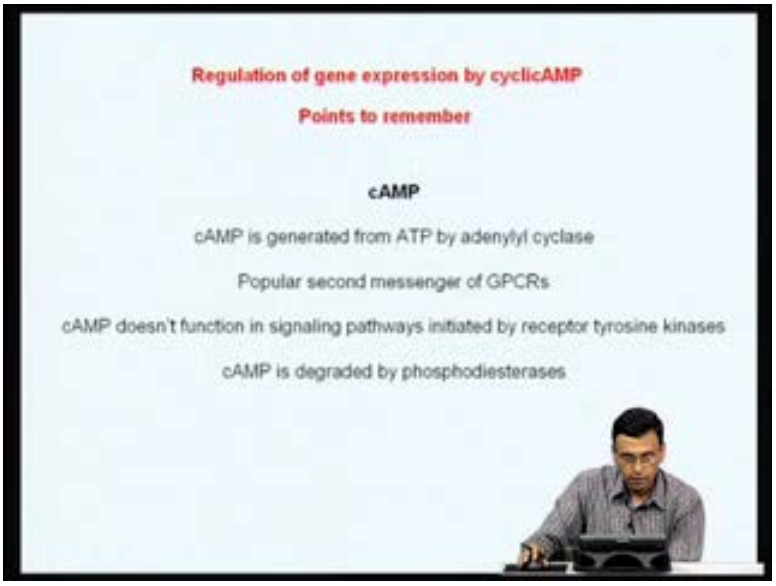

Now, cyclicAMP, the important thing that we have remembered cyclicAMP is that cyclicAMP is generated from ATP by adenylyl cyclase. It is a very popular messenger involved in the G-protein couple receptor pathway and cyclicAMP does not function in signaling pathway initiated by receptor tyrosine kinases. It is very, very important point we are going to discuss about the receptor tyrosine in the future classes. Remember, cyclicAMP is not a 2nd messenger when the receptor tyrosine kinase pathway is activated and cyclicAMP is primarily degraded by phosphodiesterases.

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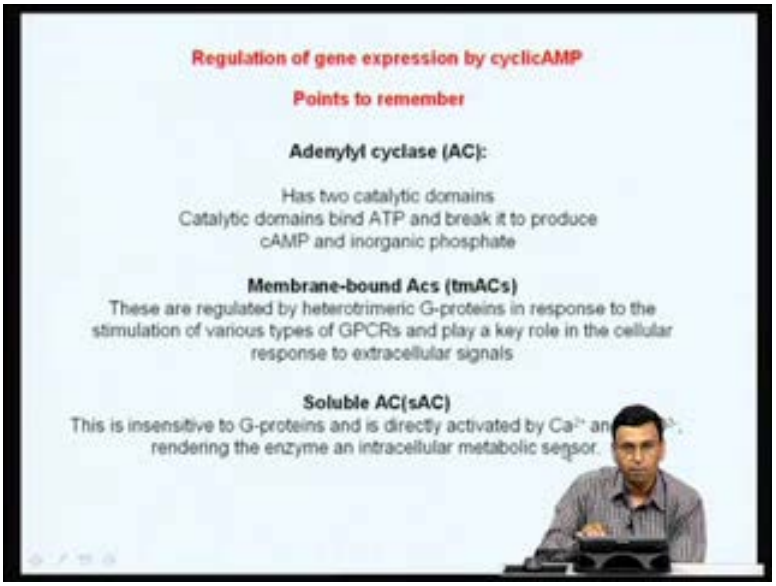

And also, I mentioned here important point, that we have to remember adenylyl cyclase' mammalian cells, there are 2 types of adenylyl cyclases - soluble adenylyl cyclase and membrane associated adenylyl cyclase. And the TM adenylyl cyclase are the ones, which are involved in the growth factors GPCRs signaling, whereas the soluble cyclases are not involved in the signal transition through the G-proteins, they are directly activated by molecules, such as calcium ions and bicarbonate ions.

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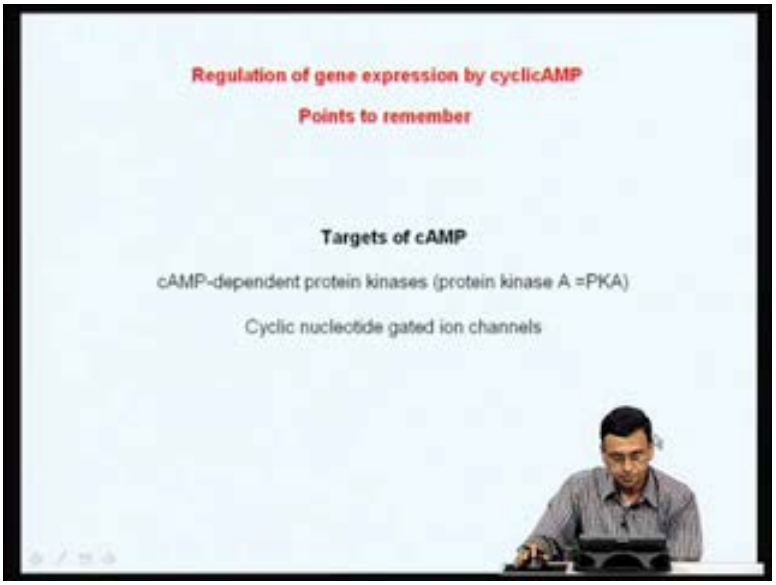

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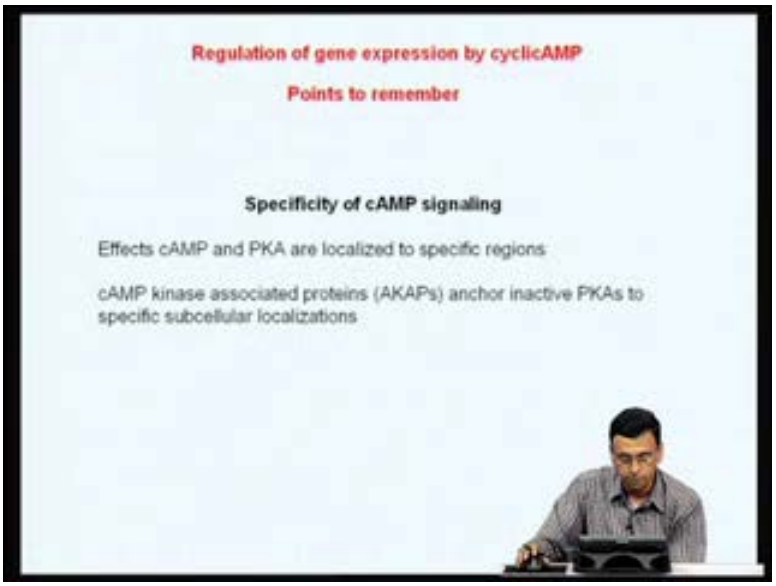

Now, what are the targets of cyclicAMP? cyclicAMP's target cyclicAMP protein kinases, as well as, cyclic nucleotide gated ion channels. We have not discussed in this aspect because it is not linked to the gene expression. Now, what is the specific of cAMP signaling? cyclicAMP in PKA are localized in specific regions and cyclicAMP kinase associated proteins of AKAPs anchor inactive PKAs to subcellular localizations, which has a very, very important regulatory role.

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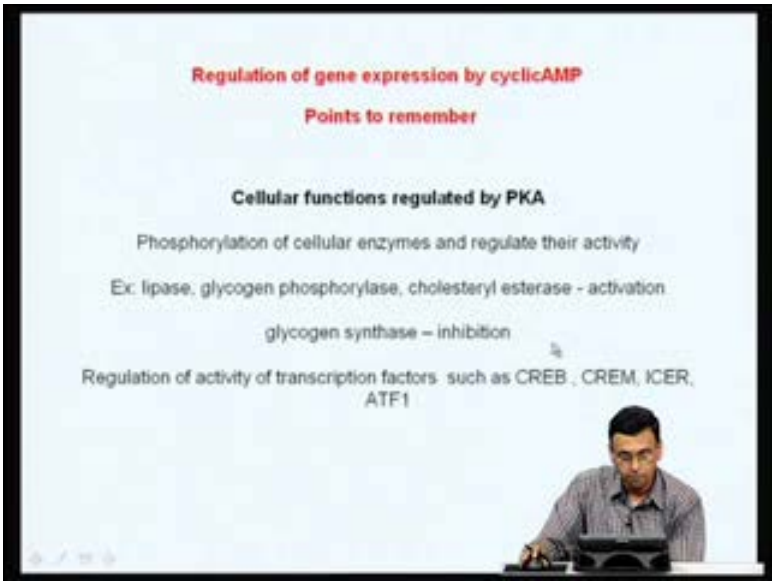

Now, what are the cellular functions of PKA? PKA can want phosphorylation number of cellular enzymes and regulate their activity. We have not discussed this particular aspect in our lecture because it is not directly linked to gene expression, like for example, cyclic PKA can activate lipases, glycogen phosphorylase, cholesteryl esterase and so on and so forth. You can inhibit this, it is very, very important in regulated roles and carbohydrate metabolism and this is like diabetes and so on and so forth. Now, what we primarily discuss in this class about the activation of transcription factors, such as CREB, CREM, ICER and ATF1 because that what is relevant to this gene lecture series.

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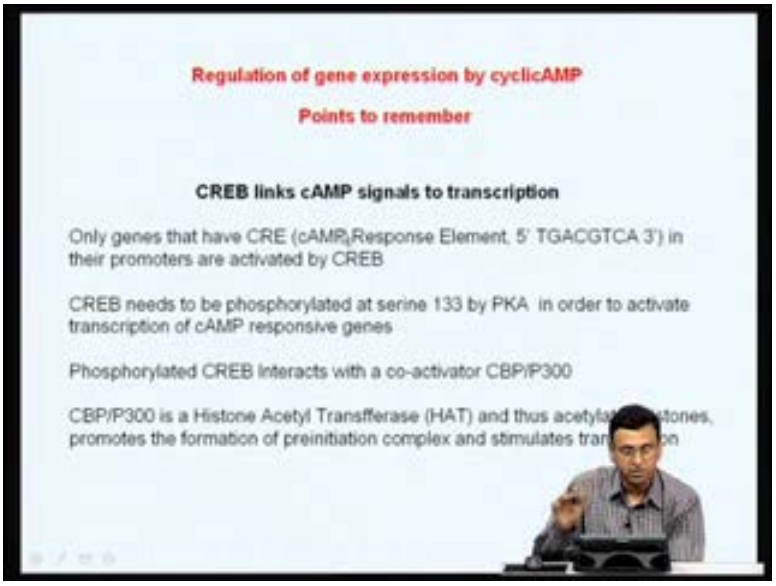

Now, CREB is the primary link between cyclicAMP and gene transcription. We discussed various aspects of CREB activation; I have listed some of the important aspects, like what is a cyclicAMP response element? What is the residue to the possible PKA? How does CREB interact with CBP P300 and how does CBP P300 by virtue of the histone acetyl transferee activity acetylates histones, leading to for stimulation of preinitiation complex formation and activation of transcription.

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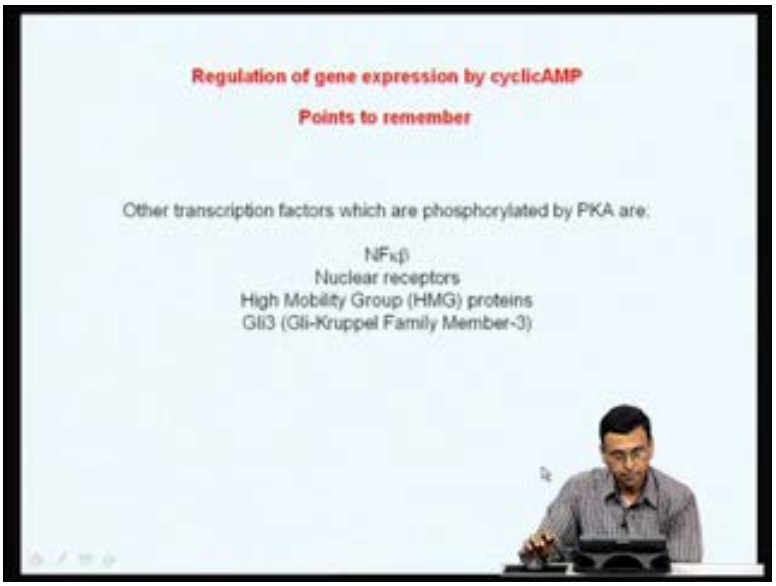

Now, in addition to the CREB ATF family of transcription factors, PKA also phosphorylates the number of other transcription factors, like NF kappa-B, nuclear receptors, high mobility group proteins and Gli-Kruppel family members, etcetera, but we have not discussed all these aspects and if you are interested, you can go and read up some of these things later (( )).

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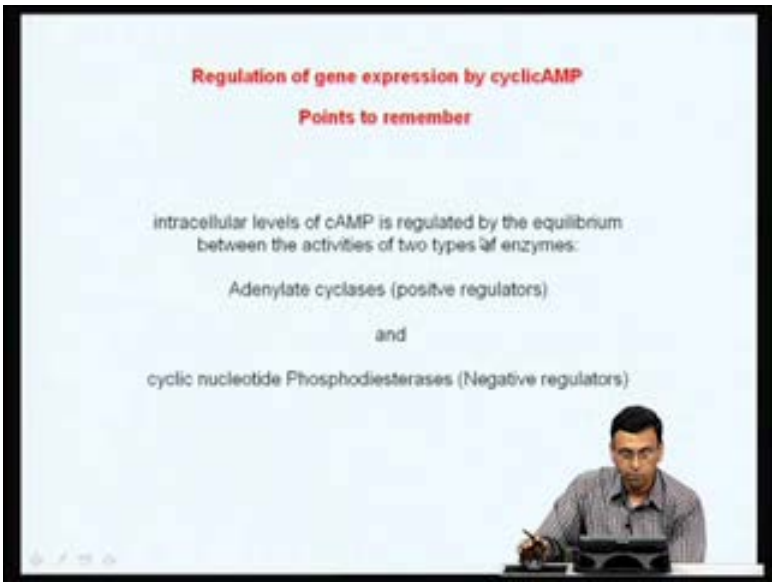

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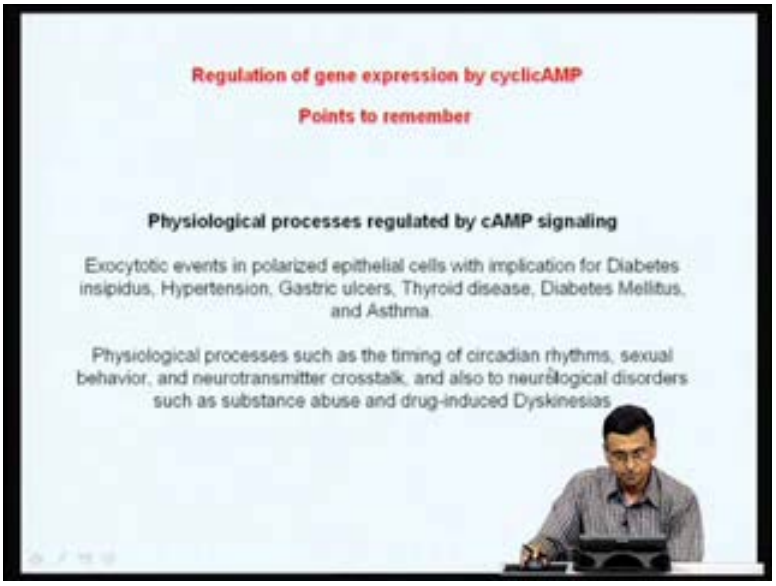

Now, intracellular levels of cyclicAMP is regulated by equilibrium between 2 activities of types of enzymes, adenylate cyclase act as positive regulators of cyclicAMP response;

cyclic nucleotide phosphodiesterases act as negative regulators. So, as soon as cyclicAMP is formed, it is also cleaved by this phosphodiesterases. Now, what are the physiological processes, which are governed by the cyclicAMP signaling? A number of events are regulated by the cyclicAMP, I have listed all these things, but we have discussed only one aspect of this, namely regulation of gene expression, but in addition to that, a number of other processes, exocytotic events, which play very important role in number of disease processes, are regulated by cyclicAMP.

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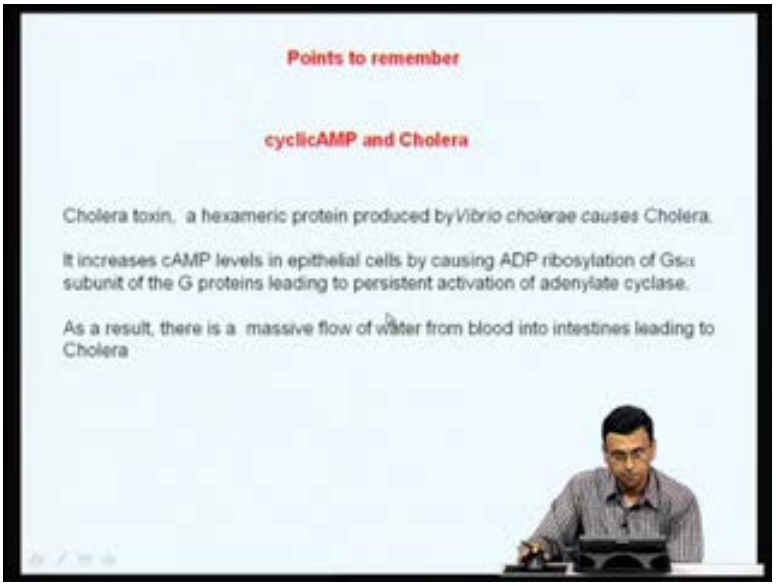

Now, just to give you that there is also very important link between cyclicAMP and cholera. Cholera toxin for example, a hexameric (( )) protein, produced by vibrio-cholerae, it causes cholera and cholera primarily causes because the cholera toxin goes and activates GPCR protein and it increase the cyclicAMP levels in epithelial cells by causing ADP ribosylation of Gs alpha subunit and therefore, there is a persistent activation of the adenylyl cyclase. There is continuous provision of cyclicAMP and as a result, there is a massive flow of water from blood into intestines leading to cholera. So, cyclicAMP is actually involved in diseases like cholera.

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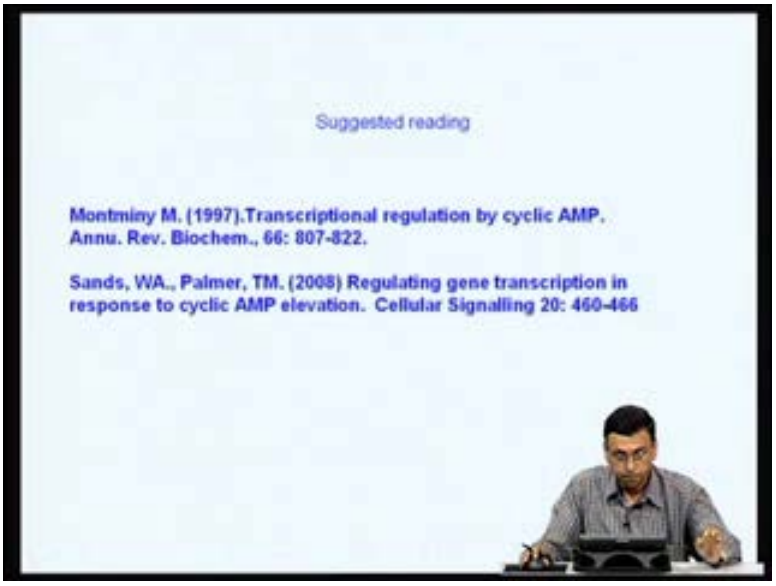

So, I suggest, that you read up at least 2 of these articles, there are number of review articles describing, that cyclicAMP activation, CREB and so on and so forth, but if you are interested in all these things, one can go ahead and read at least 2 of these papers. Montminy is the one who had discovered CREB and has done a very interesting work in the regulation of CREB, and is a very nice article in annual rev of biochemistry. And there is also a very recent review article in cellular signaling about regulating gene transcription response to cyclicAMP elevation, and these 2 papers give you much more inputs about the mechanism via cyclicAMP regulated gene expression.

I think I will stop here.